

Zoonotic Transmission of *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 *mcr-1* in fecal samples from chickens and humans. Detection of *mcr-1*-carrying bacteria in chicken samples was associated with colistin use and detection in human samples with exposure to *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 *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 *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 *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 chickens, farmers, and nearby persons for the presence of *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 *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 *mcr-1* by PCR as described previously (2). Logistic regression models were built to investigate the risk factors associated with the presence of *mcr-1* on chicken farms and in human participants. Then, we selected (using a random number table) individual *E. coli* colonies (n = 200) and extended-spectrum β -lactamase (ESBL)-producing *E. coli* colonies (n = 122) growing on different MacConkey plates and repeated PCR to confirm the presence of *mcr-1* in *E. coli* isolated from chickens and humans. We tested all *mcr-1*-positive *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 *mcr-1*-positive *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 *mcr-1* screening by PCR, respectively. The adjusted prevalence of *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).

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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

| Source | Prevalence of fecal colonization with <i>mcr-1</i> -carrying bacteria | |
|--|---|---------------------------------|
| | No. positive sweeps/total (%) | Adjusted prevalence, % (95% CI) |
| All chicken farms | 93/188 (49.5) | 59.4 (47.9–71.0) |
| Household chicken farms | 53/94 (56.4) | 59.5 (47.9–71.1) |
| Small-scale chicken farms | 40/94 (42.6) | 47.9 (35.4–60.3) |
| All human participants | 84/440 (19.1) | 20.6 (15.9–25.2) |
| All farmers | 45/179 (25.1) | 25.2 (18.3–32.0) |
| Farmers exposed to <i>mcr-1</i> -negative chickens | 16/91 (17.6) | 15.5 (7.7–23.3) |
| Farmers exposed to <i>mcr-1</i> -positive chickens | 29/88 (33.0) | 34.7 (23.9–45.5) |
| Rural persons | 31/173 (17.9) | 17.6 (11.6–23.7) |
| Urban persons | 8/88 (9.1) | 9.1 (3.1–15.1) |

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 (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 IS*ApII*, initially described as carrying the *mcr-1* gene (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

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 ST156 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.

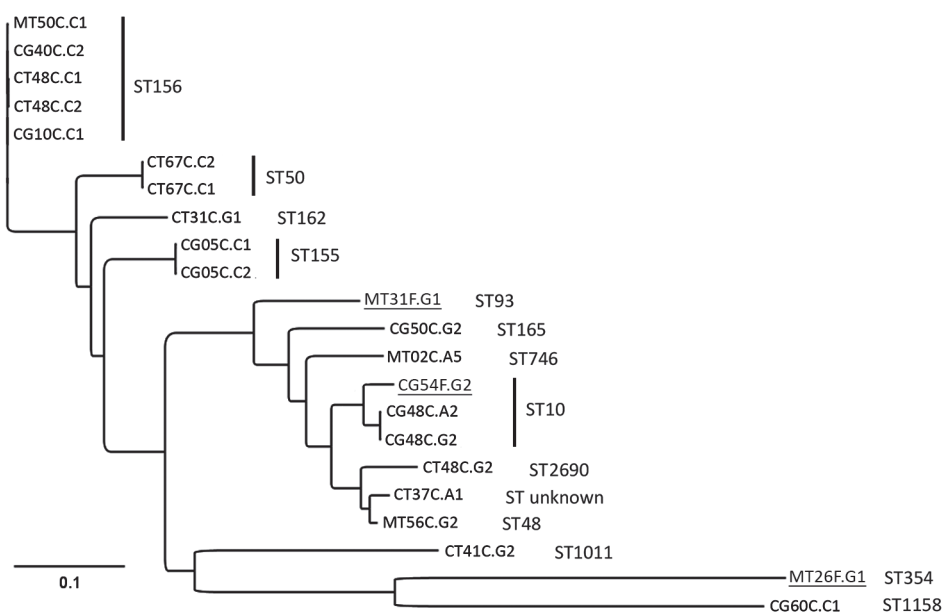


Table 2. Multivariate analysis of risk factors associated with fecal colonization with *mcr-1*-carrying bacteria in small-scale chicken farms (N = 94) and in humans (N = 440), Vietnam, 2012–2013*

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

*OR, odds ratio.

†Not involved in poultry farming.

colistin as independent risk factors for fecal colonization with *mcr-1*-carrying bacteria in chickens (odds ratios [ORs] 21.3 and 5.1, respectively) in small-scale farms (Table 2). We were unable to identify potential risk factors associated with fecal colonization with *mcr-1*-carrying bacteria in chickens in household farms. Among human participants, farmers who were exposed to *mcr-1*-positive chickens showed a significantly increased risk for colonization with *mcr-1*-carrying bacteria (OR 5.3; Table 2) in contrast with urban individuals not involved in chicken farming, rural individuals not exposed to chickens, and farmers with *mcr-1*-negative chickens.

Conclusions

Our study shows that colonization with *mcr-1*-carrying bacteria in chickens is associated with colistin usage and colonization of humans is associated with exposure to *mcr-1*-positive chickens. These findings suggest that colistin use is the main driver for the observed high prevalence (59.4%) of *mcr-1* in fecal samples from chickens, with zoonotic transmission explaining the high prevalence (34.7%) in farmers. Zoonotic transmission of colistin-resistant *E. coli* from a domesticated pig (11) and companion animals (12) to humans has been reported.

