

# Investigation into the quinolone resistant *E. coli* isolated from commercial broilers

## Abstract

Antimicrobial resistance is an overwhelming issue in human and veterinary medicine. Association between usage of antimicrobial in livestock and emergence of antimicrobial resistance has been widely discussed. Antimicrobial resistance surveillance is the only alternative to know the exact going on in the field. Quinolone is one of the common antimicrobial used in the poultry husbandry in Sri Lanka. The objectives of this study were to determine and to investigate into quantitative quinolone resistance in *E. coli* isolated in commercial broilers and to determine common quinolone resistant genes in *E. coli*.

The samples were collected from a collection of *E. coli* which were isolated and identified by a previous study. A collection of *E. coli* (n=123) were shown resistant to quinolone by disk diffusion carried out previously. All isolates were used for the current study was again confirmed as *E. coli* by Gram test, TSI, Urease test, SIM and indole test as described. The phenotypically resistant *E. coli* were selected for the MIC by agar dilution test as described (EUCAST). Conventional PCR test carried out to detect selected quinolone resistant genes in *E. coli*. EUCAST clinical breakpoint was used for interpretation of ciprofloxacin in *E. coli* and other guidelines were used for enrofloxacin and nalidixic acids.

Over 96.7% of isolates were shown high MIC as 64 µg/ml and 128 µg/ml against nalidixic acids. More than 86.2% were shown high MIC as 8 µg/ml against enrofloxacin. A 74.8% of *E. coli* were shown equivalent or more than 1 µg/ml for ciprofloxacin. *qnrB* was found in majority of isolates as 50.4% of quinolone resistant *E. coli* were positive for the gene. *gyrA* was found in 35.8% of *E. coli* and *qnrC* and *aac(6)-Ib-cr* were also found in small percentage in the study. None of isolates were shown positive for *qnrA*, *qnrD*, *aqxAB* and *qepA* in the study

Emergence of plasmid mediated quinolone resistance is an alarming finding in the poultry industry. Since quinolone is a critically importance antimicrobial in human medicine, minimizing emergence and spreading of antimicrobial resistance is vital importance in animal husbandry. Avoiding over usage and misusing quinolone and evidence based usage of antimicrobial is highly recommended to minimize risk of spreading quinolone resistance in poultry

## Introduction

Antimicrobial resistance is overwhelming issue in both human and veterinary medicine (Hricová et al., 2017). Resistant organism may spread from food to human and potential source of antimicrobial resistance in human (Roth et al., 2019). Mostly antimicrobial usage in livestock has

been criticized and related to the emergence of antimicrobial resistance in human either directly or indirectly (Hricová et al., 2017). Importantly, inappropriate usage antimicrobials has been identified as a common practices both in human and veterinary practices (Caruso et al., 2018). Although the usage of antimicrobials in livestock farming has been accepted and recommended internationally, intensive farming has accelerated over usage of antimicrobials in poultry husbandry (Caruso et al., 2018). In addition, risk of foodborne transmission of antimicrobial resistant determinants has been widely discussed (Hricová et al., 2017). Furthermore, multiple factors related to dissemination of antimicrobials from livestock to human has been investigated, existing gaps of knowledge need to be overcome through understanding molecular resistance mechanism at cellular level. Conversely, poultry products such as meat and eggs are considered as potential source of multi drug resistance in human (Hricová et al., 2017; Woźniak-Biel et al., 2018).

In Sri Lanka, over 600 000 farmers are engaged in poultry farming consisting commercial broilers, commercial layers and breeder farming. Consequently, the consumption of chicken was high as 10.2 Kg per head per year and the egg consumption was 120 eggs per capita in 2019 (DAFH, Sri Lanka). Antimicrobials are used in poultry for treating, prophylaxis and metaphylaxis purpose, quinolone is also a common antimicrobial used together with beta lactams, macrolides, tetracycline, sulfa trimethoprim, and aminoglycoside (VDCA in DAPH, Sri Lanka). Quinolone is synthetic antimicrobials which is shown broad spectrum activity against infection caused by Gram negative bacteria (Parry et al., 2010; Hao et al., 2013; Hricová et al., 2017). World health organization (WHO) has announced as quinolone as critically important antimicrobial, quinolone resistance has been significantly increased worldwide (Liu et al., 2012; Caruso et al., 2018; Woźniak-Biel et al., 2018). Only enrofloxacin is used in poultry and ciprofloxacin is not allowed while no commercial products are found for poultry farming (VDCA in DAPH, Sri Lanka). The 10% oral solution is the most common type of enrofloxacin in poultry. *E. coli* is a good indicator on antimicrobial resistance in microbial population, it is also reservoirs of resistant genes and dissemination of resistance agent from *E. coli* to other microbes has also been demonstrated (Caruso et al., 2018). In addition, *E. coli* is widespread bacterium both in human and animal, only simple technique is required to isolate and identify the organism in the laboratory. In addition, comparatively larger accessory genome with millions of mobile genetic elements provided storage of ample amount of resistant genetic determinates in the genome (Caruso et al., 2018).

