

Antimicrobial susceptibilities of *Escherichia coli* strains from a turkey operation

Sean F. Altekruse, DVM, PhD, DACVPM; François Elvinger, Dr med vet, PhD, DACVPM; Kyung-Yul Lee, PhD; Linda K. Tollefson, DVM, MPH; F. William Pierson, DVM, PhD, DACPV; Joseph Eifert, PhD; Nammalwar Sriranganathan, DVM, PhD, DACVM

Objective—To measure minimum inhibitory concentrations (MIC) of 17 antimicrobials for *Escherichia coli* isolates from a turkey operation and assess whether small samples provide precise estimates of geometric mean MIC.

Design—Prospective study.

Sample Population—105 clinical isolates from birds and 1,104 fecal isolates from 20 flocks (poults and finisher hens).

Procedure—A Mueller-Hinton broth dilution panel was used to measure MIC, and MIC of fecal and clinical isolates were compared. We drew random samples of 5, 10, 15, 20, 25, 30, 35, 40, and 45 isolates from each finisher flock and between 100 and 105 isolates from 5, 7, 10, and 20 flocks. Antimicrobial usage was determined for enrolled flocks.

Results—Six of 12 poult and 18 of 20 finisher flocks had been treated with antimicrobials, often for respiratory illnesses consistent with colibacillosis. All birds received gentamicin at the hatchery. More fecal than clinical isolates were resistant to ampicillin; however, more clinical isolates were resistant to ciprofloxacin, gentamicin, and sulfamethoxazole. Precise estimates of geometric mean MIC for flocks were obtained when ≥ 15 fecal isolates were obtained per flock and, for the operation, when 105 isolates were obtained from ≥ 7 flocks.

Conclusion and Clinical Relevance—Antimicrobial usage was common and may have contributed to the resistance patterns of isolates. With a modest allocation of laboratory resources, producers can monitor antimicrobial susceptibilities of clinical and fecal *E coli* to manage risks of antimicrobial usage and resistance. (*J Am Vet Med Assoc* 2002;221:411–416)

From the Food and Drug Administration, Center for Veterinary Medicine, 7500 Standish Pl, Rockville, MD 20895 (Altekruse, Lee, Tollefson); the Departments of Large Animal Clinical Sciences (Elvinger, Pierson) and Biomedical Sciences and Pathobiology (Sriranganathan), Virginia-Maryland Regional College of Veterinary Medicine; and the Department of Food Science and Technology, College of Agriculture and Life Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061 (Eifert). Dr. Altekruse's present address is the Division of Cancer Epidemiology and Genetics, National Cancer Institute, 6120 Executive Blvd, Room 7082 MSC#7234, Rockville, MD 20852.

Supported by the Food and Drug Administration Center for Veterinary Medicine, the Virginia Maryland Regional College of Veterinary Medicine, and Hatch Grant No. I35581.

The authors thank Ramona Hummel, Elizabeth Steele, Dawn Jones, Mary Mainous, Judy Rittenhouse, Sonya Bodeis, Linda English, and Shaohua Zhao for technical assistance.

Address correspondence to Dr. Altekruse.

Avian *Escherichia coli* infections are responsible for approximately a quarter of all disease-related losses in turkey production.^{1,3} Antimicrobial therapy is the principal control measure for avian colibacillosis. The selection of an ineffective antimicrobial agent for treatment of an outbreak of avian pathogenic *E coli* infection can lead to losses from prolonged illness and the cost of ineffective treatment.^{1,4} Pathogenic *E coli* strains are commonly resistant to aminoglycosides, β -lactams, sulfanomides, and tetracyclines.^{1,4} An increasing proportion of avian pathogenic *E coli* strains are also resistant to fluoroquinolones.^{5,6} Resistance to expanded-spectrum β -lactams⁷ is emerging in pathogenic Enterobacteriaceae that affect humans⁸ and food-producing animals.⁹ One purpose of this study was to determine the antimicrobial susceptibility patterns and geometric mean minimum inhibitory concentrations (MIC) of clinical *E coli* isolates from infected birds and fecal *E coli* isolates from an integrated turkey operation for 17 antimicrobials including ampicillin, ciprofloxacin, gentamicin, and tetracycline by use of the 1999 National Antimicrobial Resistance Monitoring System (NARMS) MIC dilution panel for gram-negative enteric bacteria.¹⁰ An additional objective was to evaluate whether geometric mean MIC values of fecal isolates within and across flocks in the operation could be precisely estimated with fewer than the 48 isolates that we collected per flock. The purpose of this analysis was to determine whether a smaller sample size could be used to monitor antimicrobial resistance in *E coli*.