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, IncHI2, and IncHI2A) might explain its

successful spread in different *E. coli* clones. We also identified the IS*AplI* 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.

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Zoonotic Transmission of *mcr-1* Colistin Resistance Gene from Small-Scale Poultry Farms, Vietnam

Technical Appendix

Selection and Recruitment of Study Subjects

Chicken Farmers and Farms

In our study, we included 102 household chicken farms (≥ 10 –200 chickens) and 102 small-scale chicken farms (> 200 –2,000 chickens) stratified by district ($n = 3$; each having 34 household-scale and 34 small-scale farms). In each district, farms were selected randomly by using different approaches for small-scale and household-scale farms. Small-scale farms and farmers were randomly selected from the list of farms available at the Sub-Department of Animal Health in Tien Giang by using a random number table. To select household-scale farms and farmers within the chosen district, a commune was selected at random, and within the commune, the farm was selected as the first farm encountered 500 m from the center of the commune (direction was designated by spinning a bottle). Farmers and farms were included in the study if they fulfilled all the following inclusion criteria: 1) living in My Tho, Cho Gao, or Chau Thanh; 2) being healthy defined as not having been hospitalized in the past month, not currently having underlying serious chronic infectious diseases (e.g., TB), and having the ability to understand the informed consent form and questionnaire; 3) engaged in small-scale or household-scale chicken production; and 4) providing informed consent. Farmers and farms were excluded from the study if they did not fulfill any of the inclusion criteria.

Matched Persons Not Involved in Poultry Farming

We included 306 persons not involved in poultry farming in our study, which were matched by age and sex to the studied farmers (Technical Appendix Table 6). These consisted of 204 persons not involved in poultry farming from the same districts with the farmers (rural

persons) and 102 persons not involved in poultry farming from the provincial city (urban persons).

For every recruited farmer, we used multiple stages of sampling to identify matched persons with the ratio of 1:1 and 1:0.5 for rural persons and urban persons, respectively. In the first stage, we listed all persons that matched the age, sex, and location of the recruited farmer by viewing the registration population data available at the Preventive Medicine Department in Tien Giang. In the second stage, we randomly selected the person from the list of all persons by using a random number table. After the person was approached, we only included them in the study if they fulfilled all of the inclusion criteria. The inclusion criteria for rural persons were 1) providing informed consent; 2) living in Cho Gao, Chau Thanh, or the rural area of My Tho city; 3) living in a household where poultry is not raised and where poultry is not raised in the contiguous, immediately adjacent households; 4) not having worked on a farm raising poultry, in a slaughter line or slaughterhouse, or engaged in selling live or dead poultry over the past 12 months; 5) being healthy defined as not having been hospitalized in the past month, not currently having underlying serious chronic infectious diseases (e.g., TB), and having the ability to understand the informed consent form and questionnaire; and 6) matching a recruited farmer by age (± 5 years), sex, and commune. The inclusion criteria for urban persons were 1) providing informed consent; 2) living in the urban area of the provincial city; 3) not having worked on a farm raising poultry, in a slaughter line or slaughterhouse, or engaged in selling live or dead poultry over the past 12 months; 4) being healthy defined as not having been hospitalized in the past month, not currently having underlying, serious chronic infectious diseases (e.g., TB), and having the ability to understand the informed consent form and questionnaire; and 5) matching a recruited farmer by age (± 5 years) and sex. Those who did not fulfill all of the inclusion criteria were excluded from the study. Those who did not wish to participate were replaced by the next-best fit.

Data Collection

Data on human antimicrobial drug use during the month before the study visit, including the product's commercial name, packaging information, dosage, and duration of use, were collected for all participants as well as for all household members by medicine cabinet surveys

(Technical Appendix Table 8), which consisted of a structured questionnaire containing both open and closed questions. Data on antimicrobial use for chickens were similarly collected during interviews with the farmers by using a questionnaire as published previously (1). The medicine cabinet survey has been shown to be efficient for getting data on antimicrobial drugs use in the community (2). Use of an antimicrobial drug was defined as the reported use in the previous month or the presence of the antimicrobial drug in the medicine cabinet. All questionnaires on antimicrobial use were administered for chickens by the staff from the Sub-department of Animal Health and for humans by the staff from the Preventive Medicine Center.

