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Journal Title: International Microbiology
Volume: Volume 18, Number 2
Publisher: Hemeroteca Científica Catalana: International Microbiology | 2015-01-01, Pages 85-90
Type of Work: Article | Final Publisher PDF
Publisher DOI: 10.2436/20.1501.01.237
Permanent URL: https://pid.emory.edu/ark:/25593/rpvsw

Final published version: http://dx.doi.org/10.2436/20.1501.01.237

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Accessed October 29, 2017 6:10 PM EDT
Unexpected distribution of the fluoroquinolone-resistance gene \textit{qnrB} in \textit{Escherichia coli} isolates from different human and poultry origins in Ecuador

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Received 15 March 2015 · Accepted 3 June 2015

\textbf{Summary.} Fluoroquinolone resistance can be conferred through chromosomal mutations or by the acquisition of plasmids carrying genes such as the quinolone resistance gene (\textit{qnr}). In this study, 3,309 strains of commensal \textit{Escherichia coli} were isolated in Ecuador from: (i) humans and chickens in a rural northern coastal area (n = 2368, 71.5%) and (ii) chickens from an industrial poultry operation (n = 827, 25%). In addition, 114 fluoroquinolone-resistant strains from patients with urinary tract infections who were treated at three urban hospitals in Quito, Ecuador were analyzed. All of the isolates were subjected to antibiotic susceptibility screening. Fluoroquinolone-resistant isolates (FRIs) were then screened for the presence of \textit{qnrB} genes. A significantly higher phenotypic resistance to fluoroquinolones was determined in \textit{E. coli} strains from chickens in both the rural area (22%) and the industrial operation (10%) than in strains isolated from humans in the rural communities (3%). However, the rates of \textit{qnrB} genes in \textit{E. coli} isolates from healthy humans in the rural communities (11 of 35 isolates, 31%) was higher than in chickens from either the industrial operations (3 of 81 isolates, 6%) or the rural communities (7 of 251 isolates, 2.8%). The occurrence of \textit{qnrB} genes in human FRIs obtained from urban hospitals was low (1 of 114 isolates, 0.9%). These results suggested that the \textit{qnrB} gene is more widely distributed in rural settings, where antibiotic usage is low, than in urban hospitals and industrial poultry operations. The role of \textit{qnrB} in clinical resistance to fluoroquinolones is thus far unknown. [\textit{Int Microbiol} 2015; 18(2):85-90]

\textbf{Keywords:} \textit{Escherichia coli} · gene \textit{qnrB} · quinolone resistance · urban hospitals · industrial poultry operations

\section*{Introduction}

Every year in the USA alone, more than two million people are infected with antibiotic-resistant bacteria, resulting in more than 23,000 deaths [3]. The antibiotic resistance of pathogens has been linked to both the medical and the agricultural usage of antibiotics [6,7,15,16]. Resistance to fluoroquinolones poses a particularly challenging health problem because these broad-spectrum antibiotics are used to treat serious bacterial infections, especially those acquired in hospitals [2,3,5]. Both the mutations in chromosomal genes [2] and the presence of conjugative or non-conjugative plasmids carrying the quinolone resistance gene \textit{qnr} or other genes [10,21,27] have been implicated in fluoroquinolone resistance. Plasmid-mediated quinolone resistance in human pathogens has been
associated with food-producing animals in some studies [8,28] but not in others [20].

Among the qnr genes, qnrB is widely distributed in South American countries [17,18,20]. In Ecuador, where there is no restriction on the use of fluoroquinolones in animal feed, the prevalence of fluoroquinolone resistance in community-acquired Escherichia coli isolates from the human urinary tract is 41% [22]. In the present study, we assessed fluoroquinolone resistance and the presence of qnrB genes in E. coli isolates obtained in Ecuador from fecal samples collected from chickens and humans in rural, low antibiotic use setting and from two higher antibiotic use settings. Specifically, we compared fluoroquinolone-resistant isolates (FRIs) from (i) healthy humans living in rural communities, (ii) chickens (broiler and free-range) raised in rural communities, (iii) humans treated at urban hospitals, and (iv) chickens from an industrial poultry operation.

