Prevalence of qnrS-positive Escherichia coli from chicken in Thailand and possible co-selection of isolates with plasmids carrying qnrS and trimethoprim-resistance genes under farm use of trimethoprim

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ABSTRACT One hundred and twenty chicken samples from feces (n = 80), the carcass surface at slaughter (n = 20), and retail chicken meat from 5 markets (n = 20) collected during 2018 and 2019 were examined for the prevalence of plasmid-mediated quinolone resistance (PMQR) in Escherichia coli. We detected qnrS-positive E. coli in a total of 74 samples from feces (n = 59), the carcass surface (n = 7), and retail meat (n = 8). These 74 qnrS-positive isolates were tested for antimicrobial susceptibility to determine the minimum inhibitory concentrations (MICs) of certain antimicrobials and genetically characterized. Ampicillin-resistance accounted for 71 of the 74 isolates (96%), followed by resistance to oxytetracycline (57/74; 77%), enrofloxacin (ERFX) (56/74; 76%), sulfoisoxazole (SUL) (56/74; 76%), trimethoprim (TMP) (49/74; 66%), and dihydrostreptomycin (48/74; 65%). All farm-borne SUL- and TMP-resistant isolates except one were obtained from samples from farm A where a combination of sulfadiazine and TMP was administered to the chickens. Concentrations of ERFX at which 50 and 90% of isolates were inhibited were 2 μg/mL and 32 μg/mL, respectively. Diverse pulsed-field gel electrophoresis (PFGE) patterns of XbaI-digested genomic DNA were observed in the qnrS-positive isolates from fecal samples. Several isolates from feces and the carcass surface had identical XbaI-digested PFGE patterns. S1-nuclease PFGE and Southern blot analysis demonstrated that 7 of 11 dfrA13-positive fecal isolates carried both the qnrS and dfrA13 genes on the same plasmid, and 2 of 3 dfrA1-positive isolates similarly carried both qnrS and dfrA1 on the same plasmid, although the PFGE patterns of XbaI-digested genomic DNA of the isolates were different. These results suggest that the qnrS gene is prevalent in chicken farms via horizontal transfer of plasmids and may partly be co-selected under the use of TMP.

Key words: chicken, Escherichia coli, plasmid-mediated quinolone resistance, qnrS, trimethoprim

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INTRODUCTION

Antimicrobial resistance occurs as a result of antimicrobial usage and is a global problem to human and animal health (McEwen and Collignon, 2018). The inappropriate use of antimicrobial medications important to humans in animals and the in-feed use of these drugs are major concerns. Quinolones, including fluoroquinolones are categorized as “highest priority critically important” antimicrobials for human medicine (WHO, 2019) and have been used in food animals in many countries (Collignon et al., 2016; Roth et al., 2019), including poultry in Thailand (Nhung et al., 2016; Wangroongsarb et al., 2021).

Although chromosomal mutations in topoisomerase genes are well-recognized for quinolone resistance, plasmid-mediated quinolone resistance (PMQR) was first described in 1998 in a clinical isolate of Klebsiella pneumoniae (Martínez-Martínez et al., 1998) and additional PMQR genes in Enterobacteriaceae have been found in isolates from various species worldwide (Jacoby et al., 2014). However, PMQR potentially facilitates the selection of higher levels of quinolone resistance in the presence of quinolones (Poirel et al., 2006; de Toro et al., 2010; Nishikawa et al., 2019). PMQR genes in Salmonella isolates from chickens (Cavaco et al., 2007; Sinwat et al., 2015) and pigs (Luk-In et al., 2017) in
Because E. coli Bacterial Isolates from meat chicken origin in Thailand have been reported. To the authors investigated using genetic analyses. To clarify prevalence of PMQR and several important antimicrobials categorized by WHO (2019) among E. coli isolates of meat chicken origin in Thailand, the antibiotic use on farms and the prevalence of the PMQR gene was determined. Because a combination of sulfadiazine and trimethoprim (TMP) was used at one of the participating farms, possible association of the use of the drug and the prevalence of the PMQR gene was investigated using genetic analyses.