Sample Analysis

Buffered peptone water (225 mL) was added to each chicken fecal sample in a different container and was manually shaken. A volume of 1 mL from each container was diluted 1:1000 in saline solution. Human rectal swabs were vortexed to release and suspend the sample in the liquid transportation medium and then 100 μ L was diluted 1:100 in saline solution. Plating 50 μ L of this dilution and incubating overnight at 37°C resulted in the growth of >100 separate colonies when plated onto MacConkey agar without antimicrobials or MacConkey agar supplemented with nalidixic acid (16 mg/L), ceftazidime (2 mg/L), or gentamicin (8 mg/L). A sweep from the full growth was collected and stored in glycerol at -20°C; afterwards, 5 randomly selected *Escherichia coli*-like colonies from the MacConkey agar without antimicrobials and 2 from each of 3 antimicrobial-supplemented agars were picked and subcultured for identification and antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed by using the disc diffusion method in accordance with the Clinical and Laboratory Standards Institute guidelines and breakpoints (3). Eleven antimicrobials were tested including tetracycline (30 mg), trimethoprim/sulfamethoxazole (1.25/23.75 mg), chloramphenicol (30 mg), gentamicin (10 mg), amikacin (30 mg), ciprofloxacin (5 mg), ampicillin (10 mg), amoxicillin/clavulanic acid (30 mg), ceftazidime (30 mg), ceftriaxone (30 mg), and meropenem (10 mg). Colistin was not included since interpretative breakpoints for disc susceptibility testing are not available with this antimicrobial. Quality controls for susceptibility testing and identification were performed every week according to the Clinical and Laboratory Standards Institute guidelines (3). Strains with an intermediate-susceptibility result were considered resistant. From each subject, all isolates with a unique, phenotypic, antimicrobial susceptibility patterns were stored for further analyses.

All *mcr-1*-positive *E. coli* isolates were tested for colistin susceptibility by using the ETEST (bioMérieux, Marcy l'Etoile, France) and interpreted in accordance with European Committee on Antimicrobial Susceptibility Testing breakpoints (4).

Real-Time PCR Detection of the *mcr-1* Gene

We designed a new forward primer (CLR5-qF1, 5'-TGACACTTATGGCACGGTCT-3') by using primer3 V4 (5,6). When combined with the reverse primer described by Liu et al. (CLR5-R, 5'-CTTGGTCGGTCTGTAGGG-3) (7), it produced a 62-bp fragment, which allowed for faster detection by real-time PCR, when we used the LightCycler 480 SYBR Green I Master mix (Roche Diagnostics, Indianapolis, IN, USA) following the manufacturer's instructions and using 60°C as the annealing temperature on a Roche LightCycler 480 instrument (Roche Diagnostics, Indianapolis, IN, USA).

Whole-Genome Sequencing Analysis

Whole-genome sequencing was performed for all *mcr-1*-positive *E. coli* isolates. Bacterial DNA was extracted from fresh pure cultures by using either the Wizard Genomic DNA purification kit (Promega, Madison, WI, USA) or the Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany). Library preparation was done in accordance with the manufacturer's instructions (Illumina, San Diego, CA, USA) and sequenced by using Illumina MiSeq technology with 150 paired-end settings (Illumina, Inc., San Diego, CA, USA). The reads were checked by using fastqc (<http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>) and trimmed using Trimmomatic V0.33 (8). De-novo genome assembly was performed with SPAdes 3.6 (9), and coverage was determined with Samtools v0.1.19 (10).

The presence of the *mcr-1* gene and the IS*AplI* transposon in the genomes was determined by using Blastn (11) with the assembled scaffolds as database and the published sequences as query (7). Online services provided by the Center for Genomic Epidemiology (<https://cge.cbs.dtu.dk/services/>) were used for multilocus sequence typing according to the scheme developed by Achtman et al. (12), for identification of plasmid incompatibility group (13) and for detection of acquired antimicrobial resistance genes other than *mcr-1* (14). We analyzed clonal complexes using e-burst V3 (http://eburst.mlst.net/v3/enter_data/single/) (15)

and the Multilocus Sequence Types (MLST) database at the University of Warwick (<http://mlst.warwick.ac.uk/mlst/dbs/Ecoli>) (16). The phylogenetic relationship between the isolates was determined by whole-genome comparison. Single-nucleotide polymorphisms (SNPs) were called by aligning the sequence reads to the genome of the commensal, nonpathogenic *E. coli* SE15 (accession no. NC_013654.1) (17) and distance between the isolates was visualized by constructing a maximum-likelihood tree on the basis of the SNP alignments with the online tool CSI Phylogeny 1.2 with the recommended default settings (18). Raw read sequences and assembled contigs have been deposited in the European Nucleotide Archive (<http://www.ebi.ac.uk/ena>) under the accession numbers ERS1262218–ERS1262239 (study accession no. PRJEB14873).

Adjustment of Prevalence Estimates for Stratified Study Design

Since the study was designed as a stratified survey with a fixed number of farms and participants in each stratum, not all the study units (farms and participants in the 3 districts) had the same probability of being selected. The prevalence of fecal colonization with *mcr-1*-carrying bacteria in chickens and humans was adjusted for the stratified survey design by assigning a stratum-specific sampling weight (W_i) to each observation unit (farm or subject) and then by using the following equation: $W_i = N_T/N_i$, where N_T = the total number of chicken farms or humans in that study district and N_i = the number of farms or participants in each stratum sampled ($i = 1 . . 7$) (Technical Appendix Tables 1 and 2). Standard errors were corrected to calculate the prevalence in each stratum. Sampling weight and sampling fraction of participants belonging to each study stratum were calculated under the assumption that chicken farmers accounted for 80% of the rural population.