In resistance mechanism, both chromosomal mutation and plasmid mediated resistance mechanism have been found in bacteria as *E coli* (Caruso et al., 2018). Plasmid mediated resistant genes cause low level resistant against quinolone (Koyama et al., 2020). The resistance to quinolone has been increased and it was 50-100% in Brazil. It was 70-90% in Poland and over 50% in UK. Importantly, resistance to quinolone has increased from 91% in 2016 in Spain and it was 16% in 2001. Since qualitative phenotypic resistance against quinolone has been increased in recent past, the objective of this study was to determine and to investigate into quantitative

quinolone resistance in *E. coli* isolated in commercial broilers. The second objective was to determine common quinolone resistant genes in *E. coli*.

### Material and methods

The samples were taken from a collection *E. coli* isolates at bacteriology division, Veterinary Research Institute, Peradeniya, Sri Lanka. All *E. coli* were shown resistant to quinolone by the disk diffusion test as described methods previously (Priyantha, et al, 2021). The isolates were stored in -40°C for one year.

The isolates were cultured on 5% sheep blood agar, MacConkey and incubated for 24 hours at 35°C. Overnight incubated cultures were used for series of biochemical test such as Gram test, TSI, Urease test, SIM and indole test according to the methods described by previously (Priyantha, et al, 2021). Only confirmed isolates (n=123) as *E. coli* was taken for the current study. The antimicrobial susceptible testing were performed for three different type of quinolone as nalidixic acid, enrofloxacin and ciprofloxacin by agar dilution method as described in EUCAST (www. EUCAST). Convectional PCR test carried out to detect selected quinolone resistant genes in *E. coli* as described previously (Parry et al., 2010; Hao et al., 2013). EUCAST clinical breakpoint was used for interpretation of ciprofloxacin in *E. coli* and other guideline were used for enrofloxacin and nalidixic acids.

### Results

Over 96.7% of isolates which shown resistant by the disk diffusion test were found high MIC as 64 µg/ml and 128 µg/ml against nalidixic acids. It was also similar in enrofloxacin and more than 86.2% were shown high MIC as 8 µg/ml. Furthermore, 74.8% of *E. coli* were shown equivalent or more than 1 µg/ml for ciprofloxacin in the collection.

In addition, *qnrB* was found in majority of isolates as 50.4% of quinolone resistant *E. coli* were positive for the gene. *gyrA* was found in 35.8% of *E. coli* and *qnrC* and *aac(6)-Ib-cr* were also found in small percentage in the study. None of isolates were shown positive for *qnrA*, *qnrD*, *aqxAB* and *qepA* in the study.

Table: 1 MIC distribution of *E. coli* for nalidixic acids, enrofloxacin and ciprofloxacin (n=123).

Substance	Resistant %	Distribution of MIC values $\mu\text{g/ml}$															
		0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512
nalidixic acid	95.1						1	1	0	1	3	6	1	2	16	58	34
enrofloxacin	96.7		1	1	2	7	8	3	8	9	20	6	20	23	11		
ciprofloxacin	98.4		0	1	1	3	26	44	14	17	15	0	0	0	0		

Table: 2 Resistant genes identified in the by conventional PCR

Name of the gene	Positive in number	%
GyrA	53	35.8
qnrA	0	0
qnrB	62	50.4
qnrC	3	2.4
qnrD	0	0
qnrS	16	13.0
aac(6')-Ib-cr	8	6.5
aqxAB	0	0
qepA	0	0
parC	0	0

parE	0	0
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## Discussion

Poultry is the most popular type of animal protein among Sri Lankans and the demand for chicken has been increased. Quinolone is one of the most commonly used antimicrobials in the poultry farming in Sri Lanka, Over 3700 Kg of quinolone sales has been reported in 2020 in the country (VDCA, DAPH). Although limited studies were found previously, resistance to quinolone is also an overwhelming issue in global poultry industry, more than 40% resistance has been reported in poultry producing regions as in Brazil, China, Africa and European Union (Roth et al., 2019; Theobald et al., 2019).

