Antimicrobial Resistance in *Escherichia coli*: a Cross Sectional Study in Chicken Poultry of Kirtipur, Nepal

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ABSTRACT

Objectives: The study was conducted to determine antimicrobial susceptibility pattern and prevalence of Extended Spectrum Beta Lactamase (ESBL) producing *E. coli* from fecal sample of different chicken poultry farm located at Kirtipur.

Methods: The cross sectional study was conducted from November 1st 2019 to February 29th 2020. The samples were collected from 27 different poultry farms and processed at Microbiology laboratory at Trichandra Multiple Campus. Identified *E. coli* were subjected to antimicrobial susceptibility test by using Kirby and Bauer Disc Diffusion technique and Combined disk method was used to determine ESBL *E. coli*.

Results: From all 27 poultry farms, *E. coli* was isolated from broiler (n=13), layer (n=10), and local (n=4) breeds, of which 23 (85.18%) were not registered. The chickens were fed with vitamin and calcium as growth promoter along with antibiotics; Piperacillin, Colistin and Doxycycline. Altogether 85.18% (n=23) isolates showed sensitivity towards Nitrofurantoin, Cefotaxime and Ceftazidime followed by Tigecycline 77.7% (n=21). Among these isolates 66.6% (n=18) were resistance towards Piperacillin followed by Ampicillin 37% (n=10). A statistically significant correlation was seen in resistance rate between broiler and layers. Among total isolates 37.03% (n=10) were Multi Drug Resistance (MDR) and 14.81% (n=4) were ESBL producer. Unregistered poultry farms were associated with MDR and ESBL Ec isolates.

Conclusion: Unregistered poultry farms and irrational use of antibiotics has influenced development of MDR and ESBL isolates. Timely monitoring and surveillance is suggested to decrease the trend of antimicrobial resistance (AMR) in poultry system.

Key words: Chicken feces, *E. coli*, AST, MDR, ESBL

INTRODUCTION

Rapid increment in poultry production in low and middle income countries with high demand for broiler and layer with massive antibiotic abuse has increased in persistence and cross transmission of Antimicrobial resistance (AMR) and Extended Spectrum Beta Lactamase (ESBL) isolates (Hedman et al. 2020; Widodo et al. 2020). Drug resistant profound in infection associated bacteria has led each of these species to give rise to antimicrobial resistance (AMR) against varied antibiotics used in treatment as seen in *E. coli* (Ec) (Dunachie et al. 2020). Since the 1st use of antibiotic drug in poultry in 1946, 20-52% of antibiotics are used only for production in poultry which has resulted in MDR and ESBL Ec colonization in poultry birds (Yang et al. 2019; Ilyas et al. 2021).

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Since the start of commercial production of poultry in 1980, these industries are known to contribute 3-4% of total Gross domestic product (GDP) of the country Nepal where programs prioritizing poultry farms with aim to alleviate poverty are increasing in different provinces of the country (Food and Agriculture Organization 2014). The chicken meat production has increased from 16,662 Metric tons to 255,001 Metric tons from 2008/09 to 2019/2020 (Ministry of Agriculture and Livestock Development 2021) also with indispensible use of antibiotics as growth promoter (Koirala et al. 2021). At one hand Kathmandu valley has been recognized as a center for poultry production where daily consumption of 350,000kg chicken is reported (Anonymous 2021) and on another hand MDR and ESBL Ec isolates from rectal swab (Subramanya et al. 2021) and meat sample (Shrestha et al. 2017) from Kathmandu valley have been reported.

ESBL Ec has been recognized as an indicator organism for ESBL producing Enterobacteriaceae which is regarded major global threat indwelling in and between human, animal and environment hosts (World Health Organization 2021). Consumption of antibiotics is known to be highest in poultry industries aiding in increment of AMR bacteria (Agyare et al. 2019). As the poultry industries are flourishing in Nepal, this study was done to determine the prevalence of MDR and ESBL Ec in poultry farm of Kirtipur, Nepal.