Risk Factor Analysis

We built logistic regression models to investigate the risk factors associated with fecal colonization with *mcr-1*-carrying bacteria in chicken farms and human participants (Technical Appendix Tables 3–5). In the model for studying the risk factors in chickens, a total of 40 variables were first tested in univariate analyses, including factors describing the farms (production type and presence of other animals), farmers' demographic factors (Technical

Appendix Table 6), husbandry factors (Technical Appendix Table 7), and antimicrobial usage (Technical Appendix Table 8). We then excluded variables with <10 outcome events (20,21). Continuous variables such as the total number of chickens on the farm, the age of the chickens, the density of chickens on the farm, the age of the farmer, and the number of years experienced in chicken farming were stratified into 2 predefined categories by using the median of the obtained values as a cutoff value.

Similarly, for identifying risk factors associated with *mcr-1*-carrying bacteria in humans, a total of 9 variables were tested in univariate analyses, and participants were stratified into 2 categories on the basis of median age. Based on their biologic plausibility and a p value <0.15 in the univariate analyses, variables were considered for multivariable analysis and were included by using a stepwise forward approach (22). Variables were retained in the final models if the p value was <0.05. All biologically plausible variables were included in the final model. All interactions between final significant variables were tested. We performed all statistical analyses using R packages epicalc, survey, and adegenet (<http://www.r-project.org>).

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Technical Appendix Table 1. Sampling weight and sampling fraction of chicken farms, Tien Giang province, Vietnam, 2012–2013*

| Stratum | NT† | Ni | Fraction that should be sampled | Fraction sampled | Wi |
|---------------------------|--------|----|---------------------------------|------------------|-----|
| Chau Thanh household farm | 10,762 | 34 | 0.3697 | 0.00117 | 317 |
| Cho Gao household farm | 16,101 | 34 | 0.5532 | 0.00117 | 474 |
| My Tho household farm | 2,026 | 34 | 0.0696 | 0.00117 | 60 |
| Chau Thanh small farm | 36 | 34 | 0.0012 | 0.00117 | 1 |
| Cho Gao small farm | 147 | 34 | 0.0051 | 0.00117 | 4 |
| My Tho small farm | 34 | 34 | 0.0012 | 0.00117 | 1 |

*Ni, no. of farms sampled per stratum; NT, no. of farms per stratum; Wi, sampling weight.

†Tien Giang statistical office (19).

Technical Appendix Table 2. Sampling weight and sampling fraction of participants, Tien Giang province, Vietnam, 2012–2013*

| Stratum | NT† | Ni | Fraction that should be sampled | Fraction sampled | Wi |
|--------------------|---------|-----|---------------------------------|------------------|-------|
| My Tho, rural | 16,621 | 68 | 0.027 | 0.000111 | 244 |
| My Tho, farmer | 66,486 | 68 | 0.108 | 0.000111 | 978 |
| Chau Thanh, rural | 46,067 | 68 | 0.075 | 0.000111 | 677 |
| Chau Thanh, farmer | 184,266 | 68 | 0.301 | 0.000111 | 2,710 |
| Cho Gao, rural | 33,594 | 68 | 0.055 | 0.000111 | 494 |
| Cho Gao, farmer | 134,375 | 68 | 0.219 | 0.000111 | 1,976 |
| My Tho, urban | 131,650 | 102 | 0.215 | 0.000166 | 1,291 |

*Ni, no. of farms sampled per stratum; NT, no. of farms per stratum; Wi, sampling weight.

†Tien Giang statistical office (19).

Technical Appendix Table 3. Univariate analyses of risk factors associated with *mcr-1*-carrying bacteria in small-scale chicken farms (N = 94), Vietnam, 2012–2013*

