Research Article

ISOLATION AND ANTIBIOTIC SENSITIVITY OF *Salmonella typhimurium* ISOLATES FROM POULTRY FARMS AND SLAUGHTERHOUSES OF CHITWAN, NEPAL

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ABSTRACT

Nepalese poultry sector is progressing rapidly contributing around 4% of GDP with flourishing impact to provide sustainable and cheapest protein as source of human food. However, with the accelerating pathway, this industry is shed-back by outbreak of several infectious and zoonotic diseases impacting huge economic losses. Salmonella also cause infection in poultry birds and constitutes the largest reservoir of Salmonella organisms in nature. S. typhimurium is an unadopted serotype of Salmonella that may be transmitted to human, animals (poultry) and environment from contaminated food, feed and water. Use of antibiotics, good farm management practices and biosecurity are the treatment and control measures of this disease in poultry production. Development of antibiotic resistant strains of S. typhimurium thus not only pose considerable threat to clinicians, but is also of public health concern as these resistant strains after ingestion are capable of transferring resistance to other pathogens. The present study was undertaken to determine prevalence and antibiotics sensitivity of S. typhimurium strains isolated from domestic poultry from Chitwan, Nepal. Out of 79 farm and 23 chicken slaughterhouse sampling sites, the 107 (19.45%) out of 550 cultured specimens were confirmed for S. typhimurium. Amongst various antibiotics used for in vitro sensitivity testing, the two antibiotics: Colistin (Cl) and Enrofloxacin (Ex), were found to be 100% effective, whereas out of 22 antibiotics, 18 antibiotics showed weak to strong resistance pattern against S. typhimurium isolate. In the present study, Polymyxin and fluoroquinolone antibiotics were found to be most effective against S. typhimurium. Understanding the source of exposure to pathogens (especially resistant pathogens) provides some insight into antibiotic use and misuse in the region. Looking through the lens of One Health approach, the regulation of antibiotic use in animal medicine has direct implications for human health through the increased resistance of foodborne pathogens to vital antibiotic therapy. Thus, judicious use of antibiotics based on their sensitivity pattern should be practiced.

Keywords: Antibiotic, isolation, poultry, resistance, *S. typhimurium*

INTRODUCTION

Poultry industry is agriculture based emerging economic sector of Nepal with flourishing impact to provide sustainable and cheapest protein as source of human food (Singh, 2018). This industry is progressing rapidly throughout the nation with contribution of around 4% of GDP and has been rapidly expanding in the last decade, with an annual growth rate between 17% and 18% and a 261% increase in meat production from 2008 to 2018 (FAO, 2018). Chitwan is one of the largest poultry egg and broiler producing districts in the country and dubbed as "Poultry Capital" of Nepal (Singh, 2018).

Despite the booming poultry business in Nepal, there is a periodic outbreak of common poultry diseases such as Colibacillosis, Salmonellosis, Chronic Respiratory Disease (CRD), Newcastle Disease (ND), Infectious Bursal Disease (IBD), Avian Influenza (AI), Infectious Bronchitis (IB) in different parts of Nepal that created a great loss to poultry industry (Gompo et al., 2019).

Currently, there are more than 2500 Salmonella serotypes prevalent in the world; a few of them are highly host-specific, while majority of them are unadopted and can cause infection in a wide variety of animal species (Gupta and Verma, 1993). Salmonella is widely distributed throughout the world and the most important bacterial agent implicated in outbreaks of the foodborne disease. A few serotypes like *S. typhimurium* and *S. enteritidis* can cause infection in humans, with poultry being a major reservoir (Dar et al., 2017). Different countries show varying salmonella prevalence in poultry. In certain developed countries, about 1% of the poultry flock are positive for Salmonella. However, conditions can affect Salmonella prevalence, as countries having complicated epidemiological situations show 10% of the flock being positive (Rychlik et al., 2014). According to Rabsch et al. (2002), *S. typhimurium*, in comparison to other serovars, is able to cause pathogenesis in broad range of hosts. In order to prevent salmonella infection and propagation there is a need for salmonella-negative breeding flocks and to employ early detection measures, which can be achieved using traditional bacteriological methods. It is a direct occupational anthropozoonosis disease of great economic and public health significance. Poultry egg and food products containing egg are the primary vehicle of infection caused by *Salmonella typhimurium* and *Salmonella enteritidis*, which causes egg-borne human salmonellosis (Messens et al., 2007). Salmonella also cause infection in poultry birds and constitutes the largest reservoir of Salmonella organisms in nature. Several factors contribute to the spread of Salmonella in poultry; of these feed and water contaminated with Salmonella are important sources of infection (Frederick & Huda, 2011).