Materials and Methods

Specimens—Six composite fecal specimens were collected from the floors of 20 finisher units with flocks of birds between 10 and 14 weeks of age in an integrated turkey operation during the summer of 1999. Two flocks were randomly selected from each of the 10 service routes. The median placement was 11,480 birds/flock. Two additional composite fecal specimens were collected from the floor of the brooder unit when poults were present in the building. Specimens were placed in sealed sterile plastic bags containing 10 ml of buffered peptone water, transported to the laboratory at 4 C, and processed within 4 hours.

Isolation and identification—A 10^{-3} dilution of each specimen was made in peptone water and 50 μ l was plated on MacConkey agar and incubated at 37 C. After 18 hours of incubation, 8 lactose-positive colonies were transferred to nutrient agar plates, incubated for 18 hours at 37 C, and tested for indole production, oxidase activity, and gram staining characteristics. Indole-producing, oxidase-negative, gram-

negative rods were classified as presumptive *E coli*. Presumptive *E coli* isolates were transferred to 4-methylumbelliferyl- β -D-glucuronide-MacConkey agar plates,^a incubated for 18 hours at 37 C, and tested for β -D-glucuronidase activity with an ultraviolet transilluminator (peak excitation of 365 nm [ultraviolet A] and peak emission of 455 nm [blue fluorescence]).¹¹ Nine hundred eighty-three of 1,104 (89%) isolates were confirmed to be *E coli* on the basis of β -D-glucuronidase activity, while 119 (11%) isolates did not have β -D-glucuronidase activity. Biochemical tests^b conducted on a random sample of 20 isolates without detectable β -D-glucuronidase activity confirmed that 18 isolates were *E coli*, 1 isolate was classified as probable *E coli*, and the last isolate was *Klebsiella pneumoniae*. The clinical isolates included in this study had been plated on EMB agar at the time of specimen submission, tested for indole production, and gram stained. One hundred five clinical isolates from flocks with colibacillosis were obtained from the integrated operation between 1997 and 1999. These clinical isolates had been stored at -70 C at a regional veterinary diagnostic laboratory.

Antimicrobial usage—At the time of the farm visit, information on all therapeutic antimicrobial usage in the flock (eg, type of drug, rationale, and time of treatment) was recorded from interviews with the field representatives. The field representatives' responses were based on review of written records for each flock. The therapeutic antimicrobials in the company formulary were chlortetracycline, enrofloxacin, erythromycin, oxytetracycline, penicillin, sulfamethazine, sulfaquinoxalin, and tetracycline.

Antimicrobial susceptibility—The 1998 NARMS MIC panel¹² was used to measure antimicrobial resistance patterns for all 1,104 fecal isolates and 105 clinical *E coli* isolates.¹⁰ The MIC panel was a microplate-based panel with serial 2-fold broth dilutions over established ranges (Table 1). The MIC breakpoints were set according to National Committee for Clinical Laboratory Standards¹³ (NCCLS).

Analyses of antimicrobial susceptibility data—Analyses regarding MIC were conducted on all 1,104 presumptive fecal *E coli* isolates and 105 clinical isolates. The Table 1—Minimum inhibitory concentration (MIC) ranges of the 1999 National Antimicrobial Resistance System gram-negative enteric bacteria broth dilution panel and National Committee for Clinical Laboratory Standards (NCCLS) intermediate and resistant MIC breakpoints for *Escherichia coli* isolates from veterinary sources

| Antimicrobial | MIC panel range (m g/ml) | MIC breakpoints (m g/ml) | |
|-------------------------------|--------------------------|--------------------------|-----------|
| | | Intermediate | Resistant |
| Amikacin | 4-32 | 32 | 64 |
| Ampicillin | 2-32 | 16 | 32 |
| Apramycin | 2-32 | — | — |
| Amoxicillin/clavulanic acid | 0.5/0.25-32/16 | 16/8 | 32/16 |
| Ceftiofur | 0.5-16 | — | — |
| Ceftriaxone | 0.25-64 | 16-32 | 64 |
| Cephalothin | 1-32 | 16 | 32 |
| Chloramphenicol | 4-32 | 16 | 32 |
| Ciprofloxacin | 0.015-4 | 2 | 4 |
| Florfenicol | 2-16 | — | — |
| Gentamicin | 0.25-16 | 8 | 16 |
| Kanamycin | 16-64 | 32 | 64 |
| Nalidixic acid | 4-256 | — | 32 |
| Streptomycin | 32-256 | — | — |
| Sulfamethoxazole | 128-512 | — | 512 |
| Tetracycline | 4-32 | — | 32 |
| Trimethoprim/sulfamethoxazole | 0.12/2.3-4/76 | — | 4/76 |

— = MIC breakpoint not defined.

percentages of *E coli* isolates in the susceptibility, intermediate susceptibility, and resistance ranges were determined for each antimicrobial according to NCCLS interpretive criteria.⁴ Geometric mean MIC (log 2) were calculated for clinical and fecal isolates.⁶ Deviations from normal distributions were encountered for several antimicrobials, and ANOVA for all antimicrobials was performed on ranked data to test 2 hypotheses. The first null hypothesis was that the rank of MIC for isolates from clinical specimens was equal to the rank of MIC for isolates from fecal specimens. The second null hypothesis was that the rank of MIC for fecal isolates from finisher flocks was equal to the rank of MIC for fecal isolates from brooder flocks. Minimum inhibitory concentrations greater than the highest concentration of the panel were set at 2 times that concentration.

