NOTE *Public Health*

Isolation and antimicrobial susceptibility of *Plesiomonas shigelloides* from great cormorants (*Phalacrocorax carbo hanedae*) in Gifu and Shiga Prefectures, Japan

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ABSTRACT. *Plesiomonas shigelloides* is a causal agent of gastroenteritis, sepsis and meningitis in humans. We examined the prevalence of *P. shigelloides* among great cormorants (*Phalacrocorax carbo hanedae*) in Japan and the antimicrobial susceptibility of isolates. *P. shigelloides* was isolated from 33 (47.8%) of 69 fecal samples from great cormorants in 2014. All 33 isolates were subjected to antimicrobial susceptibility testing using broth microdilution methods, which showed resistance to ampicillin (31 isolates, 93.9%), tetracycline (two isolates, 6.1%) and trimethoprim (one isolate, 3.0%). The high prevalence of *P. shigelloides* in the great cormorants implicates the possible microbiological risk to public health.

KEY WORDS: antimicrobial susceptibility, great cormorant, *Plesiomonas shigelloides*

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Plesiomonas shigelloides is a gram-negative, rod-shaped and facultative anaerobic bacterium that has been isolated from aquatic environments [1, 12, 17], wild and domestic animals [1, 7, 8], and humans [3, 9, 17, 18]. This bacterium is a causal agent of gastroenteritis, sepsis and meningitis in humans [3], and was classified as a food poisoning-inducing organism in 1982 in Japan. In Japan, diarrhea caused by *P. shigelloides* is frequently found among travelers returning from the Southeast Asia [18]. Antimicrobial resistance profiles of pathogenic bacteria are essential information for antimicrobial therapy. Different profiles of *P. shigelloides* have been reported using isolates obtained from various origins, such as human patients [4, 7, 9, 15], fresh water [7, 15], fish [7], amphibians and reptiles [8] and mammals and birds [7]. Most of *P. shigelloides* isolates are resistant to penicillin antibiotics due to β-lactamase production, but not to cephalosporin antibiotics [4].

The great cormorant (*Phalacrocorax carbo*; cormorant) is a known host species of *P. shigelloides* [14]. The population of *Phalacrocorax carbo hanedae*, a subspecies that inhabits Japan, has been dramatically increasing for the last 3 decades in Japan [16]. These birds cause severe damage to aquatic industries by consuming large quantities of fishes, including farmed freshwater species meant for release (e.g., ayu, *Plecoglossus altivelis*). In addition, the birds also damage woods adjacent to waterside by forming roosts and colonies [16], and large amounts of their dropped excreta (guano) gets accumulated on the ground [10]. Such accumulations of feces from an ever increasing number of birds may also escalate the microbiological risk and result in a possible public health hazard due to the enterobacteria present in feces.

Therefore, the aim of the present study was to assess the prevalence of *P. shigelloides* in fecal matter from great cormorants in Japan and test the isolates for antimicrobial resistance to elucidate their possible microbiological impacts on the environment.

We obtained 69 cormorant fecal samples from lake Biwa (n=46) in Shiga Prefecture and from Iwaya dam (n=23) in Gifu Prefecture in Japan (Fig. 1). Lake Biwa is the largest freshwater lake in Japan, and the Iwaya dam is a rock-fill dam, which stores water from the Maze River. These 2 water bodies are about a hundred kilometers apart. All cormorants were culled at each region in 2014 by the sharp-shooting method [16]. Data for individual cormorants regarding the sex and growth stage (adult: individuals with the adult plumage; juvenile: yearling that got off from the nest; and chick: yearling in the nest) were noted. Cloacal swabs of individual cormorants were sampled and stored at 4°C. Each sample was cultured on deoxycholate hydrogen sulfide lactose agar within 3 days of collection. Candidate colonies, indicated by a pale pink color, were tested using a cytochrome oxidase disk, triple sugar iron medium and lysine indole motility medium, and identified using the Api 20E bacterial identification system (BioMerieux, Tokyo, Japan).

The minimum inhibitory concentrations (MICs) were determined using broth microdilution methods according to Clinical and Laboratory Standards Institute (CLSI) guidelines [5]. The isolates were tested for resistance to the following 11 antimicrobial agents: ampicillin (AMP), cefazolin

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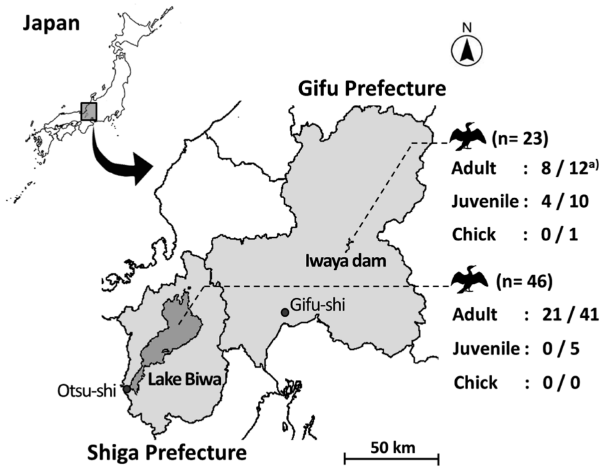

Fig. 1. Locations of lake Biwa and the Iwaya dam, and prevalences of *P. shigelloides* in cormorant fecal samples from each sampling area. We collected 46 samples from lake Biwa and 23 from the Iwaya dam. a) Numbers of *P. shigelloides*-positive samples/tested.