MATERIALS AND METHODS

Sample Collection

Cloacal swabs were obtained from 50 chickens in August 2018 and 20 chickens in March 2019 at farm A, and 10 chickens in March 2019 at farm B. A combination of sulfadiazine and TMP was orally administered to the chickens at farm A; the indication and dose were not available. In the slaughter facilities situated in close proximity to each farm, the surfaces of 10 carcasses each from farms A and B were sampled in August 2018 and March 2019, respectively, by rubbing with cotton applicators over a total area of 5 × 5 cm. Twenty pieces of chicken meat were obtained from 3 supermarkets and 2 fresh markets in July 2018, and 2 supermarkets and 2 fresh markets in May 2020. All the samples were kept at 4°C and during transportation to the laboratory for 4 to 6 h.

Bacterial Isolates

Cloacal swab samples were suspended in saline and a loopful of suspension was spread onto deoxycholate hydrogen sulfide lactose (DHL) agar plates (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) supplemented with or without 0.05 µg/mL enrofloxacin (ERFX) and incubated at 37°C for 20 h. Each cotton applicator that was applied to a carcass was immersed in 3 mL heart-infusion broth and incubated at 37°C for 20 h. Approximately 3 g of each meat sample was added to 30 mL of heart-infusion broth and incubated as described above. The broth cultures were plated onto DHL agar and incubated as above. Two potential colonies per plate were picked and identified as E. coli using polymerase chain reaction (PCR) targeting the beta-glucuronidase gene (uidA) (McDaniels et al., 1996).

Antimicrobial Susceptibility Testing

Minimum inhibitory concentrations (MICs) of ERFX were determined using an agar dilution method based on Clinical Laboratory Standards Institute (CLSI) document M7-A8 (Clinical Laboratory Standards Institute 2009). E. coli ATCC 25922 was used for quality control. MICs have been interpreted using previously defined resistance breakpoints (2 µg/µL) (Kojima et al., 2009). One isolate harboring the qnrS gene (see RESULTS) was arbitrarily selected from each sample set. For these representative isolates, the MICs of other antimicrobials were determined based upon the following resistance breakpoints: ampicillin (AMP), 32 µg/mL; cefiofur (CTF), 8 µg/mL; dihydrostreptomycin (DSM), 32 µg/mL; oxytetracycline (OTC), 16 µg/mL; chloramphenicol (CHL), 32 µg/mL; and trimethoprim (TMP), 16 µg/mL. For sulfisoxazole (SUL), 512 µg/mL was adopted as the breakpoint according to CLSI document M100-S20 (Clinical Laboratory Standards Institute 2010).

PCR Detection of Antimicrobial Resistance Genes

Isolates exhibiting an EFRX MIC >0.25 µg/mL were screened for eight PMQR genes using multiplex (qnrD and oqxAB) or simplex (qnrS, qnrA, qnrB, qnrC, qepA, and aac(6’)-Ib-cr) PCR (Park et al., 2006; Robicsek et al., 2006b; Chmelnitsky et al., 2009; Ciesielczuk et al., 2013). For isolates resistant to TMP, 5 genes (dfrA1, dfrA5, dfrA7, dfrA9, and dfrA13) responsible for this resistance were screened using PCR with primer pairs described by Maynard et al. (2004). For isolates exhibiting CTF MICs above the breakpoint (8 µg/mL), PCR detection of CTX-M-type beta-lactamase genes was performed using specific primer sets (Saishu et al., 2014).

Pulsed-Field Gel Electrophoresis

Isolates positive for the qnrS gene were subjected to XbaI-digested pulsed-field gel electrophoresis (PFGE) as previously described (Ozaki et al., 2011).

