Zoonotic Transmission of \textit{mcr-1} Colistin Resistance Gene from Small-Scale Poultry Farms, Vietnam

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We investigated the consequences of colistin use in backyard chicken farms in Vietnam by examining the prevalence of \textit{mcr-1} in fecal samples from chickens and humans. Detection of \textit{mcr-1}-carrying bacteria in chicken samples was associated with colistin use and detection in human samples with exposure to \textit{mcr-1}-positive chickens.

Colistin resistance is a gradually emerging problem among gram-negative bacteria in clinical settings in many countries (1). A transferable plasmid-derived colistin resistance gene \textit{mcr-1} discovered in China and subsequently found worldwide could be mediating this emergence (2,3). Use of colistin in animal production has been suggested as the most likely factor contributing to the emergence of the \textit{mcr-1} gene (2). However, systematic studies applying the One Health approach to investigate the epidemiologic link between the use of colistin in agriculture and colonization with \textit{mcr-1}-carrying bacteria in the community are lacking (4).

Colistin use in humans is negligible (5), but it is one of the most commonly used antimicrobial drugs in animal production in Vietnam (6). We investigated the consequences of colistin use in chicken farms by assessing chicken, farmers, and nearby persons for the presence of \textit{mcr-1}-carrying bacteria and performing epidemiologic analyses to assess the risk for subsequent transmission to unexposed human populations in southern Vietnam.

The Study

From March 2012 to April 2013, we conducted a systematic, cross-sectional study examining antimicrobial drug use and colonization with antimicrobial-resistant \textit{E. coli} in chickens and humans in Tien Giang Province, Vietnam. Fecal samples from 204 chicken farms and rectal swabs from 204 chicken farmers (1 farmer/farm) were collected as described (online Technical Appendix 1, https://wwwnc.cdc.gov/EID/article/23/3/16-1553-Techapp1.pdf) (7,8). We additionally collected rectal swabs from age- and sex-matched persons not involved in poultry farming from the same districts (rural persons, n = 204) and from their provincial capitals (urban persons, n = 102) (8).

Samples were cultured on MacConkey plates with and without antimicrobial drugs. A sweep of the full growth on plain MacConkey plates was collected and screened for the presence of \textit{mcr-1} by PCR as described previously (2). Logistic regression models were built to investigate the risk factors associated with the presence of \textit{mcr-1} on chicken farms and in human participants. Then, we selected (using a random number table) individual \textit{E. coli} colonies (n = 200) and extended-spectrum β-lactamase (ESBL)–producing \textit{E. coli} colonies (n = 122) growing on different MacConkey plates and repeated PCR to confirm the presence of \textit{mcr-1} in \textit{E. coli} isolated from chickens and humans. We tested all \textit{mcr-1}–positive \textit{E. coli} isolates for colistin susceptibility using Etest (bioMérieux, Marcy l’Etoile, France) and interpreted test results in accordance with the European Committee on Antimicrobial Susceptibility Testing breakpoints (9). In addition, whole-genome sequencing was performed on all \textit{mcr-1}–positive \textit{E. coli} isolates as described (online Technical Appendix 1).

From a total of 204 chicken and 510 human fecal specimens, 188 and 440 MacConkey sweeps were available for \textit{mcr-1} screening by PCR, respectively. The adjusted prevalence of \textit{mcr-1} was 59.4% (95% CI 47.9%–71.0%) in chicken and 20.6% (95% CI 15.9%–25.2%) in human fecal samples (Table 1).

\begin{table}
\centering
\begin{tabular}{|c|c|}
\hline
Sample Type & Prevalence (95% CI) \\
\hline
Chicken & 59.4% (47.9%–71.0%) \\
Human & 20.6% (15.9%–25.2%) \\
\hline
\end{tabular}
\caption{Prevalence of \textit{mcr-1} in chicken and human fecal samples.}
\end{table}

\textsuperscript{1}These authors contributed equally to this article.
Among 200 *E. coli* isolates, *mcr-1* was detected in 10/78 (12.8%) isolates from chickens, 2/50 (4.0%) isolates from farmers, and 0/72 isolates from persons who did not farm. Similarly, *mcr-1* was detected in 9/38 (23.7%) and 1/44 (2.3%) of ESBL-producing *E. coli* isolated from chickens and farmers, respectively.

