The Journal of Veterinary Medical Science

NOTE

Avian Pathology

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

Yasuhiro SUENAGA[1)], Takeshi OBI[1,2)], Moe IJIRI[3)], Takehisa CHUMA[2,3)] and Yoshikazu FUJIMOTO[2,3)]*

[1)]Laboratory of Veterinary Microbiology, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Kagoshima-shi, Kagoshima 890-0065, Japan
[2)]Joint Graduate School of Veterinary Medicine, Kagoshima University, 1-21-24 Kagoshima-shi, Kagoshima 890-0065, Japan
[3)]Transboundary Animal Diseases Research Center, Joint Faculty of Veterinary Medicine, Kagoshima University, 1-21-24 Kagoshima-shi, Kagoshima 890-0065, Japan

J. Vet. Med. Sci.
81(9): 1291–1293, 2019
doi: 10.1292/jvms.19-0305

Received: 4 June 2019
Accepted: 2 July 2019
Advanced Epub: 11 July 2019

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

The Hooded crane (*Grus monacha*) and White-naped crane (*Grus vipio*) are categorized as vulnerable species on the International Union for Conservation of Nature Red List, with a global population of approximately 15,000 and 7,000, respectively [9]. Annually, from mid-October, approximately 90% of the world's population for Hooded cranes and half of the world's population for White-naped cranes winter on the Izumi plain in Kagoshima prefecture, Japan. This region is an important overwintering area for Hooded and White-naped cranes, and these birds and their habitats are designated special natural treasures of Japan.

Since winter 1996, our laboratory has conducted surveillance of antibiotic-resistant *Escherichia coli* (*E. coli*) and *Salmonella* spp. in migrating wild cranes on the Izumi plain. Although wild cranes are generally unexposed to antibiotics, we identified antibiotic-resistant bacteria in their fecal samples [12, 13, 15]. We also showed that antibiotic-resistant *E. coli* isolates were more detectable than those of *Salmonella* spp. in wild cranes [12, 13]. *E. coli* is generally used as an indicator bacterium for monitoring antibiotic resistance in livestock and wild animals. In the present study, to determine the antibiotic susceptibility of recent bacterial isolates from wild cranes, we investigated the prevalence of antibiotic-resistant *E. coli* in migrating wild cranes during winter 2016 and 2017.

We collected 200 fresh fecal samples from wild cranes wintering on the Izumi plain (32.11°–32.12° N and 130.27°–130.28° E) in November 2016 and 2017, respectively. Sample collections were conducted in the early morning. Each collected sample that was not soaked by environmental water was put into sterile 35 mm petri dish, stored in a cooler box, and brought to our laboratory. Subsequently, the fecal material was suspended in buffered peptone water (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) and incubated overnight at 37°C. Then, the supernatant was spread on deoxycholate hydrogen sulfide lactose agar plates (Nissui Pharmaceutical Co., Ltd.) and incubated for 24 hr at 37°C. One or two colonies of suspected *E. coli* per sample were picked and subcultured on XM-G agar (Nissui Pharmaceutical Co., Ltd.). Blue-color colonies forming on XM-G agar were identified as *E. coli* according to the manufacture's instruction, and blue/purple- or red/purple-color colonies were tested by matrix-assisted laser desorption ionization time-of-flight mass spectrometry/time-of-flight mass spectrometry (Bruker, Billerica, MA, U.S.A.) to identified as *E. coli*. Isolated bacterial strains with sterile glycerol to the final concentration of 25% were stored at −80°C until analyzed.

*Correspondence to: Fujimoto, Y.: k7639981@kadai.jp

©2019 The Japanese Society of Veterinary Science

This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial No Derivatives (by-nc-nd) License. (CC-BY-NC-ND 4.0: https://creativecommons.org/licenses/by-nc-nd/4.0/)

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/m*l*)	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/m*l*)		
	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	-	-
17005	>512	128	>512
17014	>512	-	-
17019	>512	-	-
17024	>512	-	-
17028	-	-	256
17030	-	-	128
17054	512	-	-
17058	512	-	-
17059	>512	-	-
17067	32	-	-
17088	-	-	128
17091	>512	-	-
17093	-	-	>512
17095	>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.

ACKNOWLEDGMENTS. The authors gratefully acknowledge Dr. Kozo Takase (professor emeritus, Kagoshima University) and Tsutomu Matsui (Izumi City Crane Museum, Crane Park Izumi, Kagoshima, Japan) for the excellent advice to proceed our research.

