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

Abstract

Antimicrobial resistance is an over whelming issue in human and animal 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 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.*

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 before used 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). Convectional 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 guideline 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* was shown equivalent or more than 1 µg/ml for ciprofloxacin. *qnr*B 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 this 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

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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, livestock products such as poultry are considered as potential source of multi drug resistance in human (Hricová et al., 2017; Woźniak-Biel et al., 2018).

The commercial poultry is considered as an important livestock product in Sri Lanka, the consumption of chicken was high as 10.2 Kg per head per year in 2019 (DAPH., Sri Lanka). The egg consumption was 120 eggs per capita per year in 2019 (DAPH., Sri Lanka). Over 600 000 farmers are engaged in poultry farming consisting commercial broilers, commercial layers and breeder farming in Sri Lanka (DAPH., 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 bacteria (Parry et al., 2010; Hao et al., 2013; Hricová et al., 2017). Quinolone is one of the common antimicrobials used in poultry treating Gram negative bacterial infections in poultry farming(Caruso et al., 2018). The annual usage of quinolone was 16851 Kg in 2020 in the country (VDCA in DAPH, Sri Lanka). 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 also been demonstrated (Caruso et al., 2018). In addition, *E. coli* is widespread distribution both in human and animal, simple technique to isolates and identification in the laboratory and comparatively larger accessary genome with millions of mobile genetic elements (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). Importantly, 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 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

A samples (n=123) were collected randomly from isolates collection at bacteriology division, Veterinary Research Institute, Peradeniya, Sri Lanka. The all isolates were shown resistant to quinolone by disk diffusion test using ciprofloxacin previously (Priyantha, e al, 2021). 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 previously (Priyantha, e al, 2021). The phenotypically resistant *E. coli* were selected for the MIC by agar dilution test as described (EUCAST). Convectional 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 guideline were used for enrofloxacin and nalidixic acids(Parry et al., 2010; Hao et al., 2013).

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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, *qnr*B 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% if 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.

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

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Substance	Resistant %	**Distribution of MIC values** µg/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

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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

Discussion

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Chicken is the most popular meat among Sri Lankan and the demand for chicken has been increased every year except the period of COVID 19 pandemic in the country in 2020-21. Quinolone resistance is also an overwhelming issue in current poultry industry, more than 40% resistance to quinolone has been reported in poultry producing regions as in Brazil, China, Africa and European Union (Roth et al., 2019; Theobald et al., 2019). Europe. Importantly, 100% phenotypic resistance had been shown Poland (Liu et al., 2012; Roth et al., 2019). Simultaneously 81% of quinolone resistance was observed in layers, Taiwan previously (Yeh et al., 2017). The all isolated were shown resistant to quinolone by disk diffusion test using ciprofloxacin 5µ*g*disk (Parry et al., 2010; Woźniak-Biel et al., 2018). Conversely, quinolone resistance in E. coli has been reduced in France as a result of reduction of quinolone in poultry (Perrin-Guyomard et al., 2020). The efficacy was satisfied in usage of ciprofloxacin in determination of quinolone resistance in *E. coli* only few percentages were shown different susceptibility in MIC test by agar dilution. 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). Importantly, over 90% of resistance was obsvered against gatifloxacin and levofloxacin(Del Rio-Avila et al., 2016). However, 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. The ranges of MICs were shown low in nalidixic acids and enrofloxacin comparing to enrofloxacin 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 in difficult to conclude, high usage of quinolone in commercial poultry may be

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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 week 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 .

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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, *qnr*B 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, respectively, with QnrA (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, *qnr*B and *qnr*S 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 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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References

Caruso, G., Giammanco, A., Cardamone, C., Oliveri, G., Mascarella, C., Capra, G., Fasciana, T., 2018. Extra-Intestinal Fluoroquinolone-Resistant Escherichia coli Strains Isolated from Meat. BioMed Research International 2018, 1–7. doi:10.1155/2018/8714975

Del Rio-Avila, C., Rosario, C., Arroyo-Escalante, S., Carrillo-Casas, E.M., Díaz-Aparicio, E., Suarez-Güemes, F., Silva-Sanchez, J., Xicohtencatl-Cortes, J., Maravilla, P., Hernández-Castro, R., 2016. Characterisation of quinolone-resistant *Escherichia coli* of 1997 and 2005 isolates from poultry in Mexico. British Poultry Science 57, 494–500. doi:10.1080/00071668.2016.1187716

Hao, H., Pan, H., Ahmad, I., Cheng, G., Wang, Y., Dai, M., Tao, Y., Chen, D., Peng, D., Liu, Z., Huang, L., Yuan, Z., 2013. Susceptibility Breakpoint for Enrofloxacin against Swine Salmonella spp. J Clin Microbiol 51, 3070–3072. doi:10.1128/JCM.01096-13

