



Bacterial flora of free-living Double-crested cormorant (*Phalacrocorax auritus*) chicks on Prince Edward Island, Canada, with reference to enteric bacteria and antibiotic resistance

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Abstract

Cloacal and pharyngeal swabs from 100 tree-nesting Double-crested cormorant (DCC) chicks were examined by culture for commensal and potentially pathogenic bacteria. No *Salmonella* or *Erysipelothrix* were isolated from the cloacal swabs. Twenty-two cloacal swabs were positive for *Campylobacter*, of which 14 were *C. jejuni*, *C. coli*, and 1 *C. lari*. None belonged to common serotypes isolated from humans or animals in recent years in Canada. Tests for antimicrobial drug resistance among 187 commensal *Escherichia coli* isolates from the cloacal swabs indicated that ≤5% were resistant to any of the 12 antibiotics tested. This contrasts with the frequently high resistance rates among *E. coli* isolates from poultry. Pharyngeal swabs from DCC were negative for *Pasteurella multocida*. Culture of cloacal swabs from 100 ground-nesting DCC chicks resulted in the recovery of 19 *Salmonella* isolates, all of which were *S. enterica* serotype Typhimurium. None of

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these isolates were resistant to any of the 12 antibiotics tested. Altogether, these findings suggest that DCC from this region are not being colonized with commensal or potentially pathogenic enteric bacteria from agricultural or human sources and that enteric bacteria isolated from these birds are unlikely to contribute to a gene pool of antimicrobial drug resistance.

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Keywords: Antibiotic resistance; *Campylobacter*; Cormorant; *Erysipelothrix*; *Escherichia coli*; Intestinal flora; Pharyngeal flora; *Salmonella*

Résumé

Des écouvillons du cloaque et du pharynx de 100 jeunes cormorans à aigrettes (CA) d'une colonie arboricole ont été cultivés pour déterminer la présence de bactéries commensales ou potentiellement pathogènes. Aucun *Salmonella* ou *Erysipelothrix* ne fut isolé des écouvillons du cloaque. *Campylobacter* fut isolé de 22 écouvillons du cloaque; les souches isolées incluaient 14 *C. jejuni*, 7 *C. coli* et 1 *C. lari*. Aucune de ces souches n'appartenait à des sérotypes communs récemment isolés d'humains ou d'animaux au Canada. Des tests de résistance aux antibiotiques faits sur 187 souches commensales de *Escherichia coli* isolées des écouvillons du cloaque ont révélé que $\leq 5\%$ étaient résistantes à l'un ou l'autre de 12 antibiotiques testés. Ces résultats diffèrent du taux de résistance souvent élevé des souches de *E. coli* isolées de la volaille. Les écouvillons du pharynx des jeunes CA étaient négatifs pour la présence de *Pasteurella multocida*. La culture d'écouvillons du cloaque de 100 jeunes CA d'une colonie au sol révéla la présence de 19 souches de *Salmonella*, toutes identifiées comme étant *S. enterica* serotype Typhimurium. Aucune de ces souches n'était résistante à aucun des 12 antibiotiques testés. La somme de ces observations suggère que les CA de cette région ne sont pas porteurs de bactéries intestinales commensales ou potentiellement pathogènes d'origine humaine ou agricole et qu'il est improbable que les bactéries intestinales isolées de ces oiseaux puissent contribuer à un réservoir génétique de résistance aux drogues antimicrobiennes.

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Mots clé: *Campylobacter*; Cormoran; *Erysipelothrix*; *Escherichia coli*; Flore intestinale; Flore pharyngienne; Résistance aux antibiotiques; *Salmonella*

1. Introduction

Defining the bacterial flora of clinically healthy animals is an important step in understanding the epidemiology of bacterial diseases that may affect their populations and those of sympatric species. With migratory bird species covering wide geographic areas, interpretation of this information represents an added challenge. Knowledge of the presence of bacteria of potential zoonotic importance among free-living animals also has public health significance. In the past, a variety of pathogenic bacterial species have been isolated from wild birds. Such bacteria include *Pasteurella* species (notably *P. multocida*, the causative agent of avian cholera), *Salmonella* species of zoonotic significance, and *Erysipelothrix rhusiopathiae*, all of which are potentially important sources of mortality and reduced fitness in wild bird populations [1]. These bacteria may also cause disease in domestic animals including poultry. Free-living birds can also serve as reservoirs of

campylobacters pathogenic to humans, and coliform bacteria, such as *Escherichia coli*, carrying resistance genes to antimicrobial drugs. In one study, more than half of the *E. coli* strains isolated from waterfowl were found to be positive for conjugative resistance plasmids [2]. Such antibiotic resistance is clearly of public health significance, and migratory birds in particular are potential long-range carriers of enteropathogenic bacteria with antibiotic resistance or virulence factors [3]. Antibiotic resistance analysis of enteric bacteria may also help to determine the source of bacteria that pollute water sources because of unique resistance patterns related to the source of origin [4,5].