Multiple antimicrobial resistance patterns—Multiple antimicrobial resistance patterns were determined for ampicillin, ciprofloxacin, gentamicin, sulfamethoxazole, and tetracycline by use of NCCLS resistance breakpoint criteria (Table 1). There were 32 mutually exclusive resistance types for these 5 antimicrobials, and the frequency of each multiple antimicrobial resistance type was determined. Associations between multiple resistance patterns and the source of *E coli* isolates (ie, clinical versus fecal) were evaluated by use of the Fisher exact test.

Extended-spectrum β -lactam resistance mediated by *cmv2* genes—Two sets of published polymerase chain reaction (PCR) primers¹³ were used to assess whether 3 clinical isolates with MIC for ceftriaxone > 16 mg/ml contained cephamycinase *blaCMY* (*cmv2*) genes, which are associated with resistance to extended-spectrum β -lactams. Polymerase chain reaction analyses were conducted in the laboratories of the Food and Drug Administration, Center for Veterinary Medicine, Division of Animal and Food Microbiology in Laurel, Md.

Geometric mean MIC within flocks—To determine the effect of the number of fecal isolates that were examined within a flock on estimated geometric mean MIC, we drew random samples of 5, 10, 15, 20, 25, 30, 35, 40, and 45 isolates from each flock. We calculated the sample geometric mean MIC for ampicillin, ciprofloxacin, gentamicin, and sulfamethoxazole within each flock and the geometric mean MIC for all isolates from that flock. Instances in which sample and overall geometric mean values differed by ≥ 2 -fold were noted. Because of insufficient sample size, 1 flock was excluded from analyses in which 35 or more isolates were drawn, another was excluded from analyses of 40 or more isolates, and a third was excluded from analyses of 45 isolates.

Geometric mean MIC across flocks—Four separate bootstrap analyses were conducted to examine whether samples of 100 to 105 fecal isolates drawn from 5, 10, 15, and 20 flocks would provide precise and unbiased estimates of the overall geometric mean MIC of fecal isolates across 20 finisher flocks in the operation. The bootstrap analysis was used to assess the stability of the geometric mean MIC by repeatedly sampling from the dataset. For each analysis, a sample of flocks was randomly selected, and isolates were randomly drawn from across these flocks in equal number. Thus, in 1 bootstrap simulation we drew 20 isolates from each of 5 flocks, in another 10 isolates were drawn from each of 10 flocks, in a third simulation 7 isolates were drawn from each of 15 flocks, and in the fourth simulation 5 isolates were drawn from each of 20 flocks. One thousand iterations were conducted for each bootstrap simulation.¹ Mean MIC and 95% bootstrap intervals for ampicillin, ciprofloxacin, gentamicin, and sulfamethoxazole were compared with overall geometric mean MIC for the 923 fecal isolates from 20 finisher flocks. For all analyses, a value of $P < 0.05$ was considered significant.

Results

Antimicrobial administration—Aminoglycosides were not used therapeutically; however, 1-day-old poults were routinely injected with gentamicin at the hatchery. Two growth promoters, bacitracin and virginiamycin, were added to turkey feed at the company mill on a rotating schedule. Six of 12 brooder flocks and 18 of 20 finisher flocks had been treated with an antimicrobial drug (Table 2). Antimicrobial therapy was administered to treat birds with clinical signs consistent with colibacillosis in 12 finisher flocks and 2 brooder flocks, to treat birds with signs of gastrointestinal tract disease in 8 finisher flocks and 2 brooder flocks, and to treat birds with signs attributable to various body systems in 6 finisher and 4 brooder flocks.

Antimicrobial MIC distributions—Resistance to ampicillin was observed in 53% of fecal and 14% of clinical isolates ($P < 0.001$) and 25% of clinical isolates had intermediate susceptibility to ampicillin (Table 3). One percent of all isolates had resistance to the extended-spectrum β -lactam ceftriaxone. Eight percent of clinical isolates had resistance to ciprofloxacin, compared with 2% of fecal isolates ($P < 0.001$). Resistance to gentamicin was observed in most clinical isolates and fewer than a quarter of fecal isolates ($P < 0.001$). Nalidixic acid resistance was seen in 43% of clinical and 26% of fecal isolates ($P < 0.001$). Sulfamethoxazole resistance was detected in 84% of clinical isolates and 58% of fecal isolates ($P < 0.001$). The proportion of isolates with resistance to tetracycline was approximately 90%, regardless of source ($P = 0.9$).