| Risk factor | <i>mcr-1</i> -positive farm | Total | % | Odds ratio | 95% CI | p value |
|---|-----------------------------|-------|-------|--------------|--------------------------|---------|
| Male farmer | 28 | 51 | 54.9 | 1.94 | 0.61–6.14 | 0.261 |
| Age of farmer | | | | | | |
| <44 years | 22 | 45 | 48.9 | 1.67 | 0.61–4.56 | 0.322 |
| ≥44 years | 18 | 49 | 36.7 | Ref | Ref | Ref |
| Experience in chicken farming | | | | | | |
| <5 years | 19 | 37 | 51.4 | Ref | Ref | Ref |
| ≥5 years | 21 | 57 | 36.8 | 0.64 | 0.23–1.77 | 0.391 |
| Level of education attained by the farmer | | | | | | |
| Secondary school or less | 6 | 17 | 35.3 | Ref | Ref | Ref |
| Higher than secondary school | 34 | 77 | 44.2 | 1.03 | 0.29–3.7 | 0.968 |
| Location of farm | | | | | | |
| My Tho city | 11 | 32 | 34.4 | Ref | Ref | Ref |
| Cho Gao district | 17 | 32 | 53.1 | 2.16 | 0.79–5.96 | 0.138 |
| Chau Thanh district | 12 | 30 | 40.0 | 1.27 | 0.45–3.59 | 0.649 |
| Type of chicken production | | | | | | |
| Eggs | 13 | 56 | 23.2 | Ref | Ref | Ref |
| Meat | 27 | 38 | 71.1 | 10.36 | 3.29–32.69 | <0.0001 |
| Total number of chickens | | | | | | |
| 200–1400 | 19 | 45 | 42.2 | Ref | Ref | Ref |
| 1400–2000 | 21 | 49 | 42.9 | 1.13 | 0.42–3.05 | 0.814 |
| Chicken density | | | | | | |
| <10 chickens/m ² | 21 | 49 | 42.9 | Ref | Ref | Ref |
| ≥10 chickens/m ² | 19 | 45 | 42.2 | 1.17 | 0.44–3.15 | 0.752 |
| Age of chickens | | | | | | |
| <20.5 weeks | 32 | 47 | 68.1 | 19.39 | 5.8–4.8 | <0.0001 |
| ≥20.5 weeks | 8 | 47 | 17.0 | Ref | Ref | Ref |
| Chickens confined in pen/house 24 h/day | 35 | 84 | 41.7 | 0.64 | 0.13–3.22 | 0.589 |
| All-in-all-out system | 25 | 60 | 41.7 | 1.21 | 0.43–3.44 | 0.716 |
| Farms that bought day-old chickens | 38 | 81 | 46.9 | 4.35 | 0.68–28.04 | 0.125 |
| Source of day-old chickens | | | | | | |
| Hatched in farm | 5 | 11 | 45.5 | Ref | Ref | Ref |
| Local hatchery | 11 | 16 | 68.8 | 6.27 | 1.02–38.59 | 0.051 |
| Company hatchery | 23 | 55 | 41.8 | 3.34 | 0.79–14.09 | 0.104 |
| Presence of other animals on farms | | | | | | |
| Any | 39 | 88 | 44.3 | 2.72 | 0.27–27.01 | 0.395 |
| Fighting cocks | 5 | 10 | 50.0 | 2.07 | 0.43–10.01 | 0.369 |
| Ducks | 8 | 24 | 33.3 | 0.58 | 0.18–1.92 | 0.376 |
| Pigs | 20 | 41 | 48.8 | 1.45 | 0.53–3.97 | 0.472 |
| Cattles | 7 | 15 | 46.7 | 0.9 | 0.26–3.08 | 0.861 |
| Dogs | 34 | 74 | 45.9 | 4.35 | 1.18–16.09 | 0.03 |
| Cats | 20 | 46 | 43.5 | 0.93 | 0.34–2.51 | 0.882 |
| Fish pond | 21 | 48 | 43.8 | 1.17 | 0.43–3.18 | 0.753 |
| Farms that used antimicrobials | 31 | 68 | 45.6 | 0.97 | 0.33–2.89 | 0.958 |
| How often the farmer read guideline of antimicrobials | | | | | | |
| Always | 36 | 83 | 43.4 | Ref | Ref | Ref |
| Sometimes | 4 | 8 | 50.0 | 2.75 | 0.55–13.65 | 0.22 |
| Never | 0 | 3 | 0 | 0 | 0–0 | <0.001 |
| Farms that used disinfectants | 40 | 94 | 42.6 | 0.92 | 0.56–1.51 | 0.741 |
| Wild bird seen at farm | | | | | | |
| Never | 11 | 20 | 55.0 | Ref | Ref | Ref |
| Sometimes | 27 | 68 | 39.7 | 0.45 | 0.14–1.5 | 0.198 |
| Always | 2 | 6 | 33.3 | 0.11 | 0.01–0.91 | 0.0429 |
| Rodent seen at farm | | | | | | |
| Never | 11 | 21 | 52.4 | Ref | Ref | Ref |
| Sometimes | 25 | 61 | 41.0 | 0.57 | 0.17–1.92 | 0.365 |
| Always | 4 | 12 | 33.3 | 0.14 | 0.03–0.77 | 0.025 |
| Farms that used commercial feed | 40 | 93 | 43.0 | 1,600,119 | 211,647.3–12,097,395 | <0.001 |
| Farms with the presence of ante-room | 4 | 4 | 100.0 | 19,058,486.6 | 5,121,705.8–70,918,933.3 | <0.001 |
| Change boot/shoes before entering pen/house | 38 | 84 | 45.2 | 1.12 | 0.26–4.78 | 0.881 |
| Foot bath/foot dip at entrance | 29 | 73 | 39.7 | 0.7 | 0.21–2.33 | 0.565 |
| Outsiders allowed in farm | 0 | 2 | 0 | 0 | 0–0 | <0.001 |
| Use of specific antimicrobials at farm | | | | | | |

| Risk factor | <i>mcr-1</i> -positive farm | Total | % | Odds ratio | 95% CI | p value |
|-----------------|-----------------------------|-------|-------|--------------|-----------------------------|---------|
| Male farmer | 28 | 51 | 54.9 | 1.94 | 0.61–6.14 | 0.261 |
| Aminoglycosides | 3 | 5 | 60.0 | 1.33 | 0.15–11.99 | 0.802 |
| Penicillins | 11 | 18 | 61.1 | 2.39 | 0.74–7.71 | 0.148 |
| Lincosamides | 1 | 1 | 100.0 | 1,895,525.65 | 250,674.27– 14,333,411.8 | <0.001 |
| Macrolides | 13 | 29 | 44.8 | 0.53 | 0.18–1.57 | 0.257 |
| Colistin | 14 | 21 | 66.7 | 3.7 | 1.18–11.58 | 0.026 |
| Phenicol | 3 | 11 | 27.3 | 0.88 | 0.18–4.4 | 0.875 |
| Quinolones | 5 | 9 | 55.6 | 2.39 | 0.45–12.64 | 0.308 |
| Sulfonamides | 4 | 6 | 66.7 | 4.04 | 0.59–27.76 | 0.159 |
| Tetracyclines | 11 | 31 | 35.5 | 0.32 | 0.11–0.97 | 0.047 |

*Ref, referent.