Various antimicrobials are commonly used in poultry production in Nepal to treat and prevent this disease. Indiscriminate use of antibiotics in treatment of infections in poultry industry has led to the emergence of drug resistant Salmonella strains. The significant increase in production coupled with the likely misuse and overuse of antimicrobials in poultry production in Nepal might be accelerating the development of drug resistant Salmonella in the region (Nelson et al., 2020). Antimicrobial therapy is the first choice of treatment for this bacterial infection; however, antimicrobial resistance has become a problem due to the misuse of antibiotics both in human medicine and animal production (Castro-Vargas et al., 2020)

Meanwhile, antimicrobial resistance is another global threat in animal and human medicine. It's dangers lie mainly in the failure to successfully treat patients infected with antibiotic-resistant pathogens and in the high risk of transmission of such resistant pathogens (Roca et al., 2015). The development of this resistance is related to the misuse of antibiotics, including their use in animal production systems as growth promoters and their excessive use in clinical treatments (Zwe et al., 2018). This is a great concern because much of the antibiotic-resistant *Salmonella* have been acquired through the consumption of contaminated food of animal origin, resulting in health risk to humans and increasing the cost of health care (Eng et al., 2015).

The present study was undertaken to determine prevalence and the antibiotics sensitivity of *S. typhimurium* strains isolated from domestic poultry from Chitwan, Nepal. This study aims to provide baseline data about the prevalence of *S. typhimurium* and characterize the AMR patterns against *S. typhimurium* isolates collected from poultry farms and slaughterhouses in the Chitwan district of Nepal. This data can immediately help guide policies to improve antibiotic stewardship among stakeholders in the district. In addition, this data will lay the groundwork necessary to assess resistance patterns of AMR- *S. typhimurium* in poultry and the potential impact on human health in the region.

MATERIALS AND METHODS

This descriptive cross-sectional study was conducted in the Chitwan district during January to June 2020. 79 poultry farms and 23 chicken slaughterhouses were selected across Chitwan, Nepal's leading poultry producing district. A combination of purposive and judgment sampling was used for the selection of sites to ensure adequate representation and geographic distribution. Sampling farms of different sizes and types, and slaughterhouses in proximity to those farms were representative of regional distribution of poultry and related products. *Salmonella typhimurium* was looked for from 550 specimens collected from poultry birds and dressed carcasses, and identified according to the procedures of Edwards and Ewings (1972).

Collected samples were placed in separate sterile containers in selenite F medium and placed in a cooler with ice for transportation to the laboratory following standard methods. Culture and isolation of *Salmonella typhimurium,* biochemical characterization, and antimicrobial susceptibility testing were carried out at the Vet. Medicine Laboratory, FAVF, AFU, Rampur.

All samples were subjected to selective culture and biochemical testing for the presence of *Salmonella typhimurium.* The broth culture was aseptically streaked on *Salmonella-Shigella* agar (SS) plates for the isolation of Salmonella and incubated at 37 °C for 24 h, after which they were examined for colonies typical of Salmonella. The plates examined for typical colonies of Salmonella (bacterial colonies will appear colorless with black centers). Suspected colonies were sub-cultured and isolated colonies were cultured on nutrient agar plates to obtain pure cultures which were subjected to oxidase testing, gram-staining, and motility testing, as further identification and biochemical characterization. Biochemical identification was done using Gram's stain and oxidase test; all isolates showing Gram's stain positive and/or oxidase-positive were discarded. Further confirmation of *S. typhimurium* isolated were subjected to biochemical tests: Indole, Methyl red, Voges–Proskauer, Citrate utilization, Triple sugar iron (TSI), and urease tests as per the protocol described by Ewing, 1986.