Multiple antimicrobial resistance—Five multiple antimicrobial resistance patterns accounted for at least 5% of either clinical or fecal isolates, 78% of clinical isolates, and 72% of all fecal isolates combined (Table 4). No other multiple resistance type accounted for > 5% of isolates from either source; however, 7% of clinical and 5% fecal isolates were susceptible to all 5 antimicrobials.

Detection of the *cmv2* gene in clinical isolates—One of 3 clinical isolates with MIC for ceftriaxone > 16 $\mu\text{g/ml}$ was confirmed to contain the *cmv2* gene.

Geometric mean MIC within flocks—Mean MIC of randomly selected isolates within flocks differed from overall means for flocks by 1 logarithmic unit (base 2) in 23 of 696 (3%) analyses and by 2 logarithmic units in 3 (< 1%) analyses. Fourteen of the 23 (61%) instances occurred when 5 isolates were drawn, 6 (26%) when 10 were drawn, and 3 (13%) when 15 were drawn. No such differences were observed when ≥ 20 isolates were drawn. Two of 3 instances in which

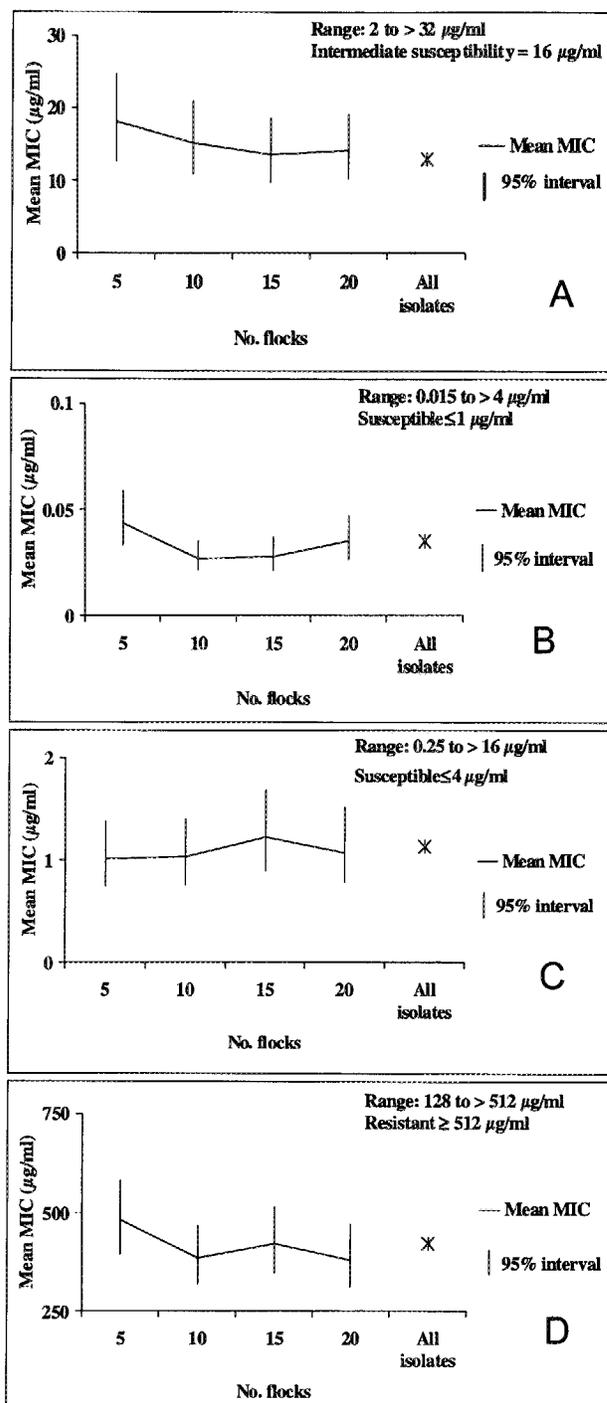


Figure 1—Geometric mean minimum inhibitory concentration (MIC) for ampicillin (A), ciprofloxacin (B), gentamicin (C), and sulfamethoxazole (D) for a fixed number of presumptive fecal *Escherichia coli* isolates obtained from finisher flocks in a turkey operation, with 95% bootstrap intervals and overall geometric mean MIC for all 923 isolates.