A variety of potentially pathogenic bacteria can be carried asymptotically by animals, including wild birds. However, several factors, including concurrent disease and social or environmental stress, can contribute to changes in the physiology of the host that allow these bacteria to cause disease. For example, the capture, transportation and captivity of free-living wild animals can represent a source of severe stress for them. In an experimental study of infection of Double-Crested Cormorants (DCC) (*Phalacrocorax auritus*) by the intestinal trematode *Cryptocotyle lingua*, carried out in 1998 and 1999, approximately 34% of 2–3-week-old nestlings brought into captivity died within 2 weeks of arrival from infection with either *S. enterica* serotype Typhimurium (*S. Typhimurium*) or *E. rhusiopathiae* [6]. As these birds were kept in isolation from other animals on the holding premises, it was hypothesized that some of them were already subclinical carriers of the pathogenic bacteria at their colony. In order to test this hypothesis and also to determine more broadly the bacterial flora of this species, birds from the colony of origin of these nestlings and from a nearby colony were sampled in 2001–2003 for our study. Additionally, antimicrobial resistance properties among potential bacterial pathogens as well as commensal *E. coli* were tested to determine if DCC constitute a reservoir of drug-resistant bacteria, and if the resistance patterns are unique to this source.

2. Materials and methods

Malpeque Bay, Prince Edward Island (PEI) (46°32'N, 63°45'W), contains two colonies of DCC. One, located on Ram Island (17.1 ha), is a tree-nesting colony of approximately 4000 breeding pairs spread throughout the island. The other, on Little Courtin Island (15.4 ha), a treeless island barely above the high tidal mark and approximately 3 km south of Ram Island, is a ground-nesting colony of approximately 300 breeding pairs, distributed in clusters of 50–100 nests, which share the island with large numbers of Herring Gulls (*Larus argentatus*) and a lesser number of Great Black-backed Gulls (GBG) (*L. marinus*). Nestlings of DCC brought into captivity in 1998 and 1999 had been captured on Little Courtin Island. Double-crested cormorants on PEI belong to the Atlantic population, which overwinters along the Atlantic coast from North Carolina southwards and in the eastern Gulf of Mexico, although there is considerable overlap with the population from the interior of the continent that overwinters in the lower Mississippi Valley and western Gulf of Mexico [7]. Every year, adults come back to PEI around mid April. Hatching starts in late May, but egg laying and hatching often extend into mid to late June.

2.1. Field sampling

On both islands, 2–4-week old DCC nestlings were selected for sampling. Determination of their age was based on previous visits to the colonies to assess the approximate laying and hatching times of the majority of the nests and on the presence of primary wing feathers in individual birds, which normally start to appear at 12–14 days of age [8]. This age group was selected to allow adequate exposure of the birds to the bacterial flora of their environment, and for ease of capture. In the ground-nesting colony, GBG chicks were also sampled. Their identity as GBG was based on the strong predominance of adults of this species in the part of the island sampled as compared to Herring Gulls, and their age was also estimated to be 2–4 weeks based on previous visits.

In the tree-nesting colony in summer 2001, the largest chick in each of 100 nests from a total of 59 trees, chosen on the basis of their accessibility, was caught, and a swab-transport system containing Amies medium (BD Sciences, Oakville, Ont.) was used to sample the cloaca (two swabs) and the pharynx (one swab). The bird was then immediately returned to its nest. The swabs were packed in crushed ice and processed within 2 h of collection.

In the ground-nesting colony in summer 2002, 50 DCC chicks from a cluster of approximately 100 nests were sampled in a single session lasting approximately 1 h. The cloaca (but not the pharynx) of each bird was sampled in a manner similar to that in the tree-nesting colony in 2001. Sampled birds were released after marking. The cloaca of 30 GBG chicks was also sampled from the same area 2 weeks earlier.