Antimicrobial susceptibility testing was performed on all *Salmonella typhimurium* positive cultures using Kirby– Bauer disc diffusion method outlined by the Clinical and Laboratory Standards Institute (Lalitha, 2004). Antibiotics were selected to represent a broad range of classes which are commonly used in the region in both human and animal medicine. 23 antibiotics tested include: Amoxicillin (Am), Ampicillin (A), Cephalexin (Cp), Chloramphenicol (C), Ciprofloxacin (Cf), Clindamycin (Cli), Cloxacillin (Cx), Colistin (Cl), Co-trimoxazole (Co), Doxycycline (Do), Enrofloxacin (Ex), Furazolidone (Fr), Gentamicin (G), Kanamycin (K), Norfloxacin (N), Oxacillin (O), Penicillin (P), Streptomycin (S), Sulphamethazole (Sx), Tetracycline (T), Trimethoprim (Tr) and Vancomycin (V). Antibiotics sensitivity and resistance prevalence data were summarized.

The prevalence of resistance against specific antimicrobials in individual studies was compiled in tabular form and antibiotic resistance was classified as either sensitive, intermediate and resistant.

All media, antibiotic disc and biochemical testing reagents used in this research were manufactured by HiMedia (HiMedia Laboratories, India). Microsoft Excel 2016 was used for data entry and management and IBM SPSS v25 was used for data analysis.

RESULTS AND DISCUSSION

Out of 79 farms and 23 chicken slaughterhouse sampling sites, the 107 (19.45%) out of 550 cultured specimens (Figure 1) were confirmed for *Salmonella typhimurium* by conventional biochemical tests, which included 85 (18.047%) of all farm samples and 22 (27.848%) of all slaughterhouse swabbed samples, as shown in Table 1.

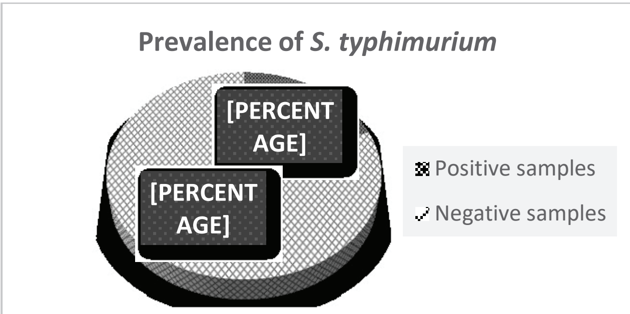

Figure 1. Prevalence of *S. typhimurium* in total samples of farms and slaughterhouse

The overall prevalence of *S. typhimurium* (19.45%) in the poultry farm and slaughterhouse means the high evidence of Salmonellosis, which needs to be treated with the antibiotics. Similarly, the slaughterhouse (27.84%) showed the high significant occurrence of *S. typhimurium* isolates than the prevalence at poultry farm (18.04%).

Table 1. Sample details and result tabulation

Sample Sites	No. of sites	Samples (n)	Positive	Percent
Farm	79	471	85	18.047
Slaughterhouse	23	79	22	27.848
Total	**102**	**550**	**107**	**19.455**

According to Porwollik et al. (2004), *Salmonella typhimurium*, *enteritidis* and *newport* are the most common serotypes existing in food products and are responsible for 50% of salmonellosis. The isolation of invasive Salmonella serotypes such as *S. typhimurium* in this study indicate the public health significance as poultry farms and contaminated chicken meat products may pose health hazards. Also, according to Shivaning et al. (2020) prevalence of Salmonella serotypes in poultry was 3.35%; predominant serotypes isolated were *S. enteritidis* (68.1%) and *S. typhimurium* (31.8%) in India. Similarly, Singh et al. (2013) has reposted that out of 26 isolates of Salmonella, 14 serotyped as *S. typhimurium* indicating a prevalence rate of 53.85% in north India, and this result is very high as compared to finding of this research. In addition to this, Dhakal et al. (2016) found 30.77% *S. typhimurium* isolates from chicken meat of Pokhara, Nepal. With comparision of the previous finding and the finding of this research, the current prevalence of *S. typhimurium* is less which might be due to improvement in farm management, biosecurity and hygiene. However, the finding of Shivhare et al. (2000) isolated 5% *S. typhimurium* from domestic poultry of Madhya Pradesh, India, which is less than this result. *S. typhimurium*, in comparison to other serovars, is able to cause pathogenesis in broad range of hosts. Usually asymptomatic in poultry, infection from meat and eggs causes extensive human illness (Rabsch et al., 2002).