### Materials and methods

**Samples and bacterial isolates.** Escherichia coli was isolated from 1,167 human fecal samples and 1,201 chicken cloacal swabs (from 1,134 chickens) cultured on MacConkey agar. Five lactose-fermenting colonies were selected from each sample and tested for glucuronidase activity on Chromocult agar. Glucuronidase-positive colonies were subjected to antibiotic susceptibility testing. One FRI was selected from each sample. In these FRIs, an inhibition zone ≤ 20 mm was produced in response to discs containing 5 µg of ciprofloxacin. Kirby-Bauer antibiotic susceptibility testing was carried out in accordance with the guidelines of the Clinical and Laboratory Standards Institute [3b].

**Isolates from chickens in rural communities.** The 1,201 E. coli isolates were obtained between January and March 2009 from chickens raised in small-scale poultry farming operations in a rural community in northwestern Ecuador. The majority of these isolates (955; 80%) were from broiler chickens that had been purchased from a local distributor and fed with commercial poultry feed. Of these 955 isolates, 831 were from 30 chickens sampled weekly for 6 weeks at three farms in one community and 124 were from 25 chickens sampled once cross-sectionally at another farm in the same community. An additional 246 (20%) isolates were obtained from household varietals (other breeds of chickens also purchased from a local distributor). Isolates from all chickens in the rural communities were labeled RemCHK.

In addition, one isolate (Rem6) was obtained from water drawn from a well in the same community as the chickens.

**Isolates from humans in rural communities.** The 1,167 commensal E. coli isolates were obtained from healthy humans (controls) residing in 24 communities in northwestern Ecuador who participated in a case-control study of diarrheal diseases between February 2009 and February 2010. Details about the region, study design, and sampling strategy were described previously [4]. The human isolates were labeled RemHUM. All interactions with human subjects were approved by the University of Michigan’s Institutional Review Board and the Universidad San Francisco de Quito’s Bioethics Committee.

### Table 1. Nucleotide sequences of qnrB genes from fluoroquinolone resistance isolates (FRIs) of Escherichia coli from humans and chickens inhabiting rural communities located on the northern coast of Ecuador, from chickens from an industrial operation, and from humans treated at an urban hospital in Quito

<table>
<thead>
<tr>
<th>Amplicon</th>
<th>GenBank accession number</th>
</tr>
</thead>
<tbody>
<tr>
<td>RemHUM1</td>
<td>JN714812</td>
</tr>
<tr>
<td>RemHUM2</td>
<td>JN714813</td>
</tr>
<tr>
<td>RemHUM3</td>
<td>JN714814</td>
</tr>
<tr>
<td>RemHUM4</td>
<td>JN714815</td>
</tr>
<tr>
<td>RemHUM5</td>
<td>JN714816</td>
</tr>
<tr>
<td>RemCHK6</td>
<td>JN714817</td>
</tr>
<tr>
<td>RemCHK7</td>
<td>JN714818</td>
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<tr>
<td>RemCHK8</td>
<td>JN714819</td>
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<td>RemCHK9</td>
<td>JN714820</td>
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<td>RemCHK10</td>
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<td>IndCHK26</td>
<td>JN714837</td>
</tr>
<tr>
<td>Quito Hospital</td>
<td>JN714838</td>
</tr>
</tbody>
</table>

**Isolates from chickens in an industrial operation.** The 827 E. coli isolates from an industrial poultry operation (located on the Ecuadorian coast, ~300 km from the study region) were obtained from broiler chickens sampled between March and November 2010. These animals had been kept in coops and received oxytetracycline (10 mg/l) in their drinking water. These isolates were labeled IndCHK.