In summer 2003, a single cloacal swab was taken from 50 DCC chicks in the tree-nesting colony and from 50 DCC chicks and 49 GBG chicks in the ground-nesting colony. Cary-Blair transport medium, which is formulated for optimal recovery of enteric organisms [9] was used this time for transport of the samples. All cloacal swabs collected in 2002 and 2003 were cultured for detection of *Salmonella* only.

2.2. Bacteriology

Cloacal swabs were cultured on blood agar (BA) (Oxoid, Nepean, Ont., Canada), and MacConkey agar (MAC) (BD Sciences), and then inoculated into Rappaport medium (Oxoid). For isolation of *Salmonella*, subcultures from Rappaport medium were done on modified semi-solid Rappaport Vassiliadis medium (MSRV) after incubation at 42 °C for 3–4 days as per the methods described by Dusch and Altwegg [10]. Suspected *Salmonella* isolates were presumptively identified on the basis of API-20E (BioMérieux Canada, Inc., St Laurent, Que., Canada) results, after initial tests for negative urease reaction and agglutination with *Salmonella* Poly-O antisera (BD Sciences). Confirmed isolates were serotyped and phage typed as per published methods [11] at the OIÉ Reference Laboratory for Salmonellosis, Guelph, Ont., Canada.

For isolation of campylobacters from cloacal swabs, *Campylobacter* blood-free agar (CBF) (Oxoid) plates were inoculated, and incubated under microaerophilic conditions employing Campy-Pak (Oxoid) at 42 °C for 48 h. Colonies grown were subcultured onto BA plates after confirmation of morphology by Gram staining. The colonies grown on the BA plates were transferred into 2% sterile skim milk in cryovials (Simport Plastics, Beloeil,

Que.) and stored at -76°C . For identification, the frozen isolates were thawed and grown microaerophilically on BA plates, and subjected to oxidase, catalase and hippurate tests, and latex agglutination for *C. jejuni/coliformi* (JCL, Integrated Diagnostics, Baltimore, MD, USA). The isolates were biotyped and serotyped at the National Microbiology Laboratory, Winnipeg, Man., Canada. For the Penner (heat stable [HS]) serotyping scheme, the passive hemagglutination test with soluble HS (O) antigens [12] was used; this involved heated extracts, sensitized sheep erythrocytes, and antisera in microtitre plates. Serotyping with the Lior (heat labile [HL]) scheme was performed by slide agglutination with live bacteria and crude and absorbed antisera for the detection of HL antigens [13].

After incubation at 37°C for 24 h on MAC, as many as five lactose fermenting (LF) colonies (depending on number of different colony morphologies) were subcultured and stored for recovery of commensal *E. coli* isolates from the cloacal swabs of 2001 for drug resistance study. These isolates were presumptively identified on the basis of positive indole reaction, and negative citrate reaction.

For isolation of *E. rhusiopathiae* from cloacal swabs, the swabs were placed in *Erysipelothrix* Selective Broth (ESB) [14] and incubated for 24–48 h at 37°C . The broths were subcultured at 24 h onto Packers [14] and BA plates. Packers plates were incubated for a further 3–4 days at 37°C for isolation of *E. rhusiopathiae* which typically forms slightly convex, translucent and colorless colonies of ≤ 1 mm diameter. Suspected colonies were examined for typical morphology after Gram staining.

The pharyngeal swabs were inoculated onto BA and MAC plates, and incubated for 24 h at 37°C . On day 2, the morphology of bacterial colonies was noted, and these colonies were Gram-stained and subcultured to BA. Gram-positive isolates were grouped and identified to genus level only. Lactose-fermenting Gram-negative isolates were cultured on BA for 24 h at 37°C and then checked for oxidase, citrate, and indole reactions. Those negative for oxidase and citrate, and positive for indole were identified as *E. coli*. Gram-negative isolates that did not grow on MAC were examined for characteristics of *P. multocida* [14]. To gain an overall idea of the nature of the Gram-negative pharyngeal isolates, they were examined for colony morphology on BA, appearance on Gram-stained smear, growth on MAC, oxidase and indole reactions, and fermentation reaction in triple sugar iron agar (TSI) (Oxoid). The isolates were grouped on the basis of these reactions, and a representative isolate from each group was tested in the API Gram-negative bacterial identification (ID) strips (BioMérieux Canada, Inc.). Results were read as per the manufacturer's instructions, and the ID was classified as acceptable or not on the basis of probability.