Results on the antibiogram profile of *S. typhimurium* isolates against 22 antimicrobial agents evaluated in this study have been presented in Table 2.

Table 2. Antibiogram (sensitive/intermediate/resistant) pattern of *S. typhimurium* isolates

Antimicrobial agent	Total no. of *S. typhimurium* isolates tested (*n*)	Pattern of antibiogram of *S. typhimurium* isolates		
		Resistant (%)	Intermediate (%)	Sensitive (%)
Ampicillin (A)	107	89 (83.17)	--	18 (16.82)
Amoxicillin (Am)	107	--	33 (30.84)	74 (69.15)
Clindamycin (Cli)	107	107 (100)	--	--
Chloramphenicol (C)	107	6 (5.60)	--	101 (94.39)
Cephalexin (Cp)	107	25 (23.36)	33 (30.84)	49 (45.79)
Ciprofloxacin (Cf)	107	4 (3.73)	--	103 (96.26)
Oxacillin (O)	107	26 (100)	--	--
Colistin (Cl)	107	--	--	107 (100)
Enrofloxacin (Ex)	107	--	--	107 (100)
Gentamicin (G)	107	3 (2.80)	5 (4.67)	99 (92.52)
Kanamycin (K)	107	--	9 (8.41)	98 (91.58)
Norfloxacin (N)	107	2 (1.86)	6 (5.60)	99 (92.52)
Penicillin (P)	107	107 (100)	--	--
Co-trimoxazole (Co)	107	27 (25.23)	--	80 (74.76)
Streptomycin (S)	107	16 (14.95)	5 (4.67)	86 (80.37)
Tetracycline (T)	107	22 (20.56)	62 (57.94)	23 (21.49)
Vancomycin (V)	107	107 (100)	--	--
Doxycycline (Do)	107	11 (10.28)	60 (56.074)	36 (33.64)
Cloxacillin (Cx)	107	88 (82.24)	10 (9.34)	9 (8.41)
Furazolidone (Fr)	107	25 (23.36)	33 (30.84)	49 (45.79)
Sulphamethazole (Sx)	107	66 (61.68)	6 (5.60)	35 (32.71)
Trimethoprim (Tr)	107	88 (82.24)	10 (9.34)	9 (8.41)

Amongst various antibiotics used for in vitro sensitivity testing, the two antibiotics: Colistin (Cl) and Enrofloxacin (Ex), were found to be 100% effective, whereas varying degree of sensitivity are shown by other agents. The per cent sensitivity of strain towards other antibiotics were 94.39% for Chloramphenicol (C), 92.52% for Gentamicin (G), 92.52% for Norfloxacin (N), 91.59% for Kanamycin (K), 80.37% for Streptomycin (S), 74.77% for Co-trimoxazole (Co), 69.16% for Amoxicillin (Am), 45.79% for Cephalexin (Cp), 45.79% for Furazolidone (Fr), 33.64% for Doxycycline (Do), 32.71% for Sulphamethazole (Sx), 21.50% for

Tetracycline (T), 16.82% for Ampicillin (A), 8.41% for Cloxacillin (Cx) and Trimethoprim (Tr), as shown in Figure 2.