Table 2—History of antimicrobial treatment in a sample of 12 brooder flocks and 20 finisher flocks from an integrated turkey operation

| Antimicrobial used | Brooder flocks | | Finisher flocks | |
|--------------------|----------------|---------------------------|-----------------|---------------------------|
| | No. flocks (%) | Median age (wk) first use | No. flocks (%) | Median age (wk) first use |
| Any drug | 6 (50) | 2 | 18 (90) | 3 |
| Tetracyclines | 2 (17) | 2 | 11 (55) | 6 |
| Sulfonamide drugs | 0 (0) | — | 2 (10) | 5 |
| Enrofloxacin | 2 (17) | 2 | 8 (40) | 5 |
| Penicillin | 5 (42) | 2 | 10 (50) | 2 |

Table 3—Antimicrobial susceptibilities for 17 antimicrobials tested against clinical and fecal *Escherichia coli* isolates from an integrated turkey operation

| Antimicrobial | <i>E coli</i> source | Percentage | | | MIC (µg/ml) | | P value* |
|-----------------------------------|----------------------|-------------|--------------|-----------|-------------|-------|----------|
| | | Susceptible | Intermediate | Resistant | Median | Mean | |
| Amikacin | Clinical | 97 | 2 | 1 | 4 | 4.59 | 0.002 |
| | Fecal | | | | | | 0.97 |
| | Finisher | 99 | 0 | 1 | 4 | 4.16 | |
| | Brooder | 99 | 0 | 1 | 4 | 4.12 | |
| Amoxicillin/ clavulanic acid | Clinical | 85 | 8 | 8 | 4 | 4.65 | 0.47 |
| | Fecal | | | | | | 0.04 |
| | Finisher | 92 | 7 | 2 | 4 | 4.31 | |
| | Brooder | 82 | 16 | 2 | 8 | 4.85 | |
| Ampicillin | Clinical | 61 | 25 | 14 | 4 | 6.0 | < 0.001 |
| | Fecal | | | | | | 0.003 |
| | Finisher | 47 | 2 | 51 | 64 | 12.9 | |
| | Brooder | 35 | 2 | 63 | 64 | 19.5 | |
| Apramycin | Clinical | — | — | — | 4 | 4.45 | < 0.001 |
| | Fecal | | | | | | 0.57 |
| | Finisher | — | — | — | 4 | 3.31 | |
| | Brooder | — | — | — | 4 | 3.25 | |
| Ceftriaxone | Clinical | 97 | 2 | 1 | 0.25 | 0.311 | 0.002 |
| | Fecal | | | | | | 0.09 |
| | Finisher | 99 | 0 | 1 | 0.25 | 0.268 | |
| | Brooder | 99 | 1 | 1 | 0.25 | 0.261 | |
| Ceftiofur | Clinical | — | — | — | 0.5 | 0.643 | < 0.001 |
| | Fecal | | | | | | 0.88 |
| | Finisher | — | — | — | 0.5 | 0.516 | |
| | Brooder | — | — | — | 0.5 | 0.526 | |
| Cephalothin | Clinical | 52 | 30 | 18 | 8 | 10.2 | 0.03 |
| | Fecal | | | | | | 0.60 |
| | Finisher | 64 | 24 | 12 | 8 | 9.54 | |
| | Brooder | 54 | 25 | 20 | 8 | 9.48 | |
| Chloramphenicol | Clinical | 94 | 3 | 3 | 4 | 4.84 | 0.025 |
| | Fecal | | | | | | 0.67 |
| | Finisher | 98 | 1 | 1 | 4 | 4.35 | |
| | Brooder | 98 | 1 | 1 | 4 | 4.37 | |
| Ciprofloxacin | Clinical | 90 | 3 | 8 | 0.015 | 0.066 | < 0.001 |
| | Fecal | | | | | | 0.008 |
| | Finisher | 98 | 1 | 2 | 0.015 | 0.035 | |
| | Brooder | 94 | 4 | 2 | 0.015 | 0.049 | |
| Florfenicol | Clinical | — | — | — | 4 | 3.37 | 0.23 |
| | Fecal | | | | | | 0.06 |
| | Finisher | — | — | — | 2 | 3.10 | |
| | Brooder | — | — | — | 4 | 3.30 | |
| Gentamicin | Clinical | 32 | 13 | 55 | 16 | 6.8 | < 0.001 |
| | Fecal | | | | | | < 0.001 |
| | Finisher | 76 | 4 | 20 | 0.5 | 1.1 | |
| | Brooder | 62 | 9 | 29 | 1 | 1.9 | |
| Kanamycin | Clinical | 53 | 2 | 45 | 16 | 40.85 | < 0.001 |
| | Fecal | | | | | | 0.14 |
| | Finisher | 67 | 2 | 31 | 16 | 30.5 | |
| | Brooder | 75 | 2 | 24 | 16 | 26.5 | |
| Nalidixic acid | Clinical | 57 | — | 43 | 4 | 24.0 | < 0.001 |
| | Fecal | | | | | | 0.005 |
| | Finisher | 76 | — | 24 | 4 | 11.1 | |
| | Brooder | 65 | — | 35 | 4 | 16.4 | |
| Streptomycin | Clinical | — | — | — | 128 | 104.5 | 0.04 |
| | Fecal | | | | | | 0.09 |
| | Finisher | — | — | — | 64 | 81.3 | |
| | Brooder | — | — | — | 64 | 92.8 | |
| Sulfamethoxazole | Clinical | 16 | — | 84 | > 512 | — | < 0.001 |
| | Fecal | | | | | | 0.8 |
| | Finisher | 42 | — | 58 | > 512 | — | |
| | Brooder | 45 | — | 55 | > 512 | — | |
| Tetracycline | Clinical | 9 | 2 | 90 | > 32 | — | 0.23 |
| | Fecal | | | | | | 0.01 |
| | Finisher | 9 | 0 | 91 | > 32 | — | |
| | Brooder | 14 | 0 | 86 | > 32 | — | |
| Trimethoprim/ sulfamethoxazole | Clinical | 9 | 2 | 90 | 0.12 | 0.17 | > 0.001 |
| | Fecal | | | | | | 0.12 |
| | Finisher | 9 | 0 | 91 | 0.12 | 0.14 | |
| | Brooder | 14 | 0 | 86 | 0.12 | 0.13 | |