2.3. Antibiotic susceptibility testing

Antibiotic susceptibility testing for isolates of *Salmonella* spp., and intestinal *E. coli* was done by the disk diffusion assay [15] employing disks from Oxoid. E-test strips (AB Biodisk, Solna, Sweden) were used to determine Minimum Inhibitory Concentration (MIC) of metronidazole for *Campylobacter* isolates and MIC of polymyxin B for *Salmonella* isolates.

The inoculum for all organisms was prepared from a 24-h culture on BA. Bacterial suspensions in normal saline were adjusted to approximate the MacFarland No. 1 turbidity standard. For testing *Salmonella* isolates, Mueller–Hinton agar (Oxoid) was used. An *E. coli* strain ATCC 25922 (American Type Culture Collection, Manassas, VA, USA)

was used as a susceptible control. Antibiotics tested included: amikacin (AK), ampicillin (AP), ceftriaxone (CT), chloramphenicol (C), ciprofloxacin (CP), gentamicin (GN), nalidixic acid (NA), neomycin (N), streptomycin (S), sulfa-trimethoprim (SM), tetracycline (TE), and polymyxin B. The disk contents and growth inhibition zones were in accordance with criteria set by the National Committee for Clinical Laboratory Standards (NCCLS) for bacteria isolated from animals [16]. For neomycin, the NCCLS recommended zone sizes [17] were used. All isolates were tested for MIC to polymyxin B using E-test. The break point for resistance to polymyxin B was set at 5 µg/ml since resistance to this antibiotic is uncommon among *S. Typhimurium*, and most susceptible isolates have a MIC of 5 µg/ml or less [18,19].

Campylobacter isolates were tested for resistance to metronidazole only using E-test strip as per the manufacturer's instructions. The isolates were swabbed on Mueller-Hinton agar with 5% sheep red blood cells (Oxoid) and the strip was applied. The plates were incubated for 48 h at 37 °C in an anaerobic jar with a Campy-Pak (Oxoid). The break point for resistance to metronidazole was set at 16 µg/ml [20].

For the *E. coli* isolates, the antibiotics tested included: amoxicillin (AX), AP, cephalothin (KF), C, CP, enrofloxacin (EN), GN, N, S, SM, and TE. The tests were done according to NCCLS standards [16]. *E. coli*, ATCC 25922, which gave reproducible growth inhibition zones, was used as a control.

3. Results

3.1. Cloacal swabs

The isolates from cloacal swabs included *Salmonella*, *E. coli*, and *Campylobacter*. *Erysipelothrix* was not isolated from any sample. No *Salmonella* spp. was isolated from any of the 150 DCC chicks sampled in the tree-nesting colony in 2001 and 2003. In

Table 1

Serotypes and phage types of 19 *Salmonella* isolates cultured from cloacal swabs of 2–3-week-old Double-crested cormorants (DCC) (15 of 100 birds sampled) and three *Salmonella* isolates cultured from cloacal swabs of 2–3-week-old Great Black-backed gulls (GBG) (three of 79 birds sampled) on Little Courtin Island, Prince Edward Island, Canada

Host species	Year	Serotype	Antigen	Phage type	No. isolated
DCC	2002	<i>S. Typhimurium</i> , variant Copenhagen	4:i:2	120	2
DCC	2002	<i>S. Typhimurium</i>	4,5:i:2	39	3
DCC	2002	<i>S. Typhimurium</i>	4,5:i:2	99	2
DCC	2002	<i>S. Typhimurium</i>	4,5:i:2	120	1
DCC	2002	I:4,12:i:–	4:i:–	99	7
DCC	2002	I:4,12:i:–	4:i:–	39	1
DCC	2003	<i>S. Typhimurium</i>	4,5:i:2	1	1
DCC	2003	I:4,5,12:i:–	4:i:–	1	1
DCC	2003	I:4,12:i:–	4:i:–	191	1
GBG	2002	I:4,5,12:i:–	4:i:–	193	1
GBG	2003	I:4,5,12:i:–	4:i:–	U284 Variant	2

Table 2

Biotypes and serotypes of *Campylobacter* isolates cultured from cloacal swabs of 2–3-week-old Double-crested cormorants (22 of 100 birds sampled) on Ram Island, Prince Edward Island, Canada

Species	Biotype	Serotype	No. isolated	Percentage
<i>C. jejuni</i> subsp. <i>jejuni</i>	I	HL ^a 18, HS ^a 0:45	1	4.54
<i>C. jejuni</i> subsp. <i>jejuni</i>	III	HL 18, HS 0:41	5	22.73
<i>C. jejuni</i> subsp. <i>jejuni</i>	III	HL 18, HS 0:55	4	18.18
<i>C. jejuni</i> subsp. <i>jejuni</i>	IV	HL 18, HS 0:41	4	18.18
<i>C. coli</i>	I	HL Untypable, HS Untypable	7	31.82
<i>C. lari</i>	I	HL Untypable ^b	1	4.54

^a HL, heat labile; HS, heat stable.

