Surveillance of antibiotic resistance in *Escherichia coli* isolated from wild cranes on the Izumi plain in Kagoshima prefecture, Japan

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**ABSTRACT.** The prevalence of antibiotic resistance in 376 *Escherichia coli* (*E. coli*) isolates from fecal samples of Hooded and White-naped cranes was investigated on the Izumi plain in Kagoshima prefecture, Japan, during winter 2016 and 2017. Resistance to oxytetracycline, ampicillin, and nalidixic acid were observed in 10.9%, 3.1–4.4%, and 2.1–7.7% of isolates, respectively. Since the previous surveillance in 2007, isolation rates of antibiotic-resistant *E. coli* recovered from wild cranes have remained at significantly low levels compared with those in Japanese livestock. Our results indicate that surveillance of antibiotic-resistant *E. coli* from wild cranes wintering in the Izumi plain could be a useful strategy to indicate natural environmental pollution by antibiotic-resistant bacteria in the environment.

**KEY WORDS:** antimicrobial resistance, *Escherichia coli*, Izumi plain, wild crane
Antibiotic susceptibility tests were performed via the agar dilution method to determine the minimum inhibitory concentrations (MICs) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines for 11 antibiotics: oxytetracycline (OTC), ampicillin (ABPC), nalidixic acid (NAL), cefazolin (CEZ), chloramphenicol (CP), colistin (CL), minocycline (MINO), kanamycin (KM), gentamicin (GM), fosfomycin (FOM), and enrofloxacin (ERFX). The antimicrobial breakpoints used as the MIC for the antibiotics were based on the CLSI criteria [5] or a previous study [14].

We recovered 193 and 183 E. coli isolates from wild crane feces during winter 2016 and 2017, respectively. E. coli isolates resistant to OTC, ABPC, and NAL were identified in 2016 and 2017 samples, and all isolates were susceptible to MINO, KM, ERFX, CP, CEZ, GM, FOM, and CL (Table 1). The prevalence of resistant isolates was 10.9%, 3.1–4.4%, and 2.1–7.7% for OTC, ABPC, and NAL, respectively. Thus, OTC-resistant E. coli isolates were the most common. Furthermore, we identified three types of multiple-resistant E. coli isolates (resistant to OTC-ABPC-NAL, OTC-ABPC, or OTC-NAL), demonstrating that all of the resistance phenotypes were combined with OTC-resistance (Table 2). Since the beginning of the 21st century, tetracycline antibiotics have been the most commonly used in food-producing animals in Japan [1, 2, 8, 17]. Tetracycline resistance was the most frequent type of antibiotic resistance found among E. coli isolates in cattle (average resistance rate: 31.4%), pigs (65.5%), and broiler chickens (63.2%) during 2000–2007 [8], and the isolation rates of tetracycline-resistant strains in these animal species have remained stable until recent years [17]. Our surveillance data in 2007 [13], 2016, and 2017 demonstrated that OTC-resistant E. coli isolates from wild cranes were also the most frequently detected (15.9% for 2007, 10.9% for 2016 and 2017) among the antibiotics tested. However, the isolation rates of OTC-resistant E. coli have remained at low levels compared with that of food-producing animals in Japan. Furthermore, the types of antibiotic-resistant E. coli isolates observed in wild cranes in 2016 and 2017 (OTC, ABPC, and NAL) were less than those in 2007 (OTC, ABPC, NAL, MINO, KM, and ERFX). Thus, a low prevalence of antibiotic resistance has been maintained among Hooded and White-naped cranes wintering on the Izumi plain.

Extended-spectrum β-lactamase (ESBL)-producing E. coli

### Table 1. Antimicrobial resistance of Escherichia coli isolated from wild cranes in each ficial year

| Antimicrobial agents | Break point (µg/ml) | No. of resistance (%) | 2016 | 2017 |
|---------------------|---------------------|-----------------------|------|------|
| Oxytetracycline     | 16                  | 21 (10.9)             | 20 (10.9) | |
| Ampicillin          | 32                  | 6 (3.1)               | 8 (4.4) | |
| Cefazolin           | 32                  | 0 (0)                 | 0 (0) | |
| Nalidixic acid      | 32                  | 4 (2.1)               | 14 (7.7) | |
| Minocycline         | 16                  | 0 (0)                 | 0 (0) | |
| Chloramphenicol     | 32                  | 0 (0)                 | 0 (0) | |
| Kanamycin           | 64                  | 0 (0)                 | 0 (0) | |
| Gentamicin          | 16                  | 0 (0)                 | 0 (0) | |
| Fosfomycin          | 256                 | 0 (0)                 | 0 (0) | |
| Colistin            | 16                  | 0 (0)                 | 0 (0) | |
| Enrofloxacin        | 2                   | 0 (0)                 | 0 (0) | |