*P value for ANOVA of ranks for clinical and fecal isolates and for fecal isolates from finisher and brooder flocks. Farm of origin was incorporated into models as a source of variation.

— = No NCCLS breakpoints for apramycin, ceftiofur, florfenicol, or streptomycin, and no intermediate susceptibility breakpoint for nalidixic acid or sulfamethoxazole.

Table 4—Antimicrobial resistance types accounting for at least 5% of either clinical or fecal isolates from an integrated turkey operation^a

| Resistance type ^a | Clinical (%) | Fecal ^b (%) | P value ^c |
|------------------------------|--------------|------------------------|----------------------|
| AGST | 3 (3) | 102 (9) | 0.03 |
| AST | 5 (5) | 235 (21) | < 0.001 |
| AT | 2 (2) | 199 (18) | < 0.001 |
| GST | 50 (48) | 96 (9) | < 0.001 |
| ST | 21 (20) | 152 (14) | 0.08 |
| Susceptible | 7 (7) | 59 (5) | 0.57 |

^aMIC greater than the NCCLS resistance breakpoint. ^b1,104 presumptive fecal *E coli* (923 isolates from finisher and 181 isolates from brooder flocks).

^cχ²Test of equal proportions for clinical and fecal isolates.

A = Ampicillin. G = Gentamicin. S = Sulfamethoxazole. T = Tetracycline. Susceptible = Susceptible to all 5 antimicrobial combinations. There were 32 possible mutually exclusive antimicrobial resistance types for these antimicrobials.

See Table 1 for MIC breakpoint key.

a 2-logarithmic-unit difference was seen occurred when 5 isolates were drawn (ampicillin and gentamicin) and the other when 10 isolates were drawn (gentamicin).

Geometric mean MIC across flocks—When 100 to 105 isolates were drawn from across flocks, mean MIC were within 1 log-2 unit of the overall geometric mean MIC for all 923 isolates (Fig 1), with the exception of ampicillin when isolates were drawn from 5 flocks (mean MIC > 16 µg/ml).

Discussion

Antimicrobial therapy was common in the flocks that were studied and was often administered to treat respiratory syndromes consistent with avian colibacillosis. Most avian pathogenic *E coli* strains were resistant to sulfamethoxazole, tetracycline, and gentamicin. Random samples of as few as 15 fecal isolates yielded precise and unbiased estimates of overall geometric mean MIC within a flock. Similarly, accurate and unbiased estimates of overall geometric mean MIC across flocks were obtained when 105 fecal isolates were drawn in equal proportion from as few as 7 flocks. These results suggest that the MIC of fecal *E coli* MIC can be estimated with a modest allocation of laboratory resources. Monitoring MIC of fecal *E coli* isolates in food animal operations provides producers with an indicator organism to make informed decisions regarding therapeutic options and to manage risks of antimicrobial usage and resistance.

Differences in MIC of ciprofloxacin, gentamicin, and sulfamethoxazole between clinical and fecal isolates may relate to selective pressures on these bacterial populations. Results of other studies also indicate that a large proportion of avian pathogenic *E coli* strains are resistant to fluoroquinolones,^{3,6} sulfonamides,^{7,14,15} and tetracyclines.^{7,14-16} Individual and multiple antimicrobial resistance patterns of avian pathogenic *E coli* strains both reflect antimicrobial usage patterns in the poultry industry.^{14,18} Although gentamicin was not administered after poults left the hatchery, most clinical isolates had resistance to this drug.^{7,14,17,18} Dipping of eggs and injection of day-old poults with gentamicin are likely to drive gentamicin resistance in *E coli* isolates from turkeys.¹⁸ Conversely, a larger pro-

portion of fecal than clinical isolates had resistance to ampicillin, suggesting that use of penicillin in sampled flocks may have exerted selective pressure on the fecal flora. While clinical isolates were collected over a longer timespan than fecal isolates and isolates from 2 sources may differ,¹⁵ MIC data for fecal and clinical *E coli* isolates provided separate indications of the effect of antimicrobial pressures within this operation.