^b *Campylobacter lari* is not represented in the Penner Typing Scheme.

the ground-nesting colony in 2002 and 2003, 19 isolates of *Salmonella* spp. were cultured from 15 of 100 (15%) DCC chicks and three isolates were cultured from three of 79 (3.8%) GBG chicks. No established serotype other than *S. Typhimurium* was identified among the 19 isolates from DCC. However, 10 of these isolates were monophasic variants of serotype *Typhimurium* (Table 1). Phage type 99 was the most common (9/19) among the 5 phage types of DCC isolates. The GBG isolates belonged to phage types that are different from those from DCC.

In the disk diffusion assay none of the 19 *Salmonella* isolates from DCC was found to be resistant to AP, AK, C, CT, CP, GN, N, NA, S, SM, or TE. The MICs for polymyxin in the E-test ranged from 0.5 to 1.0 µg/ml, showing sensitivity of all isolates to this drug.

Twenty-two isolates of *Campylobacter* species were cultured from 22 of 100 DCC chicks in the tree-nesting colony (Table 2). Of these, 14 were *C. jejuni*, seven *C. coli*, and one *C. lari*. The MICs for metronidazole in the E-test ranged from 0.25 to 2.00 µg/ml with an average of 0.64 µg/ml. With the break point for resistance fixed at 16 µg/ml, none of the 22 isolates was resistant to metronidazole.

One hundred and eighty-seven Gram-negative, LF organisms were identified as *E. coli* in DCC from the tree-nesting colony. The results of the disk diffusion assay of these 187 isolates showed that all were susceptible to C, CP, EN, KF, and SM (Table 3). Of the remaining antibiotics, resistance was seen only in 9 (4.8%) of the isolates. Resistance to more than one antibiotic (multiple resistance) was seen in six (3.2%) isolates (Table 4).

Table 3

Kirby-Bauer antibiotic resistance patterns of 187 isolates of *Escherichia coli* cultured from cloacal swabs of from 100, 2–3-week-old Double-crested cormorants on Ram Island, Prince Edward Island, Canada

	AP ^a	AX	C	CP	EN	GN	KF	N	S	SM	TE
No. of resistant isolates	4	4	0	0	0	1	0	1	3	0	7
Percent	1.9	1.9	0	0	0	0.5	0	0.5	1.6	0	3.7

^a AP, Ampicillin (10); AX, amoxicillin (30); C, chloramphenicol (30); CP, ciprofloxacin (5); EN, enrofloxacin (30); GN, gentamicin (10); KF, cephalothin (30); N, neomycin (30); S, streptomycin (10); SM, sulfamethoprim (25); TE, Tetracycline (30). Antibiotic concentration in µg.

Table 4

Escherichia coli isolates with multiple drug resistance (6 of 187) cultured from cloacal swabs of 100 2–3-week-old Double-crested cormorants on Ram Island, Prince Edward Island, Canada

Number of isolates	Resistant drugs
1	AP ^a , AX, N, S, TE
3	AP, AX, TE
2	S, TE

^a AP, ampicillin; AX, amoxicillin; N, neomycin; S, streptomycin; TE, tetracycline.

3.2. Pharyngeal swabs

Pharyngeal isolates were grouped as Gram-positive cocci and Gram-negative bacilli. The rare Gram-positive rods isolated belonged to *Bacillus* group, and were not further identified. Of a total of 231 isolates of Gram-positive cocci, which included different colony types recovered from each specimen, 212 (91.8%) were staphylococci (catalase positive), and of these 27% were coagulase-positive, with the remainder (73%) being coagulase-negative. Only 8% were identified as streptococci. Gram-positive cocci were not studied further.

A total of 176 isolates of Gram-negative rods/coccobacilli were recovered on the basis of colony type, growth characteristics, hemolysis on BA, ability to grow on MAC, and oxidase, indole and TSI reactions. None of the isolates were identified as *P. multocida*, and only 64 (36.4%) isolates were identifiable satisfactorily to a genus or species level. Very good identifications were limited to *E. coli* ($N=25$), *Acinetobacter* sp. ($N=4$), *Vibrio alginolyticus* ($N=1$), and *Proteus* ($N=1$) sp. These identifications had 99% acceptability in API identification system. Thirty three isolates were identified as *Weeksella virosa*, with 80% acceptability with API NE strips.