METHODS

Excreted fresh fecal sample of chicken were collected aseptically in wide mouth container from the ground inside of the poultry farm (Ybanez et al. 2018; Cox et al. 2015). Samples were kept in ice box with ice during transportation and reached to laboratory within 3 hours of collection (Zhuang et al 2017).

All the samples were processed for identification of *E. coli* at microbiology laboratory of Trichandra Multiple College, Kathmandu. The fecal samples were suspended in buffered peptone water in 1:10 (w/v) for enrichment and incubated at 37°C for 24hr (Stromberg et al. 2017). After 24 hr, a loop full of sample was streaked onto Mac Conkey Agar plate and incubated for 24hr at 37°C. After 24hr the Lactose fermenting pink colonies were further streaked onto Nutrient Agar plate and incubated for 24hr at 37°C. The colonies from NA plates were taken for Gram Staining, enzymatic (catalase and oxidase) and biochemical (IMViC), TSI, Citrate, O/F and Urease tests and also streaked onto Eosin Methylene Blue Agar (EMB) (Quinn et al 2011). Antibiotic susceptibility test was performed using Kirby and Bauer disc diffusion technique (Hudzicki 2016). The panel of ten different antibiotics categories was used for performing AST, as follows: Ampicillin (10µg), Piperacillin (30µg), Cefoxitin (30µg),, Ceftriaxone (30 µg), Ceftazidime (30 µg), Gentamicin (10 µg), Ciprofloxacin (5 µg), Nitrofurantoin (300 µg), Nalidixic acid (30 µg) and Tigecycline (15 µg). Multi Drug Resistance (MDR) isolates were categorized according to European Centre for Disease Prevention and Control (ECDC) guidelines (Wolfensberger et al. 2019). The ESBL was detected by using combined disk method using Cefotaxime (30µg) and Ceftazidime (30µg) alone and in combined with Clavulanic acid (10µg) as in Clinical Laboratory Standards Institute guidelines (2020).

The correlation coefficient was calculated by IBM SPSS Statistics 21 software as a test for significance. The level of significance was set al $p \leq 0.05$ with 95% confidence intervals.

RESULTS

All of the 27 chicken fecal samples collected showed growth of *E. coli*. Among this 13 were from boiler, 10 from layers and 4 local breed. Out of 27 poultry farms, 23 (85.18%) were not registered whereas 4 (14.82%) were registered (Fig1).

Vitamins and calcium were used as growth promoter in 25 (92.5%) poultry farms, whereas 2 (7.4%) local poultry farm fed zinger and garlic. The local and layer poultry farms 23 (85.15%) fed chickens with antibiotics viz., Pipericillin, Colistin and Doxycycline. Antibiotic Susceptibility test profile showed *E. coli* were highly sensitive towards Nitrofurantoin, Ceftriaxone and Ceftazidime (85.18%). Highest resistance rate was seen against Piperacillin (66.6%) followed by Ampicillin (37.03%). The results showed 22.2% (n=6) isolate resistance to Tigecycline (Table1).

E. coli isolate from Broiler and Layer showed resistance against Piperacillin 76.9% and 70% followed by Ampicillin 53.8% and 60% respectively. The resistance percentage showed by broiler and layer for different antibiotics showed statistically significant pairwise correlation at 0.01 level of significance (p=0.03) (Table 2).

E. coli isolate from broiler 69.2% (n=9) and Layer 10% (n=1) were found to be MDR whereas 23.7% (n=3) and 7.6% (n=1) *E. coli* isolate from broiler and Layers were found to be ESBL producers respectively. The *E. coli* isolates

from the poultry farm showed 10 (37.03%) MDR and 4 (14.81%) were ESBL producers. The MDR isolates in unregistered farm (33.33%) were comparatively higher than registered farm (3.71%). All the ESBL isolates (n=4) were identified from unregistered farm only. None of the isolates from local breeds were MDR or ESBL producer (Table 3). A significant association is seen in between MDR and ESBL isolated and unregistered poultry ($p<0.05$). The MDR isolates showed resistance against various types of antibiotics as shown in Table 4.