In this study, 3 clinical isolates were resistant to ceftriaxone and 1 carried the *cmv2* gene that confers resistance to extended-spectrum β-lactams.^{8,9,13} Furthermore, while only 8% of clinical isolates were resistant to ciprofloxacin, 43% were resistant to nalidixic acid. This trait, generally conferred by a mutation in the *gyrA* subunit, is often a first step toward expression of fluoroquinolone resistance.¹⁹ These findings add to concerns that the use of antimicrobials to control avian colibacillosis infections is costly,² marginally effective, and can contribute to antimicrobial-resistant infections in humans, poultry, and other animals.²⁰

Our findings regarding the effect of sample size on estimates of geometric mean MIC within and across flocks may be of interest to food animal producers and others who seek to manage the risk of antimicrobial resistance in food animal production.²¹⁻²³ A modest allocation of laboratory resources may permit informed decisions regarding antimicrobial usage in food animal populations. For example, as the geometric mean MIC of an antimicrobial agent approaches a threshold (eg, intermediate susceptibility MIC breakpoint), the veterinarian can limit its use in favor of another drug. Our findings that a small sample of fecal *E coli* isolates provides a precise estimate of central MIC is consistent with findings of a study conducted in Colorado dairy herds.⁸ In that study, estimates of central MIC remained stable when as few as 5 isolates were examined per animal or herd. We note that the MIC of individual strains can provide additional and often early evidence of the emergence of a resistance problem such as *cmv2*-mediated resistance to extended spectrum β-lactams, as detected among clinical isolates in our sample.

The selection of presumptive fecal *E coli* as an indicator organism for monitoring antimicrobial resistance in food animal production is supported by other studies.²³ These organisms are common and easy to grow and identify in the laboratory. In another study, > 95% of presumptive fecal *E coli* isolates from finisher swine were found to be *E coli* when complete biochemical testing was conducted.²⁴ In our study, composite fecal specimens were collected rather than specimens from individual birds. Results of a study of finisher pigs indicate that composite and individual fecal specimens produce equally precise and unbiased estimates of the prevalence of antimicrobial-resistant fecal *E coli*.²⁴ Drag swabs are often used to sample poultry environments because they are safe, efficient, and reflect the common exposure profiles of production flocks.²⁵ A limitation of composite fecal swabs is that because saturated swabs are not absorbent, strains that are clustered within a house may be missed.²⁶ To mitigate this, we collected composite fecal specimens from small, equally sized areas throughout the house.

In our study, most avian pathogenic *E coli* isolates

were resistant to gentamicin, sulfamethoxazole, and tetracycline. Nearly half of pathogenic isolates were resistant to nalidixic acid, and a quarter had intermediate resistance to ampicillin. Five multiple antimicrobial resistance patterns accounted for > 70% of clinical and fecal isolates. Our data suggest that precise and unbiased estimates of geometric mean MIC of fecal *E coli* within and across flocks in a poultry operation can be obtained with a modest allocation of laboratory resources. This type of monitoring may be used to make informed decisions regarding therapeutic options and manage risks of antimicrobial resistance.

*Remel Laboratories, Lenexa, Kan.

^bVitek Systems, Hazelwood, Mo.

^cTrek Diagnostics, Westlake, Ohio.

^dProc FREQ, SAS, version 8, SAS Institute, Cary, NC.

^eProc MEANS, SAS, version 8, SAS Institute, Cary, NC.

^fResampling Stats, add-in for Microsoft Excel v.2b, Institute for Professional Education, Arlington, Va.

^gMorley PS, Bolte D, Wittum TE. Effect of varying sampling strategies within-animals on population estimates for antimicrobial drug resistance in non-type-specific *E coli* (abstr), in *Proceedings. Int Soc Vet Epidemiol Econ*, 2000.