Hricová, K., Röderová, M., Pudová, V., Hanulík, V., Halová, D., Julínková, P., Dolejská, M., Papoušek, I., Bardoň, J., 2017. Quinolone-resistant Escherichia coli in Poultry Farming. Cent Eur J Public Health 25, 163–167. doi:10.21101/cejph.a4328

Koyama, S., Murase, T., Ozaki, H., 2020. Research Note: Longitudinal monitoring of chicken houses in a commercial layer farm for antimicrobial resistance in Escherichia coli with special reference to plasmid-mediated quinolone resistance. Poultry Science 99, 1150–1155. doi:10.1016/j.psj.2019.09.005

Laarem, M., Barguigua, A., Nayme, K., Akila, A., Zerouali, K., El Mdaghri, N., Timinouni, M., 2017. Occurrence of plasmid-mediated quinolone resistance and virulence genes in avian Escherichia coli isolates from Algeria. J Infect Dev Ctries 11, 143–151. doi:10.3855/jidc.8643

Liu, B.-T., Liao, X.-P., Yang, S.-S., Wang, X.-M., Li, L.-L., Sun, J., Yang, Y.-R., Fang, L.-X., Li, L., Zhao, D.-H., Liu, Y.-H., 2012. Detection of mutations in the gyrA and parC genes in Escherichia coli isolates carrying plasmid-mediated quinolone resistance genes from diseased food-producing animals. Journal of Medical Microbiology 61, 1591–1599. doi:10.1099/jmm.0.043307-0

Parry, C.M., Thuy, C.T., Dongol, S., Karkey, A., Vinh, H., Chinh, N.T., Duy, P.T., Thieu Nga, T.V., Campbell, J.I., Van Minh Hoang, N., Arjyal, A., Bhutta, Z.A., Bhattacharya, S.K., Agtini, M.D., Dong, B., Canh, D.G., Naheed, A., Wain, J., Tinh Hien, T., Basnyat, B., Ochiai, L., Clemens, J., Farrar, J.J., Dolecek, C., Baker, S., 2010. Suitable Disk Antimicrobial Susceptibility Breakpoints Defining *Salmonella enterica* Serovar Typhi Isolates with Reduced Susceptibility to Fluoroquinolones. Antimicrob Agents Chemother 54, 5201–5208. doi:10.1128/AAC.00963-10

Perrin-Guyomard, A., Jouy, E., Urban, D., Chauvin, C., Granier, S.A., Mourand, G., Chevance, A., Adam, C., Moulin, G., Kempf, I., 2020. Decrease in fluoroquinolone use in French poultry and pig production and changes in resistance among E. coli and Campylobacter. Vet Microbiol 243, 108637. doi:10.1016/j.vetmic.2020.108637

Priyantha, e al, 2021. Determination of Carbapenem and Extended Spectrum Beta Lactamases in E. Coli from Commercial Broilers, Sri Lanka. Asian Journal of Research in Animal and Veterinary Sciences, Asian Journal of Research in Animal and Veterinary Sciences 7, 16–22.

Roth, N., Käsbohrer, A., Mayrhofer, S., Zitz, U., Hofacre, C., Domig, K.J., 2019. The application of antibiotics in broiler production and the resulting antibiotic resistance in Escherichia coli: A global overview. Poult Sci 98, 1791–1804. doi:10.3382/ps/pey539

Seo, K.W., Lee, Y.J., 2019. Detection of plasmid-mediated quinolone resistance genes in β-lactamase-producing Escherichia coli isolates from layer hens. Poult Sci 98, 1480–1487. doi:10.3382/ps/pey545

Theobald, S., Etter, E.M.C., Gerber, D., Abolnik, C., 2019. Antimicrobial Resistance Trends in Escherichia coli in South African Poultry: 2009-2015. Foodborne Pathog Dis 16, 652–660. doi:10.1089/fpd.2018.2612

Woźniak-Biel, A., Bugla-Płoskońska, G., Kielsznia, A., Korzekwa, K., Tobiasz, A., Korzeniowska-Kowal, A., Wieliczko, A., 2018. High Prevalence of Resistance to Fluoroquinolones and Tetracycline *Campylobacter* Spp. Isolated from Poultry in Poland. Microbial Drug Resistance 24, 314–322. doi:10.1089/mdr.2016.0249

Yeh, J.-C., Lo, D.-Y., Chang, S.-K., Chou, C.-C., Kuo, H.-C., 2017. Prevalence of plasmid-mediated quinolone resistance in Escherichia coli isolated from diseased animals in Taiwan. J Vet Med Sci 79, 730–735. doi:10.1292/jvms.16-0463

Yue, L., Jiang, H.-X., Liao, X.-P., Liu, J.-H., Li, S.-J., Chen, X.-Y., Chen, C.-X., Lü, D.-H., Liu, Y.-H., 2008. Prevalence of plasmid-mediated quinolone resistance qnr genes in poultry and swine clinical isolates of Escherichia coli. Veterinary Microbiology 132, 414–420. doi:10.1016/j.vetmic.2008.05.009