Technical Appendix Table 4. Univariate analyses of risk factors associated with *mcr-1*-carrying bacteria in household chicken farms (N = 94), Vietnam, 2012–2013*

| Risk factor | <i>mcr-1</i> -positive farm | Total | % | Odds ratio | 95% CI | p value |
|---|-----------------------------|-------|-------|--------------|-----------------------|---------|
| Male farmer | 24 | 40 | 60.0 | 1.79 | 0.65–4.98 | 0.264 |
| Age of farmer | | | | | | |
| <46.5 years | 23 | 47 | 48.9 | Ref | Ref | Ref |
| ≥46.5 years | 30 | 47 | 63.8 | 1.49 | 0.56–3.98 | 0.428 |
| Experience in chicken farming | | | | | | |
| <8 years | 25 | 45 | 55.6 | Ref | Ref | Ref |
| ≥8 years | 25 | 49 | 51.0 | 0.57 | 0.21–1.49 | 0.252 |
| Level of education attained by the farmer | | | | | | |
| Secondary school or less | 19 | 32 | 59.4 | Ref | Ref | Ref |
| Higher than secondary school | 34 | 62 | 54.8 | 1.16 | 0.42–3.22 | 0.774 |
| Location of farm | | | | | | |
| My Tho city | 20 | 32 | 62.5 | Ref | Ref | Ref |
| Cho Gao district | 17 | 30 | 56.7 | 1.67 | 0.61–4.54 | 0.32 |
| Chau Thanh district | 16 | 32 | 50.0 | 1.31 | 0.48–3.58 | 0.603 |
| Type of chicken production | | | | | | |
| Eggs | 0 | 1 | 0 | Ref | Ref | Ref |
| Meat | 53 | 93 | 57.0 | 11,225,671 | 1,489,675–84,592,729 | <0.001 |
| Total no. of chickens | | | | | | |
| <75 | 27 | 47 | 57.4 | 1.14 | 0.42–3.09 | 0.792 |
| 75–199 | 26 | 47 | 55.3 | Ref | Ref | Ref |
| Chicken density | | | | | | |
| <1 chickens/m ² | 31 | 57 | 54.4 | Ref | Ref | Ref |
| ≥1 chickens/m ² | 22 | 37 | 59.5 | 1.42 | 0.54–3.75 | 0.478 |
| Age of chickens | | | | | | |
| <16 weeks | 23 | 45 | 51.1 | Ref | Ref | Ref |
| ≥16 weeks | 30 | 49 | 61.2 | 1.41 | 0.53–3.73 | 0.487 |
| Chickens confined in pen/house | 1 | 1 | 100.0 | 1,052,607.31 | 139,818.2–7,924,448.9 | <0.001 |
| 24h/day | | | | | | |
| All-in-all-out system | 20 | 29 | 69.0 | 1.98 | 0.71–5.57 | 0.197 |
| Farms that bought day-old chickens | 51 | 92 | 55.4 | 0 | 0–0 | <0.001 |
| Source of day-old chickens | | | | | | |
| Hatched in farm | 26 | 54 | 48.1 | Ref | Ref | Ref |
| Local hatchery | 14 | 21 | 66.7 | 2.87 | 0.86–9.6 | 0.09 |
| Company hatchery | 6 | 8 | 75.0 | 2.27 | 0.38–13.59 | 0.373 |
| Market/neighbor | 6 | 10 | 60.0 | 1.35 | 0.29–6.4 | 0.704 |
| Presence of other animals on farm | | | | | | |
| Any | 53 | 94 | 56.4 | NC | NC–NC | NC |
| Fighting cocks | 17 | 28 | 60.7 | 0.95 | 0.32–2.78 | 0.926 |
| Ducks | 28 | 42 | 66.7 | 2.44 | 0.89–6.72 | 0.087 |
| Pigs | 30 | 48 | 62.5 | 1.86 | 0.7–4.92 | 0.217 |
| Cattles | 13 | 21 | 61.9 | 1.47 | 0.43–4.99 | 0.539 |
| Dogs | 51 | 89 | 57.3 | 2.46 | 0.26–23.61 | 0.438 |
| Cats | 30 | 53 | 56.6 | 0.62 | 0.23–1.68 | 0.352 |
| Fish pond | 32 | 59 | 54.2 | 0.73 | 0.28–1.94 | 0.531 |
| Using antimicrobials on farm | 26 | 44 | 59.1 | 1.27 | 0.48–3.34 | 0.627 |
| How often the farmer read guideline of antimicrobials | | | | | | |
| Always | 41 | 71 | 57.7 | Ref | Ref | Ref |