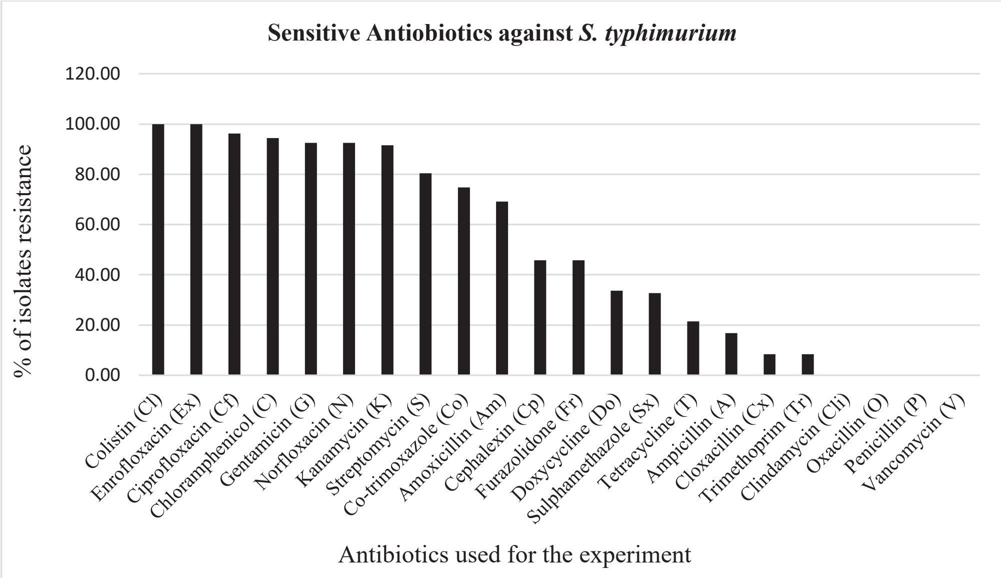

Figure 2. Antibiotics sensitive pattern against *S. typhimurium* isolates

Out of 22 antibiotics, 18 antibiotics showed weak to strong resistance pattern against *S. typhimurium* isolate. Interestingly, Penicillin (P), Clindamycin (Cli) and Vancomycin (V), were resistant to the extent of 100%, as shown in Figure 3. Threlfall et al. (1983) also reported varying degrees of resistance in *S. typhimurium* isolates to Gentamicin, Ampicillin, Tetracyclines, Chloramphenicol and Sulphonamides. Similarly, according to Bhatta et al. (2007), the resistance to Ceftrixiazone was observed in a few isolates of Salm. Typhimurium and Salm. Enteritidis. However, contrary to present report, *S. typhimurium* was highly sensitive to Chloramphenicol and moderately sensitive to Nitrofurantoin and Ampicillin whereas resistant to many antibiotics and the resistance is often plasmid-coded (Taylor et al., 1982). Previous studies conducted in the north India demonstrated 100% resistance to colistin (Singh et al., 2010). However, this research has found 100% sensitivity to Colistin against *S. typhimurium* isolates. The finding of Sunita et al., (2000) also support this finding with their result that all isolates were resistant to Penicillin, Furazolidone, Sulphonamides and Trimethoprim, were found to be the resistant whereas Fluoroquinolones were found to be the most effective against *S. typhimurium*.