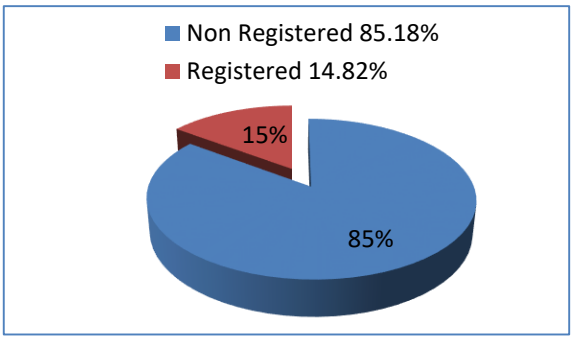

Figure 1: Distribution of poultry farms

Table 1: Antibiotic Susceptibility profile of *E. coli*

Antibiotics	Sensitive		Intermediate		Resistance	
	No.	%	No.	%	No.	%
Ampicillin	8	29.6	9	33.3	10	37
Cefoxitin	19	70.3	0	0	8	29.6
Ceftriaxone	23	85.18	2	7.4	2	7.4
Ceftazidime	23	85.18	2	7.4	2	7.4
Ciprofloxacin	14	51.8	5	18.5	8	29.6
Gentamicin	19	70.3	0	0	8	29.6
Nalidixic acid	17	62.9	5	18.5	5	18.5
Nitrofurantoin	23	85.18	2	7.4	2	7.4
Piperacillin	5	18.51	4	14.81	18	66.6
Tigecycline	21	77.7	0	0	6	22.2

Table 2: Antibiotic sensitivity and resistant profile among breeds of chicken

Antibiotics	Broiler				Layer				Local			
	Sensitive		Resistance		Sensitive		Resistance		Sensitive		Resistance	
	No	%	No.	%	No.	%	No.	%	No.	%	No.	%
Ampicillin	3	23	7	53.8	3	30	6	60	3	75	0	0
Cefoxitin	7	53.8	6	46	8	80	2	20	4	100	0	0
Ceftriaxone	9	69.2	2	15.3	8	80	1	10	4	100	0	0
Ceftazidime	9	69.2	2	15.3	8	80	1	10	4	100	0	0
Ciprofloxacin	4	30.7	7	53.8	1	10	6	60	4	100	0	0
Gentamicin	9	69.2	4	30.7	6	60	4	40	4	100	0	0
Nalidixic acid	5	38.4	5	38.4	8	80	0	0	4	100	0	0
Nitrofurantoin	9	69.2	2	15.3	10	100	0	0	3	75	1	25
Piperacillin	1	7.6	10	76.9	1	10	7	70	3	75	1	25
Tigecycline	9	69.2	4	30.7	9	90	1	10	3	75	1	25

Table 3: MDR and ESBL profiles among breeds in two types of farms

Breed/ Farm	Isolate n (%)		MDR n (%)		ESBL n (%)	
	Registered (n=4)	Unregistered (n=23)	Registered (n=1)	Unregistered (n=9)	Registered (n=0)	Unregistered (n=4)
Broiler	3 (11.11)	10 (37.04)	1 (3.71)	8 (29.62)	0	3 (11.1)
Layer	1 (3.71)	9 (33.33)	0	1 (3.71)	0	1 (3.71)
Local	0	4 (14.81)	0	0	0	0

Table 4: MDR isolate showing resistance against various types of antibiotics

SN	Sample no.	Breed	Antibiotics
1	15	Broiler	CN, CX, PI
2	27	Broiler	CN, CIP, TGC
3	10	Broiler	CN, CIP, NX, PI
4	19	Broiler	CN, CX, CTX, TGC
5	5	Broiler	CN, CIP, NX, PI, AMP
6	12	Layer	CN, CX, CTX, PI, AMP
7	14	Broiler	CN, CX, CIP, NIT, PI
8	18	Broiler	CN, CX, CIP, PI, AMP, TGC
9	23	Broiler	CN, CX, CIP, NX, PI, AMP, TGC
10	24	Broiler	CN, CX,CTX, CIP, NIT, NX, PI, AMP, TGC

AMP: Ampicillin, CIP: Ciprofloxacin, CN: Gentamicin, CX: Cefoxitin, NX: Nalidixic acid, NIT: Nitrofurantoin, CTX:Ceftriaxone, PI: Piperacillin, TGC: Tigecycline