Plasmid DNA Analysis

The chromosomal or plasmid location of qnrS and TMP-resistant genes was determined in isolates with antimicrobial resistance. Southern blot analysis was performed using S1 nuclease-digested genomic DNA of selected isolates separated by PFGE according to previously described methods (Shahada et al., 2011). DNA from the PFGE gel was transferred onto a Hybond-N+ membrane (Amersham Biosciences UK Ltd., Little Chalfont, UK) and PCR-amplified qnrS, dfrA1, dfrA13, or CTX-M group 4 beta-lactamase gene fragments from each of the qnrS-, dfrA1-, dfrA13-, or CTX-M group 4 beta-lactamase gene-positive isolates were labeled with digoxigenin using a DIG High Prime Labeling and Detection Starter Kit (Roche Diagnostics Corp., Indianapolis, IN) and used as a specific probe for each gene.
**Table 1. Isolation of plasmid-mediated quinolone resistance (PMQR)-positive *Escherichia coli* (EC) from DHL plates with or without enrofloxacin (ERFX).**

| DHL plates supplemented with ERFX | Number of samples that yielded qnrS-positive EC | DHL plates supplemented with ERFX | Number of samples that yielded qnrS-positive EC |
|----------------------------------|-----------------------------------------------|----------------------------------|-----------------------------------------------|
| Retail meat                      | Carcass surface                               | Fecal samples collected in Aug 2018 (n = 50) | Fecal samples collected in Aug 2018 (n = 50) |
| Yes                              | 21                                            | 5                               | 28                                            |
| No                               | 14                                            | 5                               | 35                                            |
| Both plates                      | 9                                             | 3                               | 25                                            |

1DHL, deoxycholate hydrogen sulfide lactose.

**Results**

**Isolation Rates of qnrS-Positive E. coli Using Plates Supplemented With or Without ERFX**

Among the PMQR genes tested, only *qnrS* was detected in *E. coli* isolated from the samples. Of the 40 retail meat samples, *qnrS*-positive *E. coli* were isolated from 21 and 14 samples using DHL plates supplemented with and without 0.05 μg/mL ERFX, respectively (Table 1), and the isolation rate between each of the plates was not significantly different. In 9 samples, *qnrS*-positive *E. coli* were isolated from both ERFX-supplemented and non-supplemented DHL plates. Additionally, *qnrS*-positive *E. coli* were isolated from 28 and 35 fecal samples collected in August 2018 using DHL plates supplemented with and without ERFX, respectively, and the isolation rate between each of the plates was not significantly different. Thus, DHL plates without ERFX were used thereafter for isolation of PMQR *E. coli* from fecal samples.

_Antimicrobial Resistance Genes Other Than qnrS Among E. coli Isolates-Positive E. coli Obtained During 2018 and 2019_!

Of the 45 total TMP-resistant *E. coli* isolates from farm A, *dfrA1* and *dfrA13* were detected in 4 and 13 isolates, respectively, including one isolate from a carcass surface. Additionally, an isolate from retail meat was positive for the *dfrA13* gene and another isolate was resistant to CTF (MIC, 128 μg/mL) and harbored the CTX-M group 4 beta-lactamase gene.

**XbaI-Digested PFGE Patterns of qnrS-Positive E. coli Isolates**

PFGE analysis revealed highly diverse patterns of *qnrS*-positive *E. coli* isolates obtained from farms A and B, although several isolates from each farm showed identical patterns, respectively (Supplementary Figure 1). Additionally, PFGE patterns of *qnrS*-positive *E. coli* isolates obtained from carcass surface samples from farm B were indistinguishable from those observed in a *qnrS*-positive fecal isolate from farm B. PFGE patterns of the retail meat isolates were different from each other.
Plasmid DNA Analysis Using S1 Nuclease-Digested PFGE and Southern Blot Hybridization

The S1 nuclease-digested PFGE patterns of qnrS- and dfrA13-positive E. coli isolates contained various plasmids ranging from less than 50 kilobase pairs (kbp) to approximately 200 kbp (Table 3 and Supplementary Figures 2 and 3). Southern blot hybridization revealed that plasmids of approximately 200 kbp detected in 7 of the 11 dfrA13-positive fecal isolates from farm A that carried both the qnrS and dfrA13 genes, although PFGE patterns of XbaI-digested genomic DNA of the 7 isolates differed from each other (Supplementary Figure 1). In 3 isolates from fecal samples, these genes were located on separate plasmids (Table 3 and Supplementary Figure 2). Plasmid DNA from the remaining isolate (Supplementary Figure 2, lane 23) was degraded and hybridization was not detected. Plasmids in the isolates obtained from retail meat and carcass surface samples from farm A carried both genes, although the sizes were not identical to those in the fecal isolates. In 2 of 3 dfrA1-positive isolates, both the qnrS and dfrA1 genes were located on the same plasmid (Table 3 and Supplementary Figure 3). Additionally, a retail meat isolate carried the CTX-M group 4 beta-lactamase gene and qnrS on the same plasmid (Table 3 and Supplementary Figure 3).