| Risk factor | <i>mcr-1</i> -positive farm | Total | % | Odds ratio | 95% CI | p value |
|---|-----------------------------|-------|-------|-------------------|--------------------------------|---------|
| Male farmer | 24 | 40 | 60.0 | 1.79 | 0.65–4.98 | 0.264 |
| Sometimes | 10 | 19 | 52.6 | 0.84 | 0.25–2.89 | 0.788 |
| Never | 2 | 4 | 50.0 | 0.77 | 0.08–7.83 | 0.828 |
| Farms that used disinfectants | 48 | 85 | 56.5 | 0.89 | 0.15–5.4 | 0.902 |
| Wild bird seen in farm | | | | | | |
| Never | 22 | 38 | 57.9 | Ref | Ref | Ref |
| Sometimes | 29 | 50 | 58.0 | 0.86 | 0.31–2.37 | 0.77 |
| Always | 2 | 6 | 33.3 | 0.35 | 0.04–3.44 | 0.369 |
| Rodent seen in farm | | | | | | |
| Never | 23 | 41 | 56.1 | Ref | Ref | Ref |
| Sometimes | 28 | 47 | 59.6 | 0.71 | 0.26–1.92 | 0.503 |
| Always | 2 | 6 | 33.3 | 1.93 | 0.3–12.6 | 0.493 |
| Farms that used commercial feed | 39 | 64 | 60.9 | 1.69 | 0.56–5.09 | 0.354 |
| Farms with the presence of ante-room | 2 | 2 | 100.0 | 14,362,266.5 3 | 3,328,276.24– 61,976,436.25 | <0.001 |
| Change boot/shoes before entering pen/house | 31 | 50 | 62.0 | 2.21 | 0.84–5.81 | 0.113 |
| Foot bath/foot dip at entrance | 25 | 40 | 62.5 | 1.89 | 0.71–5.07 | 0.207 |
| Outsiders allowed in farm | 7 | 13 | 53.8 | 0.73 | 0.17–3.12 | 0.67 |
| Use of specific antimicrobials on farm | | | | | | |
| Aminoglycosides | 5 | 9 | 55.6 | 0.87 | 0.15–4.99 | 0.874 |
| Penicillins | 5 | 12 | 41.7 | 0.93 | 0.24–3.65 | 0.915 |
| Lincosamides | 4 | 4 | 100.0 | 13,773,734.4 2 | 4,583,095.04– 41,394,681.58 | <0.001 |
| Macrolides | 9 | 10 | 90.0 | 41.17 | 4.74–357.48 | 0.001 |
| Colistin | 9 | 18 | 50.0 | 0.66 | 0.2–2.23 | 0.51 |
| Phenicol | 0 | 2 | 0 | 0 | 0–0 | <0.001 |
| Quinolones | 5 | 8 | 62.5 | 0.99 | 0.19–5.09 | 0.99 |
| Sulfonamides | 1 | 5 | 20.0 | 0.12 | 0.01–1.15 | 0.068 |
| Tetracyclines | 14 | 21 | 66.7 | 1.67 | 0.51–5.43 | 0.397 |

*NC, not calculated; ref, referent.

Technical Appendix Table 5. Univariate analyses of risk factors associated with *mcr-1*-carrying bacteria in humans (N = 440), Vietnam, 2012–2013*

| Risk factors | <i>mcr-1</i> -positive subject | Total | % | Odds ratio | 95% CI | p value |
|---|--------------------------------|-------|------|------------|------------|---------|
| Participant group | | | | | | |
| Farmers exposed to <i>mcr-1</i> -negative chickens | 16 | 91 | 17.6 | 1.84 | 0.72–4.69 | 0.205 |
| Farmers exposed to <i>mcr-1</i> -positive chickens | 29 | 88 | 33.0 | 5.31 | 2.23–12.65 | <0.001 |
| Rural person not exposed to chickens | 31 | 173 | 17.9 | 2.14 | 0.93–4.96 | 0.07 |
| Urban person not exposed to chickens | 8 | 88 | 9.1 | Ref | Ref | Ref |
| Household location | | | | | | |
| Cho Gao district | 25 | 143 | 17.5 | 1 | 0.51–1.96 | 1 |
| Chau Thanh district | 30 | 147 | 20.4 | 1.37 | 0.72–2.61 | 0.34 |
| My Tho city | 29 | 150 | 19.3 | Ref | Ref | Ref |
| Age of participant | | | | | | |
| <46 years | 45 | 214 | 21.0 | 1.56 | 0.88–2.76 | 0.13 |
| ≥46 years | 39 | 226 | 17.3 | Ref | Ref | Ref |
| Male participant | 55 | 283 | 19.4 | 1.06 | 0.59–1.92 | 0.84 |
| Presence of other animals | 62 | 298 | 20.8 | 2.16 | 1.17–3.99 | 0.01 |
| Presence of pig(s) | 27 | 93 | 29.0 | 2.12 | 1.16–3.86 | 0.01 |
| Participants that used antimicrobials in the past month | 15 | 69 | 21.7 | 1.02 | 0.49–2.11 | 0.96 |
| Chicken meat consumption | | | | | | |
| Often (at least twice/week) | 35 | 198 | 17.7 | 1.68 | 0.34–8.17 | 0.52 |
| Sometimes (at least twice/month) | 45 | 209 | 21.5 | 1.67 | 0.37–7.44 | 0.5 |
| Never | 4 | 33 | 12.1 | Ref | Ref | Ref |
| Egg consumption | | | | | | |
| Often (at least twice/week) | 17 | 82 | 20.7 | 2.47 | 0.61–9.94 | 0.2 |
| Sometimes (at least twice/month) | 63 | 334 | 18.9 | 3.02 | 0.75–12.11 | 0.11 |
| Never | 4 | 24 | 16.7 | Ref | Ref | Ref |

*Ref, referent.