The MIC of colistin for the 22 *mcr-1*–carrying *E. coli* isolates ranged 3–4 mg/L. Because the Etest might underestimate the true MIC \( (\times 10) \), these results indicate reduced susceptibility. Single-nucleotide polymorphism (SNP)–based phylogenetic analyses of the core genomes showed little genomic similarity between isolates, but the analyses did show many isolates belonged to the same multilocus sequence types \( (n = 14) \) (Figure). Analysis of the acquired resistance genes, reflecting the presence of an accessory genome, showed a large variation in resistance gene content, with only the tet(A) gene, encoding for tetracycline resistance, present in all genomes (online Technical Appendix 2 Table, https://wwwnc.cdc.gov/EID/article/23/3/16-1553-Techapp1.xlsx). De novo bacterial genome assembly was performed, and the contigs carrying *mcr-1* were analyzed. A replication origin could be located in 5 isolates, leading to the identification of plasmid incompatibility groups IncHI2 (1 isolate), IncI2 (2 isolates), and combined IncHI2 and IncHI2A (2 isolates). Transposon ISAp1, initially described as carrying the *mcr-1* gene \( (\times 2) \), was identified in 18 of 22 contigs.

We investigated risk factors for fecal colonization with *mcr-1*–carrying bacteria separately for small-scale farms and household farms because a joint model did not converge due to inflated sampling weight assigned to household chicken farms (online Technical Appendix 1 Table 1). Multivariate analysis identified the presence of younger chickens (<20.5 weeks old) and the use of

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**Table 1.** Prevalence of fecal colonization with *mcr-1*–carrying bacteria in chickens and humans, Tien Giang Province, Vietnam, 2012–2013

<table>
<thead>
<tr>
<th>Source</th>
<th>No. positive sweeps/total (%)</th>
<th>Adjusted prevalence, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All chicken farms</td>
<td>93/188 (49.5)</td>
<td>59.4 (47.9–71.0)</td>
</tr>
<tr>
<td>Household chicken farms</td>
<td>53/94 (56.4)</td>
<td>59.5 (47.9–71.1)</td>
</tr>
<tr>
<td>Small-scale chicken farms</td>
<td>40/94 (42.6)</td>
<td>47.9 (35.4–60.3)</td>
</tr>
<tr>
<td>All human participants</td>
<td>84/440 (19.1)</td>
<td>20.6 (15.9–25.2)</td>
</tr>
<tr>
<td>All farmers</td>
<td>45/179 (25.1)</td>
<td>25.2 (18.3–32.0)</td>
</tr>
<tr>
<td>Farmers exposed to <em>mcr-1</em>–negative chickens</td>
<td>16/81 (17.6)</td>
<td>15.5 (7.7–23.9)</td>
</tr>
<tr>
<td>Farmers exposed to <em>mcr-1</em>–positive chickens</td>
<td>29/88 (33.0)</td>
<td>34.7 (23.9–45.5)</td>
</tr>
<tr>
<td>Rural persons</td>
<td>31/173 (17.9)</td>
<td>17.6 (11.6–23.7)</td>
</tr>
<tr>
<td>Urban persons</td>
<td>8/88 (9.1)</td>
<td>9.1 (3.1–15.1)</td>
</tr>
</tbody>
</table>