References

1. Barnes HJ, Gross WB. Colibacillosis. In: Calnek BW, Barnes HJ, Beard CW, et al, eds. *Diseases of poultry*. 10th ed. Ames, Iowa: Iowa State University Press, 1997;131-141.
2. Christiansen KH, Hird DW, Snipes KP, et al. California National Animal Health Monitoring System for meat turkey flocks—1988-89 pilot study: management practices, flock health, and production. *Avian Dis* 1996;40:278-284.
3. Owings W. Colibacillosis is big health problem for Iowa turkey growers. *Poult Times* 1995;8:28.
4. Lambie N, Ngeleka M, Brown G, et al. Retrospective study on *Escherichia coli* infection in broilers subjected to postmortem examination and antibiotic resistance of isolates in Trinidad. *Avian Dis* 2000;44:155-160.
5. Blanco JE, Blanco M, Mora A, et al. Prevalence of bacterial resistance to quinolones and other antimicrobials among avian *Escherichia coli* strains isolated from septicemic and healthy chickens in Spain. *J Clin Microbiol* 1997;35:2184-2185.
6. White DG, Piddock LJ, Maurer JJ, et al. Characterization of fluoroquinolone resistance among veterinary isolates of avian *Escherichia coli*. *Antimicrob Agents Chemother* 2000;44:2897-2899.
7. Salmon SA, Watts JL. Minimum inhibitory concentration determinations for various antimicrobial agents against 1570 bacterial isolates from turkey poults. *Avian Dis* 2000;44:85-98.
8. Winokur PL, Brueggemann A, DeSalvo DL, et al. Animal and human multidrug-resistant, cephalosporin-resistant *Salmonella* isolates expressing a plasmid-mediated CMY-2 AmpC beta-lactamase. *Antimicrob Agents Chemother* 2000;44:2777-2783.
9. Bradford PA, Petersen PJ, Fingerman IM, et al. Characterization of expanded-spectrum cephalosporin resistance in

E coli isolates associated with bovine calf diarrhoeal disease. *J Antimicrob Chemother* 1999;44:607-610.

10. Tollefson L, Angulo F, Fedorka-Cray P. National surveillance for antibiotic resistance in zoonotic enteric pathogens. *Vet Clin North Am Food Anim Pract* 1998;14:141-150.

11. Pezzlo M. Aerobic bacteriology. In: Isenberg HD, ed. *Clinical microbiology procedures handbook*. Washington, DC: American Society of Microbiology, 1992;1-20.

12. National Committee for Clinical Laboratory Standards. *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. Document M31-A*. Wayne, Pa: National Committee for Clinical Laboratory Standards, 1999.

13. Zhao S, White DG, McDermott PF, et al. Identification and expression of cephamycinase bla_{CMY} genes in *Escherichia coli* and *Salmonella* isolated from food animals and ground meats. *Antimicrob Agents Chemother* 2001;45:3647-650.

14. Bass L, Liebert CA, Lee MD, et al. Incidence and characterization of integrons, genetic elements mediating multiple-drug resistance in avian *Escherichia coli*. *Antimicrob Agents Chemother* 1999;43:2925-2999.

15. Cloud SS, Rosenberger JK, Fries PA, et al. In vitro and in vivo characterization of avian *Escherichia coli*. I. Serotypes, metabolic activity, and antibiotic sensitivity. *Avian Dis* 1985;29:1084-1093.

16. Irwin RJ, McEwen SA, Clarke RC, et al. The prevalence of verocytotoxin-producing *Escherichia coli* and antimicrobial resistance patterns of nonverocytotoxin-producing *Escherichia coli* and *Salmonella* in Ontario broiler chickens. *Can J Vet Res* 1989;53:411-418.

17. Allan BJ, van den Hurk JV, Potter AA. Characterization of *Escherichia coli* isolated from cases of avian colibacillosis. *Can J Vet Res* 1993;57:146-151.

18. Dubel JR, Zink DL, Kelley LM, et al. Bacterial antibiotic resistance: frequency of gentamicin-resistant strains of *Escherichia coli* in the fecal microflora of commercial turkeys. *Am J Vet Res* 1982;43:1786-1789.

19. Hooper DC, Wolfson JS. Mechanisms of bacterial resistance to quinolones. In: Hooper DC, Wolfson JS, eds. *Quinolone antimicrobial agents*. Washington, DC: American Society for Microbiology, 1993;97-118.

20. White DG, Zhao S, Sudler R, et al. The isolation of antibiotic-resistant *Salmonella* from retail ground meats. *N Engl J Med* 2001;345:1147-1154.

21. Bager F. DANMAP: monitoring antimicrobial resistance in Denmark. *Int J Antimicrob Agents* 2000;14:271-274.

22. Moreno MA, Dominguez L, Teshager T, et al. Antibiotic resistance monitoring: the Spanish programme. *Int J Antimicrob Agents* 2000;14:285-290.

23. Wray C, Gnanou JC. Antibiotic resistance monitoring in bacteria of animal origin: analysis of national monitoring programmes. *Int J Antimicrob Agents* 2000;14:291-294.

24. Dunlop RH, McEwen SA, Meek AH, et al. Sampling considerations for herd-level measurement of faecal *Escherichia coli* antimicrobial resistance in finisher pigs. *Epidemiol Infect* 1999;122:485-489.

25. Mallinson ET, Tate CR, Miller RG, et al. Monitoring poultry farms for *Salmonella* by drag-swab sampling and antigen-capture immunoassay. *Avian Dis* 1989;33:684-690.

26. Rolfe DL, Riemann HP, Farver TB, et al. Drag swab efficiency factors when sampling chicken manure. *Avian Dis* 2000;44:668-675.