Over 96% were shown resistant by the agar dilution test to quinolone and all were shown resistant by disk diffusion test using ciprofloxacin 5 µg disk previously (Parry et al., 2010; Woźniak-Biel et al., 2018). Importantly, High MICs were found for all quinolones used in the study. In addition, 100% phenotypic resistance had been shown in Poland (Liu et al., 2012; Roth et al., 2019). Simultaneously 81% of quinolone resistance was observed in layers, Taiwan previously (Yeh et al., 2017). Therefore, quinolone resistance is a threatening issue in global poultry industry. Alternatively, reduction of usage of quinolone has responded positively, quinolone resistance in *E. coli* has been reduced in France as a result of reduction of quinolone in poultry (Perrin-Guyomard et al., 2020). In addition, the efficacy was of ciprofloxacin in determination of quinolone resistance in *E. coli* was satisfied in the study, Nalidixic acids was shown a highest MIC in the study. Similar finding has been reported in some other studies globally, the highest phenotypic resistance was observed with nalidixic acids, and all isolates were shown resistant for nalidixic acid, enrofloxacin by MIC determination of agar dilution test (Parry et al., 2010; Woźniak-Biel et al., 2018). The phenotypic quinolone resistance has been reported on the second and the third generation, over 90% of resistance was observed against gatifloxacin and levofloxacin (Del Rio-Avila et al., 2016).

The ranges of MICs were shown low in nalidixic acids and enrofloxacin comparing to enrofloxacin in the study. A 97.5% isolates were shown resistant for ciprofloxacin by agar dilution test although all were resistant by the disk diffusion test. MICs of nalidixic acids (0.5- >258 µg/ml), enrofloxacin (0.03-128 µg/ml) and ciprofloxacin (0.03-8 µg/ml) were shown high in the study. Importantly, high quantitative margin MICs were found against three types of quinolones, and which can be alarming signal on emergence of quinolone resistance in *E. coli*, commercial poultry. Although the exact reason is difficult to conclude, high usage of quinolone in commercial poultry may be considered as reason for emergence of high percentage of resistance on quinolone. Conversely, results of our study are a good indicator of high prevalence and quantification of quinolone resistance *E. coli* in gastrointestinal tract of the commercial poultry. The antimicrobial therapy on the first two weeks of commercial broilers may have effect

on phenotypic resistance of antimicrobial resistance in food animals. In addition, low level of frequency of resistance was also been reported against plasmid mediated quinolone resistance in *E.coli* from poultry (Yue et al., 2008). Although ciprofloxacin is not used in livestock Sri Lanka, presence of resistance is an indication potential risk of emerging antimicrobial resistance and dissemination resistant genes high although lack of field usage.

Plasmid mediated quinolone resistance has reported by many authors in commercial broiler and layers (Seo and Lee, 2019). In Europe and Taiwan, *qnrS* was found common than *qnrB* (Hricová et al., 2017; Yeh et al., 2017). *qnrB* was the most common plasmid mediated quinolone resistant gene in the study and *qnrS* was found in 13% of *E.coli*. Importantly, *qnrB* and *qnrS* both are usually found in plasmid, small mobile genetic element, dissemination of resistant gene among chicken may be high than chromosomal mediated quinolone resistance in *E coli*. *qnrB* was first detected in *Klebsiella pneumoniae* strain isolated in South India (Jacoby, 2006). *QnrB* and *QnrS* share 41% and 60% amino-acid identity with *QnrA* respectively, (Poirel, 2006). However, *qnrB* alone was shown low level resistance against all quinolone in *E coli* at the initial point (Woźniak-Biel et al., 2018). Our study *GyrA* were observed as the second common resistant genes found which caused chromosomal mutation in dissemination of resistant is minimum in animal's body. In China and Mexico, *GyrA* were found common in *E. coli* and over 97% in phenotypically resistant isolates of *E. coli* of chicken (Liu et al., 2012; Del Rio-Avila et al., 2016). In addition, *qnrB* and *qnrS* were found common in *E. coli* from commercial broilers and *qnrA*, *qnrC*, *qnrD* and *qnrE* were not observed in the study. In addition, *aqxAB* and *qupA* were also not shown in this collection of *E. coli*.

In addition, quinolone resistant *E coli* were shown multi drug resistance in the study although which was not quantified. *E coli*, multi drug resistance was also shown in the study although which was not the primary objective of the study. Multi drug resistance were found common in *E coli* from commercial poultry in Africa (Laarem et al., 2017; Theobald et al., 2019).

### **Conclusion**

Resistance to quinolone was shown high in *E coli* isolated from commercial broilers and both chromosomal and plasmid mediated resistance were reported high. Therefore, evidence based antimicrobial resistance is strongly encouraged in poultry farming in order to control emerging quinolone resistance in poultry and human.

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