**Isolates from humans in urban hospitals.** The 114 clinical FRIs were isolated from patients with E. coli urinary tract infections who were seen at three hospitals in urban Quito from May to July 2010. Of these isolates, 61 were from Hospital Vozandes (kindly provided by Jeannette Zurita), 42 were from Hospital Carlos Andrade Marin (kindly provided by Isabel Narváez), and 11 were from the Institute of Microbiology at the Universidad San Francisco de Quito. These clinical human isolates were labeled E. coli Quito Hospital.
Fig. 1. Maximum likelihood analysis of qnrB genes in *Escherichia coli* isolates. RemHUM: bacterial sequences obtained from humans in Ecuadorian rural communities. RemCHK: sequences from chickens in rural communities. *E. coli* Quito Hospital: *qnrB* from hospital isolates in Quito, Ecuador. IndCHK: sequences from isolates from a poultry industrial operation. The remaining sequences were obtained from GenBank: *E. coli* strain F84, accession number KM094204.1; *E. coli* strain F257 accession number KM094205.1; *E. coli* strain ECH18, accession number KP268825.1; *Haemophilus parasuis* strain SC056, accession number HQ117877.1. Numbers are bootstrap values obtained after 500 pseudoreplicates. Asterisk indicates sequences analyzed in the present work.
Polymerase chain reaction amplification and DNA sequence analysis. Bacterial DNA from a single FRI colony was extracted from the cells using a boiling technique [25]. A PCR for qnrB genes was carried out following the method described in [8] and using the following primers to amplify internal fragments of the target gene: qnrB F 5′-GG-MATHGAAATTCGCCACTG-3′ and qnrB R 5′-TTTGCGYGGTCG- CAGTCGAA-3′. The PCR conditions were as follows: 95°C for 5 min, 35 cycles of 94°C for 30 s, 56°C for 40 s and 72°C for 1 min, and a final incubation at 72°C for 10 min. Amplicons were sequenced by the University of Michigan DNA Sequencing Core. The qnrB gene sequences described in this report were deposited under the accession numbers JN714812 to JN714838 (Table 1). A subset of these amplicons (IndCHK24, IndCHK25, IndCHK26, and E. coli Quito Hospital) were sequenced a second time at Functional Biosciences, Inc. (Madison, WI) to rule out errors. They were used in the phylogenetic analysis, performed with the Mega5.1 program (Fig. 1). Two qnrB sequences from the FRIs of broiler chicken came from the same animal, from two colonies collected 2 weeks apart (RemCHKb7 and RemCHKb9).

Statistical analysis. A χ² test was used to analyze the differences in fluoroquinolone resistance and qnrB gene frequency between sample types.

Results and Discussion

Rates of resistance. The rates of fluoroquinolone resistance were significantly higher (P < 0.001) in isolates from chickens in rural communities (broilers: 214 of 961 isolates; household varietals: 37 of 246 isolates) than in isolates from chickens raised in industrial operations (81 of 827 isolates) or in isolates from humans living in rural communities (35 of 1,167 isolates) (Table 2).

Presence of qnrB gene in fluoroquinolone-resistant isolates. The percentage of human FRIs carrying qnrB genes differed substantially, depending on the origin of the isolates: 31.4% (11 of 35 isolates) of the human FRIs from rural communities carried the qnrB gene vs. 0.88% (1 of 114 isolates) of those from hospitals in Quito (P < 0.0001) (Table 2). The differences among the chicken isolates were smaller: 1.87% (4 of 214 isolates) of the FRIs from broiler chickens in rural communities 8.1% (3 of 37 isolates) of those from household varietals, and 6% (3 of 50 isolates analyzed) of those from broiler chickens raised in industrial operations (P = 0.4). The differences between FRIs from broiler chickens and household varietals in the rural community were not significant (P = 0.069).

Phylogenetic analysis of qnrB genes. The nucleotide sequences of all the amplicons showed high similarities to previously described qnrB genes (Fig. 1).