Table 2. Minimum inhibitory concentration (MIC) values in antimicrobial-resistant Escherichia coli isolated from wild cranes

| E. coli ID | MIC (µg/ml) | Oxytetracycline | Ampicillin | Nalidixic acid |
|-----------|-------------|-----------------|------------|---------------|
| 16004     | 32          | -               | -          | -             |
| 16018     | -           | 64              | -          | -             |
| 16022     | 256         | -               | -          | -             |
| 16024     | 64          | -               | -          | -             |
| 16029     | 32          | -               | -          | -             |
| 16031     | 32          | -               | -          | -             |
| 16033     | 32          | -               | -          | -             |
| 16051     | -           | 128             | -          | -             |
| 16055     | 32          | -               | -          | -             |
| 16057     | 32          | -               | -          | -             |
| 16058     | 32          | -               | -          | -             |
| 16068     | 128         | -               | -          | -             |
| 16073     | 128         | -               | -          | 128           |
| 16080     | 64          | -               | -          | -             |
| 16092     | -           | -               | 128        | -             |
| 16096     | 64          | -               | -          | -             |
| 16097     | 64          | >512            | -          | -             |
| 16098     | 128         | -               | -          | -             |
| 16099     | -           | 64              | -          | -             |
| 16101     | -           | 256             | -          | -             |
| 16105     | 128         | -               | -          | -             |
| 16106     | 32          | -               | -          | -             |
| 16143     | 64          | -               | 64         | -             |
| 16148     | 32          | -               | -          | -             |
| 16163     | -           | 256             | -          | -             |
| 16175     | 32          | -               | -          | -             |
| 16188     | -           | -               | 64         | -             |
| 16197     | 256         | -               | -          | -             |
| 16005     | >512        | 128             | >512       | -             |
| 16014     | >512        | -               | -          | -             |
| 16019     | >512        | -               | -          | -             |
| 16024     | >512        | -               | -          | -             |
| 16028     | -           | 256             | -          | -             |
| 16030     | -           | -               | 128        | -             |
| 16054     | 512         | -               | -          | -             |
| 16058     | 512         | -               | -          | -             |
| 16059     | >512        | -               | -          | -             |
| 16067     | 32          | -               | -          | -             |
| 16088     | -           | -               | 128        | -             |
| 16091     | >512        | -               | -          | -             |
| 16095     | >512        | 512             | 512        | -             |
| 17102     | >512        | -               | 128        | -             |
| 17103     | >512        | -               | 128        | -             |
| 17114     | >512        | 256             | >512       | -             |
| 17123     | >512        | -               | 128        | -             |
| 17124     | >512        | >512            | -          | -             |
| 17128     | -           | >512            | -          | -             |
| 17131     | 512         | -               | 512        | -             |
| 17136     | >512        | -               | -          | -             |
| 17137     | 512         | >512            | >512       | -             |
| 17146     | >512        | -               | 256        | -             |
| 17179     | -           | >512            | -          | -             |
| 17183     | 32          | -               | 64         | -             |
| 17196     | -           | -               | 128        | -             |

- indicate that E. coli isolates were susceptibility to each antimicrobial agent.
represent a major problem in human and veterinary medicine [4]. There are several reports of ESBL producing E. coli isolates recovered not only from human and domestic animals but also from wild animals, and most ESBL genes are mutant derivatives of the classical TEM beta-lactamases [6, 7, 20]. In our present study, almost all ABPC-resistant E. coli isolates from wild cranes possessed the TEM beta-lactamase gene (data not shown), correlating with the surveillance results from winter 2007 [13]. However, all ABPC-resistant isolates in the present study were susceptible to cefazolin, a second-generation cephalosporin, indicating that colonization by ESBL-producing E. coli is yet to be established in wild crane populations.

The overlapping of living areas between wildlife and other animal species, such as human and livestock, leads to a rapid change in the antibiotic resistance of bacteria colonized in wild animals [3, 11]. Therefore, several recent Japanese studies have begun to consider the role of wildlife as sentinels for antimicrobial resistance over a decade [10, 16, 18, 19]. Our results demonstrated that antibiotic resistance has remained low in E. coli isolates from wild cranes since 2007, indicating that the surveillance of antibiotic-resistant E. coli isolates from wild cranes wintering on the Izumi plain could be a useful strategy to indicate the natural environmental pollution by antibiotic-resistant bacteria. In addition, a total of 15 strains of Salmonella spp. was also recovered from wild crane fecal samples in the present surveillance. Using these bacterial isolates, further molecular analyses including pulsed-field gel electrophoresis may lead to elucidate whether antibiotic-resistant bacteria entered into the Izumi plain accompanied by the migration of wild cranes or not. In the future, continual and comprehensive surveillance of wild cranes should be undertaken to protect these endangered species from pathogenic epidemics.

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