(CFZ), cefotaxime (CTX), gentamicin, kanamycin, tetracycline (TET), nalidixic acid, ciprofloxacin, colistin (CST), chloramphenicol and trimethoprim (TMP). The breakpoints established by CLSI were used for each agent, except for CST [6]. We could not set a microbiological breakpoint for CST as its MICs were modally distributed. *Escherichia coli* ATCC 25922 was used as a quality control strain.

P. shigelloides was isolated from 33 (47.8%) of 69 samples (Fig. 1). There were no significant differences in isolation rates between the geographical areas, host sex or host stages. A high prevalence of *P. shigelloides* in great cormorant (*P. carbo carbo*) fecal samples was also reported in Germany (74.4%, 32/43) [14]. An epidemiological study between 1974 and 1976 in Japan showed that *P. shigelloides* was prevalent in feces sampled from dogs (3.8%, 37/967), cats (10.3%, 40/389) and freshwater fishes (10.2%, 25/246), but rarely detected in humans, cattle, swine or poultry [1]. A previous survey in Japan revealed the contamination of river water and sludge samples with *P. shigelloides* at ratios of 12.8% (64/497) and 10.5% (2/19), respectively [1]. It is believed that humans are mainly infected with *P. shigelloides* on contact with polluted aquatic environments or consumption of contaminated foods [11, 17]. Further studies are needed to clarify, if the environmental pollution of *P. shigelloides* due to colonization of great cormorants progresses in these regions.

Except for two, all isolates exhibited antimicrobial resistance (Table 1). AMP resistance was found in 31 isolates (93.9%, ≥32 μg/m*l*). The resistance to TET (≥16 μg/m*l*) and TMP (≥16 μg/m*l*) was detected in two isolates (6.1%) and one (3.1%) isolate, respectively. Three isolates exhibited resistance to multiple antimicrobials, in addition to AMP resistance. No isolate exhibited resistance to the other eight antimicrobials. The results of the present study, in terms of resistance to AMP but susceptibility to cephalosporins, CFZ and CTX, were similar to the previous observations of *P. shigelloides* isolates from humans and aquatic environments [4, 9, 13].TET resistance of *P. shigelloides* has been reported in several countries [8, 9, 11], and TMP resistance was reported in North America [13]. In addition, a fluoroquinolone-resistant *P. shigelloides* isolated from a catfish was reported in Southeast Asia [11]. Treatment of the wild great cormorant with antimicrobial agents is extremely rare, because the extent of damage to the aquatic industries and increased population of the birds makes the idea economically unfeasible. The low prevalence of antimicrobial resistance, except for AMP resistance, in isolates of *P. shigelloides* may be associated with the absence of contact to antimicrobial agents in cormorants.

The β-lactamase activity of 2 AMP-susceptible and 10 AMP-resistant isolates was tested using the acidometric method (P/Case test-N, Nissui, Tokyo, Japan). One AMP-susceptible isolate (MIC for AMP=1 μg/m*l*) was negative for both penicillinase and cephalosporinase activity, while the

Table 1. The MIC distribution of antimicrobial agents against 33 isolates of *P. shigelloides* from 69 great cormorants

Antimicrobial agents[a)]	MICs (μg/m*l*)														Resistant isolates (%)
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	>128	
AMP						1		1			2	17	11	1	93.9
CFZ							32	1							0.0
CTX					33										0.0
GEN						12	20	1							0.0
KAN						1	1	1	7	23					0.0
TET					11	18	2			2					6.1
NAL						29	2	2							0.0
CIP	30	1	1	1											0.0
CST			7	20	6										-
CHL						29	1	1		2					0.0
TMP				4	7	8	9	1	3		1				3.0

White fields represent the range of dilutions tested. MIC values equal to or lower than the lowest concentration are presented as the lowest concentration. MIC values greater than the highest concentration in the range are presented as one dilution step above the range. The values of CLSI breakpoint were indicated as vertical solid lines. a) Ampicillin (AMP), cefazolin (CFZ), cefotaxime (CTX), gentamicin (GEN), kanamycin (KAN), tetracycline (TET), nalidixic acid (NAL), ciprofloxacin (CIP), colistin (CST), chloramphenicol (CHL) and trimethoprim (TMP).

other (MIC for AMP=4 μg/m*l*) was positive for penicillinase activity, as observed for the 10 AMP-resistant isolates. Avison *et al*. [2] showed the diversity of penicillinase produced by *P. shigelloides* and the difference in its activity against each penicillin substance. A further study on susceptibility to other penicillin antibiotics and identification of β-lactamase in isolates from the cormorants is required.

In conclusion, the high prevalence of the foodborne pathogen *P. shigelloides* in wild great cormorant poses public health implications in Japan resulting from microbial environmental pollution.

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