DISCUSSION

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 (Central Bureau of Statistics 2015). The chickens (92.5%) were fed with vitamins and calcium as growth promoter. The vitamin and minerals are generally regarded as nutraceuticals which helps to boost the poultry production by promoting health and development of skeletal muscles (Manikandan et al. 2020). Local chicken breed (n=2) were fed with ginger and garlic. These natural supplements are known to substitute the commercial growth promoter enhancing the longevity and health status of chicken (Karangiya et al. 2016). The antibiotics Piperacillin, Doxycycline and Colistin are used irrationally by the farmers as fed supplement in poultry farms (85.1%) although the dose regimen for various antibiotics has been suggested for use in poultry production (Castanon 2007). Since 1940, the discovery of antibiotics as growth promoter, doxycycline is used for disease prevention, Piperacillin for increasing weight and Colistin for growth enhancement (Manikandan et al. 2020). The population feeding on such commercially grown meat products is known to suffer from obesity (Angulo et al 2005). The study done by Shrestha et al. (2020) showed increase in prevalence of obesity from 4.7% in 1975 to 13.1% in 2014; and consumption of poultry product of 5% in 1980 to 46% in 2021 (Anonymous 2021).

The *E. coli* showed broad range of AMR pattern and were highly resistant towards Piperacillin (66.6%) followed by Ampicillin (37%). Intensification of the poultry production in LMIC for economic upliftment has compromised in quality of poultry system and massive use of growth promoter and antibiotics as fed supplement has resulted in

increased burden of AMR population in poultry (Hedman 2020). A common resistance towards Ampicillin, Tetracycline, 3GC and development of wide range of resistance varieties has been reported (Food and Agriculture Organization 2016). The highly resistance against Piperacillin in our study was due to its use as feed supplement. Lower resistance rate was seen against third generation Cephalosporins (7.4%) which was similar to study done by Bushen et al (2021). Tigecycline resistance was seen in 22.2% of isolates in this study. Tigecycline is the last drug of choice for MDR producing Gram negative isolates and plasmid mediated resistance towards this antibiotic in poultry production has created baleful situation for human population in South Asia (Moshin et al 2021).

Among total isolates about one fourth were MDR among which one fourth were ESBL producer. Food animals are known to be reservoirs of ESBL and ampicillinase producing *E. coli* (FAO 2016) where MDR with 52.5% (Bushen et al. 2021), 75.06% (Rahman et al. 2020) and 43.2% (Manishimwe et al. 2017) have been reported. The lower rate of MDR in our study was due to the study in small sample. Similarly, 37.8% of ESBL Ec has been recorded in Nigeria by Mwambete and Stephen (2015) which were higher than our study but 13.91% of ESBL Ec was reported by Rahman et al. (2020) which was almost similar to our study. A significant correlation was seen in resistant rate of Broiler and Layers in our study which was related to study done by Rahman et al. (2020) and Manishiwe et al. (2017). None of the isolate from local breed was MDR or ESBL producer as antibiotics were not given as feed supplements. The major contributing factor for AMR resistant development is prolonged use of sub therapeutic level of antibiotics as growth promoter in poultry production (Food and Agriculture Organization 2016). ESBL producers are known to bear diverse antibiotic resistance gene. Meanwhile poultry products are recognized as potential source for ESBL producing bacteria burgeoning MDR related bacterial infection in human community (Falgenhauer et al 2019). The presence of an antibiotic residue in consumable poultry product above the tolerance level is known to cause health severity in human ranging from rise of MDR to cell toxicity (Trieiber and Knaeuer 2021). The poultry farms are to be regulated timely for any unforeseen hazards in near future.

CONCLUSION

The poultry industries are one of the contributors to National GDP of Nepal but lack of regulation system has increased unregistered poultry farm. The major breed in poultry industries are broiler and layers. The poultry farmers are dependent mostly on the artificial growth promoter and irrational use of antibiotics which have resulted in the increased burden of AMR with ESBL Ec in poultry system.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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