DISCUSSION

The occurrence of qnrS in the present study is higher than that in previous reports on this gene in E. coli from chicken and meat originating worldwide (Li et al., 2014; Niero et al., 2018; Nishikawa et al., 2019; Seo and Lee, 2019). Additionally, in this study, diverse PFGE patterns were found in the E. coli isolates from fecal samples, suggesting that qnrS-positive E. coli were prevalent on the farms. Interestingly, the sizes of plasmids carrying the qnrS gene were similar in isolates with different PFGE patterns from farm A. These results were possibly due to transmission of the plasmids carrying this gene among E. coli at this farm, although detailed

Table 2. Prevalence of qnrS-positive Escherichia coli (E. coli) isolates and antimicrobial resistance of PMQR-positive isolates.

| Sample type | Source  | Sampling date | No. of samples | qnrS-positive1 | AMP   | CTF   | DSM   | CHL   | OTC   | ERFX  | SUL   | TMP   |
|-------------|---------|---------------|----------------|----------------|-------|-------|-------|-------|-------|-------|-------|-------|
| Feces       | Farm A  | Aug 2018      | 50             | 38             | 36    | 0     | 27    | 12    | 35    | 30    | 37*   | 34*   |
| Feces       | Farm A  | Mar 2019      | 20             | 13             | 13    | 0     | 11*   | 4     | 13    | 10    | 13*   | 11*   |
| Feces       | Farm B  | Mar 2019      | 10             | 8              | 8     | 0     | 3*    | 0     | 6     | 5     | 0*    | 0*    |
| Feces       | Farm A  | Aug 2018      | 10             | 2              | 2     | 0     | 2     | 1     | 2     | 2     | 2     | 2     |
| Feces       | Farm B  | Mar 2019      | 10             | 5              | 5     | 1     | 4     | 0     | 0     | 5     | 3     | 2     |
| Retail meat | Markets | Jul 2018      | 20             | 18             | ND3   | 4     | ND    | ND    | ND    | 6     | ND    | 13    |

Abbreviations: AMP, ampicillin; CHL, chloramphenicol; CTF, ceftiofur; DSM, dihydrostreptomycin; ERFX, enrofloxacin; No., number; OTC, oxytetracycline; PMQR, plasmid-mediated quinolone resistance; SUL, sulfooxazole; TMP, trimethoprim.

*Significant (P < 0.05) differences in the prevalence of antimicrobial-resistant E. coli in fecal samples between farms A and B.

1No. of samples that yielded qnrS-positive E. coli.

2One isolate from each sample set was subjected to antimicrobial susceptibility testing (see MATERIALS AND METHODS).

3Not done.

Table 3. Plasmid location of antimicrobial resistance genes in qnrS-positive Escherichia coli isolates.