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**Figure.** Phylogenetic analyses of *mcr-1*–positive *Escherichia coli* isolated from chickens and chicken farmers, Vietnam, 2012–2013. Maximum-likelihood tree of 22 *mcr-1*–carrying *E. coli* isolated from 15 chicken fecal samples and 3 human fecal swab samples (underlined), constructed by using CSI Phylogeny 1.4 (https://cge.cbs.dtu.dk/services/CSIPhylogeny/), shows a genome-wide single-nucleotide polymorphism (SNP) comparison. A total of 74,585 SNPs were concatenated for pairwise comparison (difference between pairs 0–32,267 SNPs). The multilocus sequence types (ST) are indicated next to the isolate names. The ST155 isolates CG05C.C1 and CG05C.C2 differ by 1 SNP; the ST10 isolates CG48C.A2 and CG48C.G2 differ by 1 SNP and 1 antimicrobial resistance gene; the ST1056 isolates CT48C.C1 and CT48C.C2 differ by 4 SNPs and 3 antimicrobial resistance genes; and the ST50 isolates CT67C.C1 and CT67C.C2 are phenotypically different but have 0 SNP differences and originate from the same sample and are therefore likely to be highly related or identical. Scale bar indicates number of nucleotide substitutions per site.
Table 2. Multivariate analysis of risk factors associated with fecal colonization with mcr-1–carrying bacteria in small-scale chicken farms (N = 94) and in humans (N = 440), Vietnam, 2012–2013

<table>
<thead>
<tr>
<th>Variables</th>
<th>No. tested</th>
<th>No. mcr-1–positive</th>
<th>OR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Small-scale chicken farms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age of chickens</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chickens &lt;20.5 weeks old</td>
<td>47</td>
<td>32</td>
<td>21.3 (5.8–78.5)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chickens ≥20.5 weeks old</td>
<td>47</td>
<td>8</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Use of colistin</td>
<td>21</td>
<td>14</td>
<td>5.1 (1.4–18.8)</td>
<td>0.017</td>
</tr>
<tr>
<td><strong>Humans</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban persons†</td>
<td>88</td>
<td>8</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Rural persons†</td>
<td>173</td>
<td>31</td>
<td>2.1 (0.9–5.0)</td>
<td>0.075</td>
</tr>
<tr>
<td>Farmers exposed to mcr-1–negative chickens</td>
<td>91</td>
<td>16</td>
<td>1.8 (0.7–4.7)</td>
<td>0.205</td>
</tr>
<tr>
<td>Farmers exposed to mcr-1–positive chickens</td>
<td>88</td>
<td>29</td>
<td>5.3 (2.2–12.7)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

†Not involved in poultry farming.

We found that younger chickens were more likely to be colonized with mcr-1–carrying bacteria than older chickens (≥20.5 weeks), probably because of the higher antimicrobial treatment incidence in younger chickens (74.0 [interquartile range 0–278]/1,000 chickens treated daily with 1 defined daily dose) than in older chickens (46.3 [interquartile range 0–124]/1,000 chickens treated daily with 1 defined daily dose) (N.V. Trung, unpub. data). However, our study was insufficiently powered to detect such an association in multivariate analysis. In addition, the gastrointestinal tract of younger chickens might be colonized by antimicrobial-resistant bacteria more readily than older chickens (13).

The spread of the mcr-1 gene on different plasmid types (IncI2, IncH12, and IncH12A) might explain its successful spread in different E. coli clones. We also identified the ISApl1 transposon in 81.8% (18/22) of our isolates. Because this genetic element is involved in horizontal gene transfer, it is likely to be a key factor contributing to the widespread dissemination of mcr-1 (14).

Our study is subject to several limitations. First, the cross-sectional study design precludes the demonstration of direct transmission of the mcr-1 gene between chickens and humans. Second, the presence of colistin in chicken feeds could not be verified and thus misclassification of farms in terms of their colistin use was possible. Last, we did not screen for the mcr-2 gene, which is also involved in colistin resistance (15).

In summary, our results show an association between colistin use on farms and the presence of the mcr-1 gene in animals. Given the potentially serious consequences of the spread of the mcr-1 gene from food production animals to humans, prudent use of antimicrobial drugs in animal production should be enforced globally, including in small-scale and household farms.

Acknowledgment
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References


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