Technical Appendix Table 6. Comparison of key characteristics of study participants in Tien Giang province, Vietnam in 2012–2013

| Participant characteristic | Included human participants | | | Excluded human participants† | | |
|------------------------------|-----------------------------|------------------------|-----------------------|------------------------------|-----------------------|-----------------------|
| | Farmers, N = 179 | Rural persons, N = 173 | Urban persons, N = 88 | Farmers, N = 25 | Rural persons, N = 31 | Urban persons, N = 14 |
| Median age, y (IQR) | 45.0 (38.5–54.0) | 46.0 (38.0–53.0) | 46.5 (42.0–53.0) | 42.0 (36.0–52.0) | 48.0 (37.5–55.0) | 46.0 (40.8–58.5) |
| Male participant, no. (%) | 116 (64.8) | 116 (67.1) | 51 (58.0) | 16 (64.0) | 18 (58.1) | 9 (64.3) |
| Location of household | | | | | | |
| Cho Gao district, no. (%) | 57 (31.8) | 57 (32.9) | 29 (33.0) | 11 (44.0) | 11 (35.5) | 5 (35.7) |
| Chau Thanh district, no. (%) | 58 (32.4) | 58 (33.5) | 31 (35.2) | 10 (40.0) | 10 (32.3) | 3 (21.4) |
| My Tho city, no. (%) | 64 (35.8) | 58 (33.5) | 28 (31.8) | 4 (16.0) | 10 (32.3) | 6 (42.9) |

*IQR, interquartile range.

†Participants were excluded from risk factor analysis if sweep samples had not been stored (n = 45) or did not show any growth on MacConkey agar plate (n = 25) and therefore could not be tested for the presence of the *mcr-1* gene.

Technical Appendix Table 7. Comparison of key characteristics of study farms, Tien Giang province, Vietnam, 2012–2013

| Characteristic | Included farms, N = 188 | Excluded farms,* N = 16 |
|------------------------------|-------------------------|-------------------------|
| Backyard-scale farm (%) | 94 (50.0) | 8 (50.0) |
| Type of chickens | | |
| Meat, no. (%) | 108 (57.4) | 8 (50.0) |
| Egg, no. (%) | 57 (30.3) | 6 (37.5) |
| Mixed, no. (%) | 23 (12.2) | 2 (12.5) |
| Location of farm | | |
| Cho Gao district, no. (%) | 64 (34.0) | 4 (25.0) |
| Chau Thanh district, no. (%) | 60 (31.9) | 8 (50.0) |
| My Tho city, no. (%) | 64 (34.0) | 4 (25.0) |

* Farms were excluded from risk factor analysis if sweep samples had not been stored and therefore could not be tested for the presence of the *mcr-1* gene.

Technical Appendix Table 8. Use of antimicrobial drugs in chickens and humans, Tien Giang province, Vietnam, 2012–2013

| Class of antimicrobial | Chickens,* no. (%), N = 204 | Humans,† no. (%) | | |
|------------------------------|-----------------------------|------------------|----------------|----------------|
| | | Farmer, N = 204 | Rural, N = 204 | Urban, N = 102 |
| Any antimicrobial drug | 118 (57.8) | 33 (16.2) | 32 (15.7) | 17 (16.7) |
| 1st generation cephalosporin | 0 (0) | 12 (5.9) | 17 (8.3) | 7 (6.9) |
| 2nd generation cephalosporin | 0 (0) | 1 (0.5) | 2 (1.0) | 1 (1.0) |
| 3rd generation cephalosporin | 0 (0) | 5 (2.5) | 6 (2.9) | 1 (1.0) |
| Penicillins | 32 (15.7) | 11 (5.4) | 6 (2.9) | 5 (4.9) |
| Polymyxins | 39 (19.1) | 0 (0) | 0 (0) | 0 (0) |
| Macrolides | 38 (18.6) | 3 (1.5) | 4 (2) | 4 (3.9) |
| Quinolones | 19 (9.3) | 2 (1.0) | 2 (1.0) | 1 (1.0) |
| Lincosamides | 4 (2.0) | 1 (0.5) | 0 (0) | 1 (1.0) |
| Aminoglycosides | 18 (8.8) | 1 (0.5) | 0 (0) | 0 (0) |
| Chloramphenicol | 0 (0) | 1 (0.5) | 0 (0) | 0 (0) |
| Phenicol | 12 (5.9) | 0 (0) | 0 (0) | 0 (0) |
| Sulfonamides/trimethoprim | 12 (5.9) | 0 (0) | 0 (0) | 1 (1.0) |
| Tetracyclines | 51 (25.0) | 0 (0) | 0 (0) | 1 (1.0) |
| Pleuromutilins | 1 (0.5) | 0 (0) | 0 (0) | 0 (0) |

*Use during the previous 3 months for household-scale farms (≥10–200 chickens) or for the current flock for small-scale farms (>200–2000 chickens).

†Use during the month before the survey visit.