| Isolate no. | Sample type | Source  | Sampling date | Estimated size (kilo base pairs) of plasmid harboring:1 |
|-------------|-------------|---------|---------------|-------------------------------------------------------|
|             |             |         |               | qnrS | dfrA13 | dfrA1 | CTX-M group 42 |
| 1           | Retail meat | Market  | Jul 2018      | 150 | 150    |       |               |
| 12          | Feces       | Farm A  | Aug 2018      | 200 | 200    |       |               |
| 19          | Feces       | Farm A  | Aug 2018      | 200 | 200    |       |               |
| 24          | Feces       | Farm A  | Aug 2018      | <19 | 50     |       |               |
| 25          | Feces       | Farm A  | Aug 2018      | 200 | 200    |       |               |
| 28          | Feces       | Farm A  | Aug 2018      | <19 and 50 | 120 |       |               |
| 30          | Feces       | Farm A  | Aug 2018      | 200 | 200    |       |               |
| 33          | Feces       | Farm A  | Aug 2018      | 200 | 200    |       |               |
| 45          | Feces       | Farm A  | Aug 2018      | 200 | 200    |       |               |
| 47          | Carcass surface | Farm A | Aug 2018 | 60 | 60     |       |               |
| 23          | Feces       | Farm A  | Aug 2018      | ND  | ND     |       |               |
| 49          | Feces       | Farm A  | Mar 2019      | 200 | 200    |       |               |
| 54          | Feces       | Farm A  | Mar 2019      | 50  | 110    |       |               |
| 10          | Feces       | Farm A  | Aug 2018      | 80  | 80     |       |               |
| 29          | Feces       | Farm A  | Aug 2018      | 80  | 80     |       |               |
| 38          | Feces       | Farm A  | Aug 2018      | 210 | ND     |       |               |
| 4           | Retail meat | Market  | Jul 2018      | 250 |        |       |               |

1The size of plasmid harboring antimicrobial resistance genes was estimated by pulsed-field gel electrophoresis of S1 nuclease-digested genomic DNA of Escherichia coli and Southern blot hybridization with a probe prepared from the polymerase chain reaction amplicon using primer pairs specific for each of the genes (see Supplementary Figures 2 and 3).

2CTX-M group 4 beta-lactamase gene
characterization of these plasmids would be necessary to confirm this. More than half of the qnrS-positive E. coli isolates obtained from fecal samples demonstrated low-level resistance to quinolones as demonstrated by the ERFX MIC50 for these isolates being 2 μg/mL. The qnrS gene encodes protein QnrS that have been shown to protect E. coli DNA gyrase from quinolone inhibition at low concentrations (Jacoby et al., 2014). However, the selective pressure of fluoroquinolones may result in elevated resistance, which is caused by mutations to gyrA (Poirel et al., 2006; de Toro et al., 2010). Further studies for the isolates in this study are necessary to elucidate possible association of the presence of the qnrS gene with additional mechanisms for elevated resistance to fluoroquinolones including sequence analysis of the gyrA gene.

The presence of a low concentration (0.05 μg/mL) of ERFX in DHL agar plates was unlikely effective for isolation of qnrS-positive isolates because the isolation result from plates supplemented with and without ERFX for retail meat samples were contrary to that for fecal samples. High prevalence of a variety of qnrS-positive E. coli isolates on the participating farms may partly be a possible reason for the results for fecal samples that differences in the isolation rate between plates with and without ERFX were not significant.

PFGE patterns of qnrS-positive isolates from the carcass surface and fecal samples were identical to each other, suggesting that the carcasses may be contaminated with intestinal contents via meat processing. Moreover, qnrS-positive E. coli were isolated from retail meat samples, although it was not possible to trace whether the meat products from the farms participating in this study were sold in the markets where meat samples were collected. The prevalence of qnrS-positive E. coli in retail chicken meat obtained in May 2020 was almost twice what it was in July 2018. Sinwat et al. (2015) have reported that 5 of 80 Salmonella isolates from chicken meat collected from 2010 to 2013 in Thailand harbored the qnrS gene. Thus, continuous monitoring for contamination of chicken meat with Enterobacteriaceae carrying this gene is necessary. The present study additionally demonstrated that an isolate from a retail meat sample carried both qnrS gene and CTX-M group 4 beta-lactamase gene which is one of the genes encoding extended-spectrum beta-lactamases (ESBLs), on the same plasmid, although the source of the isolate was unknown. Plasmids carrying PMQR genes occasionally have genes encoding ESBLs, causing co-selection and therapeutic concerns (Robicsek et al., 2006a; Jacoby et al., 2014).