Conclusions. Based on the published literature [1] and the high prevalence of fluoroquinolone resistance previously reported in Ecuador [22], a higher frequency of qnrB genes was expected from pathogenic FRIs from hospitals in Quito, where antibiotic use is high, than in rural settings, where antibiotic use is low. However, a high proportion of commensal E. coli isolated from humans in rural communities carried qnrB genes. Conversely, despite the high prevalence of FRIs obtained from chickens raised in rural communities, the proportion carrying qnrB genes was low. FRI from urban hospitals in Quito, where antibiotic use is high, had the lowest proportion.

Table 2. Fluoroquinolone resistance isolates (FRIs) of Escherichia coli with and without the qnrB gene. Five E. coli isolates were analyzed from each fecal sample obtained from either humans or chickens inhabiting rural communities located on the northern coast of Ecuador.

<table>
<thead>
<tr>
<th>Source of the isolate</th>
<th>Number of isolates</th>
<th>FRIs (%)</th>
<th>FRIs with qnrB (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human isolates from a rural area</td>
<td>1,167</td>
<td>35 (2.9%)</td>
<td>11 (31.4%)</td>
<td></td>
</tr>
<tr>
<td>Human isolates from urban hospitals</td>
<td>ND</td>
<td>114</td>
<td>1 (0.88%)</td>
<td>P &lt; 0.0001</td>
</tr>
<tr>
<td>Isolates from broiler chickens in a rural area</td>
<td>955</td>
<td>214 (22.4%)</td>
<td>4 (1.87%)</td>
<td></td>
</tr>
<tr>
<td>Household varietals from a rural area</td>
<td>246</td>
<td>37 (15.4%)</td>
<td>3 (8.1%)</td>
<td></td>
</tr>
<tr>
<td>Isolates from broiler chickens from an industrial poultry operation</td>
<td>827</td>
<td>81 (9.8%)</td>
<td>3 (6.0%)</td>
<td></td>
</tr>
</tbody>
</table>

aND = Not determined.
bOut of 81 isolates, only 50 were analyzed for qnrB genes.
cP-value obtained from a comparison of fluoroquinolone resistant human isolates from the rural community and from the hospital in Quito.
of qnrB gene carriage. Although qnrB genes cause low-level resistance to quinolones [9], they are thought to be important in the development of the highly resistant phenotype [14]. The lack of association of the qnrB gene with clinical fluoroquinolone resistance in this study was therefore unexpected.

Other studies have found that qnrB genes are widely distributed in commensal Escherichia coli isolated from healthy humans, including children, living in urban settings in Peru and Bolivia [17,18], from humans in remote Peruvian Amazon communities [19], and from domestic and farm animals in Germany [23]. In our study, healthy humans from rural, but not urban areas of Ecuador had high rates of qnrB carriage. We also found that although chickens had high rates of fluoroquinolone resistance, the rates of qnrB carriage in the FRIs from these animals were low.

Although our findings suggest that qnrB genes are not linked to fluoroquinolone clinical resistance, they have been associated with the development of high resistance to quinolones [14,24]. Moreover, qnrB genes are one of the most common plasmid-mediated quinolone resistance genes [13]. The presence of qnrB genes in isolates from domestic animals is also a matter of concern, as these genes have been detected in food pathogens such as Salmonella [11]. The main limitation of this study was the amplified segment of DNA, which did not allow us to determine the diversity of the qnr genes. These and other studies on the role of qnr genes in fluoroquinolone resistance are needed.

Acknowledgements. The authors thank the Ecologia, Desarrollo, Salud, y Sociedad EcoDSS field team, and especially Nadia Lopez, for their invaluable contribution in collecting the data. This study was supported by grant numbers R01-AI050038 and K01-AI03544 from the National Institute of Allergy and Infectious Diseases NIAID; grant number 0811934 from the Ecology of Infectious Diseases program; the Fogarty International Center (FIC) of the USA National Institutes of Health (NIH); and the National Science Foundation (NSF).

Competing interests. None declared.

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