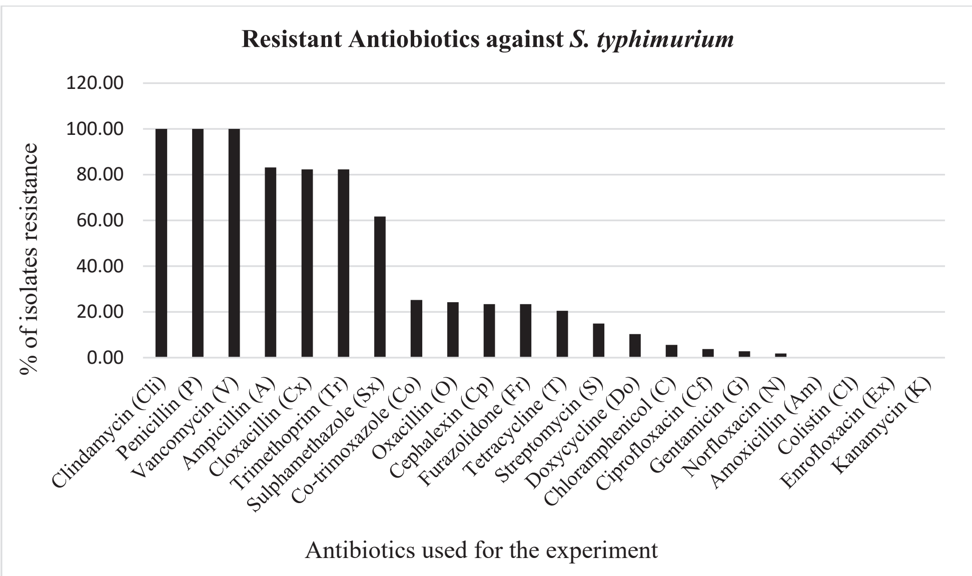

Figure 3 Antibiotics resistant pattern against *S. typhimurium* isolates

A high level of antibiotic resistance in *S. typhimurium* isolate was associated with commonly used antibiotics in poultry production, such as Penicillin, medium levels of antibiotic resistance were associated with antibiotics such as Ampicillin, Sulphamethazole and Trimethoprim. The prevalence of resistant samples to these antibiotics be explained by their frequent administration in veterinary medicine.

In the present study, Polymyxin and fluoroquinolone antibiotics were found to be most effective against *S. typhimurium*. These second-generation quinolones have rapid and prompt bactericidal action, at a very low minimum inhibitory concentration against gyrase-mediated DNA supercoiling (Hooper, 2002). Moreover, higher sensitivity of the isolated to quinolones group of antibiotics might be due to their limited use in poultry as these were introduced recently for use in poultry.

The results are in agreement with those of Okoli et al. (2006) and Chatlod (2007) where Salmonella isolates from commercial poultry feeds showed antimicrobial resistance against nitrofurantoin and tetracycline. Possible reasons for resistance against these antibiotics may have been their indiscriminate use in livestock production and animal husbandry (Cohen & Tauxe, 1986).

Poultry represents an important source of both food security and economic security for a growing number of Nepalese. To ensure the success of this industry and the continued health of the population, it is important to understand the risks posed by foodborne pathogens such as Salmonella (Flower et al., 2021). It is also important to understand and regulate the use of antibiotics within this industry. Establishing good antibiotic stewardship, through collaborative education efforts among veterinarians, para-vets, and farmers could help mitigate the resistance of pathogens to antibiotics and improve the efficacy of treatment. Regulatory oversight of water quality, antibiotic sales and use, animal slaughter practices and facility hygiene, along with consumer education about food safety are critical components to control the spillover of drug-resistant strains of Salmonella from poultry to humans (Sharma et al., 2021).

Keeping in view the change in resistance showed by Salmonella serovars from one place to another, the assessment of antibiogram of Salmonella isolates at farm level is often advantageous for identification of effective antimicrobial agent.

CONCLUSION

S. typhimurium is an unadopted serotype of Salmonella that may be transmitted to human and animals from contaminated food, feed and water. Development of antibiotic resistant strains of *S. typhimurium* thus not only pose considerable threat to clinicians, but is also of public health concern as these resistant strains after ingestion are capable of transferring resistance to other pathogens through episomal transfer of resistance factor. The use of antibiotics against severe cases of gastroenteritis can be lifesaving, but as antibiotic resistance increases among these pathogens, treatment options become scarce and expensive. Understanding the sources of pathogens as well as their antibiotic resistance patterns is important for mitigating transmission and updating treatment protocols.

As the poultry industry grows, so does the use of antibiotics. Further research may help elucidate the genetic factors leading to this resistance. On the other hand, antibiotic use within Nepal is largely unregulated and use within animal medicine does not require veterinary oversight or consultation. Understanding the source of exposure to pathogens (especially resistant pathogens) provides some insight into antibiotic use and misuse in the region. Thus, judicious use of antibiotics based on their sensitivity pattern should be practiced.

To conclude, looking through the lens of One Health approach, the regulation of antibiotic use in animal medicine has direct implications for human health through the increased resistance of foodborne pathogens to vital antibiotic therapy. Extending this network to initiate routine surveillance for AMR pathogens from livestock and implementing regulatory oversight of antibiotic sales and use within the agriculture industry will protect human health, protect animal production and health, and prevent AMR reservoirs from developing in the environment.

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