High prevalence of SUL- and TMP-resistance in isolates obtained from fecal samples from farm A might be associated with the use of the combination of sulfadiazine and TMP at this farm. Several isolates from fecal samples at this farm carried both a TMP-resistance gene (dfrA1 or dfrA13, encoding dihydrofolate reductases) and qnrS on the same plasmid, suggesting that the qnrS gene may be partly co-selected under these conditions. Because more than 30 genes conferring resistance to TMP have been identified (Wüthrich et al., 2019) and only 4 of these were examined in this study, other genes not investigated here may be involved in TMP resistance in the isolates. The co-existence of TMP-resistance and PMQR genes, including qepA in E. coli of feline origin (Chen et al., 2014), qnrB6 in Klebsiella pneumoniae and Citrobacter freundii of canine origin (Ma et al., 2009), and qnrS in avian pathogenic E. coli from broiler chickens (Yoon et al., 2020) have been reported. Chen et al. (2014) demonstrated that multidrug-resistant plasmids harboring the qepA gene had disseminated in E. coli isolates from companion animals, food animals, and farm environments in China. In the present study, the prevalence of SUL- and TMP-resistance was low in E. coli isolates from farm B, although qnrS-positive isolates were obtained from this farm at a rate comparable to that in farm A. Thus, the prevalence of E. coli with PMQR may be caused by the use of quinolones in poultry in Thailand (Nhung et al., 2016; Wangroongsarb et al., 2021) and the association of the combined use of sulfadiazine and TMP at farm A with the selection of E. coli with TMP resistance and PMQR is likely to be limited. Because only 2 farms participated in the present study, it is important to conduct large-scale studies to evaluate the prevalence of PMQR in E. coli originating from chickens in Thailand. High prevalence of TMP-resistance in qnrS-positive isolates (13/18) from retail chicken meat samples obtained in May 2020 may be taken into consideration.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2021.101538.

REFERENCES

Cavaco, L. M., R. S. Hendriksen, and F. M. Aarestrup. 2007. Plasmid-mediated quinolone resistance determinant qnrS1 detected in Salmonella enterica serovar Corvallis strains isolated in Denmark and Thailand. J. Antimicrob. Chemother. 60:704-706.

Chaisitit, C., C. Tribuddharat, C. Pulsrikarn, and S. Dejsirilert. 2012. Molecular characterization of antibiotic-resistant bacteria in contaminated chicken meat sold at supermarkets in Bangkok, Thailand. Jpn. J. Infect. Dis. 65:527-534.

Chen, X., L. He, Y. Li, Z. Zeng, Y. Deng, Y. Liu, and J.-H. Liu. 2014. Complete sequence of a F2.A-B- plasmid pH3A11 carrying rmtB and qepA, and its dissemination in China. Vet. Microbiol. 174:267-271.

Chmelitský, I., O. Hermesh, S. Navon-Venezia, J. Strahilevitz, and Y. Carmeli. 2009. Detection of aac(6’)-Ib-cr in KPC-producing
Klebsiella pneumoniae isolates from Tel Aviv, Israel. J. Antimicrob. Chemother. 64:718–722.

Ciesielczuk, H., M. Hornsey, V. Choi, N. Woodford, and N. Hirayama, and T. Murase. 2011. Antimicrobial resistance in local Escherichia coli isolated from growing chickens. Poult. Microbiol. 150:132–139.

Nieto, G., V. Bortolaia, M. Vanni, L. Intorre, L. Guardabassi, and A. Piccirillo. 2018. High diversity of genes and plasmids encoding resistance to third-generation cephalosporins and quinolones in clinical Escherichia coli from commercial poultry flocks in Italy. Vet. Microbiol. 216:93–98.

Nishikawa, R., T. Murase, and H. Ozaki. 2019. Plasmid-mediated quinolone resistance in Escherichia coli isolates from commercial broiler chickens and selection of fluoroquinolone-resistant mutants. Poult. Sci. 98:5900–5907.

Ozaki, H., H. Esaki, K. Takemoto, A. Ikeda, Y. Nakatani, A. Soneya, N. Hirayama, and T. Murase. 2011. Antimicrobial resistance in local Escherichia coli isolated from growing chickens on commercial broiler farms. Vet. Microbiol. 150:132–139.

Park, C. H., A. Robicsek, G. A. Jacoby, D. Sahm, and D. C. Hooper. 2006. Prevalence in the United States of aac(6’)-Ib-cr encoding a ciprofloxacin-modifying enzyme. Antimicrob. Agents Chemother. 50:3935–3935.