REFERENCES

1. Asai, T., Harada, K., Ishihara, K., Kojima, A., Sameshima, T., Tamura, Y. and Takahashi, T. 2007. Association of antimicrobial resistance in *Campylobacter* isolated from food-producing animals with antimicrobial use on farms. *Jpn. J. Infect. Dis.* **60**: 290–294. [Medline]
2. Asai, T., Kojima, A., Harada, K., Ishihara, K., Takahashi, T. and Tamura, Y. 2005. Correlation between the usage volume of veterinary therapeutic antimicrobials and resistance in *Escherichia coli* isolated from the feces of food-producing animals in Japan. *Jpn. J. Infect. Dis.* **58**: 369–372. [Medline]
3. Bengtsson-Palme, J., Kristiansson, E. and Larsson, D. G. J. 2018. Environmental factors influencing the development and spread of antibiotic resistance. *FEMS Microbiol. Rev.* **42**. [Medline] [CrossRef]
4. Briñas, L., Zarazaga, M., Sáenz, Y., Ruiz-Larrea, F. and Torres, C. 2002. Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. *Antimicrob. Agents Chemother.* **46**: 3156–3163. [Medline] [CrossRef]
5. Clinical Laboratory Standards Institute (CLSI). 2008. Performance standards for antimicrobial disk dilution susceptibility tests for bacteria isolated from animals; approved standard-3rd edition. M31-A3 vol. 28 No.8.
6. Costa, D., Poeta, P., Sáenz, Y., Vinué, L., Rojo-Bezares, B., Jouini, A., Zarazaga, M., Rodrigues, J. and Torres, C. 2006. Detection of *Escherichia coli* harbouring extended-spectrum beta-lactamases of the CTX-M, TEM and SHV classes in faecal samples of wild animals in Portugal. *J. Antimicrob. Chemother.* **58**: 1311–1312. [Medline] [CrossRef]
7. Cristóvão, F., Alonso, C. A., Igrejas, G., Sousa, M., Silva, V., Pereira, J. E., Lozano, C., Cortés-Cortés, G., Torres, C. and Poeta, P. 2017. Clonal diversity of extended-spectrum beta-lactamase producing *Escherichia coli* isolates in fecal samples of wild animals. *FEMS Microbiol. Lett.* **364**. [Medline] [CrossRef]
8. Harada, K. and Asai, T. 2010. Role of antimicrobial selective pressure and secondary factors on antimicrobial resistance prevalence in *Escherichia coli* from food-producing animals in Japan. *J. Biomed. Biotechnol.* **2010**: 180682. [Medline] [CrossRef]
9. International Union for Conservation of Nature. 2019. The IUCN red list of threatened species. Version 2019-1.
10. Ishihara, K., Kanamori, K., Asai, T., Kojima, A., Takahashi, T., Ueno, H., Muramatsu, Y. and Tamura, Y. 2011. Antimicrobial susceptibility of *Escherichia coli* isolates from wild mice in a forest of a natural park in Hokkaido, Japan. *J. Vet. Med. Sci.* **73**: 1191–1193. [Medline] [CrossRef]
11. Kinjo, T., Minamoto, N., Sugiyama, M. and Sugiyama, Y. 1992. Comparison of antimicrobial resistant *Escherichia coli* in wild and captive Japanese serows. *J. Vet. Med. Sci.* **54**: 821–827. [Medline] [CrossRef]
12. Kitadai, N., Ninomiya, N., Murase, T., Obi, T. and Takase, K. 2010. *Salmonella* isolated from the feces of migrating cranes at the Izumi Plain (2002–2008): serotype, antibiotic sensitivity and PFGE type. *J. Vet. Med. Sci.* **72**: 939–942. [Medline] [CrossRef]
13. Kitadai, N., Obi, T., Yamashita, S., Murase, T. and Takase, K. 2012. Antimicrobial susceptibility of *Escherichia coli* isolated from feces of wild cranes migrating to Kagoshima, Japan. *J. Vet. Med. Sci.* **74**: 395–397. [Medline] [CrossRef]
14. Kojima, A., Asai, T., Ishihara, K., Morioka, A., Akimoto, K., Sugimoto, Y., Sato, T., Tamura, Y. and Takahashi, T. 2009. National monitoring for antimicrobial resistance among indicator bacteria isolated from food-producing animals in Japan. *J. Vet. Med. Sci.* **71**: 1301–1308. [Medline] [CrossRef]
15. Maeda, Y., Tohya, Y., Nakagami, Y., Yamashita, M. and Sugimura, T. 2001. An occurrence of *salmonella* infection in cranes at the Izumi Plains, Japan. *J. Vet. Med. Sci.* **63**: 943–944. [Medline] [CrossRef]
16. Nakamura, I., Obi, T., Sakemi, Y., Nakayama, A., Miyazaki, K., Ogura, G., Tamaki, M., Oka, T., Takase, K., Miyamoto, A. and Kawamoto, Y. 2011. The prevalence of antimicrobial-resistant *Escherichia coli* in two species of invasive alien mammals in Japan. *J. Vet. Med. Sci.* **73**: 1067–1070. [Medline] [CrossRef]
17. National veterinary Assay Laboratory, Ministry of Agriculture, Forestry and Fisheries. Report on the Japanese Veterinary Antimicrobial Resistance Monitoring System 2014–2015. http://www.maff.go.jp/nval/yakuzai/pdf/JVARM_Report_2014-2015.pdf [accessed on June 3, 2019].
18. Obi, T., Chibana, M., Taira, C., Nakayama, A., Miyazaki, K., Takase, K., Nakamura, I., Miyamoto, A. and Kawamoto, Y. 2014. Antimicrobial susceptibility in *Enterobacteriaceae* recovered from Okinawa least horseshoe bat *Rhinolophus pumilus*. *Wildl. Boil.* **20**: 64–66. [CrossRef]
19. Ogawa, K., Yamaguchi, K., Suzuki, M., Tsubota, T., Ohya, K. and Fukushi, H. 2011. Genetic characteristics and antimicrobial resistance of *Escherichia coli* from Japanese macaques (*Macaca fuscata*) in rural Japan. *J. Wildl. Dis.* **47**: 261–270. [Medline] [CrossRef]
20. Parker, D., Sniatynski, M. K., Mandrusiak, D. and Rubin, J. E. 2016. Extended-spectrum β-lactamase producing *Escherichia coli* isolated from wild birds in Saskatoon, Canada. *Lett. Appl. Microbiol.* **63**: 11–15. [Medline] [CrossRef]