4. Discussion

A comprehensive survey of normal bacterial flora of DCC cannot currently be found in the literature. Pathogenic bacteria such as *Salmonella* and *Campylobacter* have not previously been characterized specifically in this species. All isolates of *Salmonella* recovered from DCC in this study were Typhimurium serotype or its variants. In fact, 10 isolates were flagellin antigen negative variants, and such isolates are considered atypical monophasic variants of serotype Typhimurium [21]. Serotype Typhimurium has previously been isolated from internal organs of DCC that were affected with Newcastle disease in Canada, and close contact of juvenile DCC with birds of other species that were more resistant to disease was suspected as the cause of infection [22]. Phage type 99, which was the most common type from DCC has not been found among the *Salmonella* isolates from humans, beef cattle or chickens on PEI [19]. Some specific phage types are known to affect certain avian species more than others (e.g. *S. Typhimurium* variant Copenhagen phage type 99 in pigeons [23], *S. Typhimurium* phage type 40 in songbirds

[24]. The possibility of host-adapted variant strains of *Salmonella* also affecting DCC should be investigated further. Wild birds are commonly infected with paratyphoid forms of *Salmonella*, notably serotype Typhimurium, which is becoming an increasingly common cause of illness and death in wild birds [25]. Isolates from DCC have included *S. agona*, *S. Typhimurium* and *S. infantis* [26]. *Salmonella* is an environmentally persistent pathogen, and individually infected birds may harbor it commensally in their intestinal tract and thus act as reservoirs for this organism, shedding it into their environment for weeks or months.

Lack of isolation of *Salmonella* in 2001 lead to a change in transport medium. Despite this change, negative bacteriology results in two different years (2001 and 2003) in the tree-nesting colony of DCC suggest that birds in this colony either do not carry, or have a very low prevalence of carriers of *Salmonella*. The carrier rate of *Salmonella* spp. in the tree-nesting colony of DCC seems to contrast with that in the ground-nesting colony. In the latter, opportunity for close and frequent contact among young DCC is much higher and, therefore, is more likely to promote cross-contamination. Herring gulls and GBG, which are in relative proximity to DCC in the ground-nesting colony, were thought originally to act as a potential source of *Salmonella* for these birds, since the prevalence of carriers of this bacterium is often high among gulls, particularly those that have access to agricultural land and/or refuse sites. Quessy and Messier [27] found 10 serovars of *Salmonella* in the feces of 8.5% of sampled gulls, with birds that fed at refuse sites having the greatest levels of this bacterium. The lower prevalence of carriers of *Salmonella* among gulls as compared to DCC in the ground-nesting colony was therefore unexpected. Moreover, the different serotypes and phage types isolated from these two species do not suggest cross-contamination between them. Further tests on these isolates, such as pulse-field electrophoresis of DNA, might demonstrate a closer relatedness than is currently evident.

While *Salmonella* isolates from humans and domestic animals, including cattle and poultry, in PEI showed multiple drug resistance [19], it was not the case with the *S. Typhimurium* isolates from DCC in this study. This suggests that these birds are not being infected with *Salmonella* from agricultural or human sources. Hudson et al. [28] noted that, despite the general susceptibility of *Salmonella* isolates from non-domestic birds in southeastern United States to most antimicrobial agents, antibiotic resistance genes were present in some of these isolates. In a study of DCC in Florida, White and Forrester [26] found strains of *S. agona* that were resistant to AP, K, N, S, and sulfonamides. None of our *S. Typhimurium* isolates were resistant to polymyxin B, a drug which has some application in control of *Salmonella* in poultry [29].

Campylobacter spp. are commonly found in many species of wild birds including several aquatic species [30]. As with the *Salmonella* strains, the *Campylobacter* strains isolated from DCC in our study do not match those which could ordinarily be associated with agricultural or human sources. However, serotype HL:18 has been recovered from humans, chickens, and cattle on a less frequent basis [31]. The seven isolates of *C. coli* from DCC were untypable in both HL and HS schemes; this suggests that they are not represented by either schemes, and that they may be found less commonly and may not originate from farm run-off. Susceptibility of *C. jejuni* strains to metronidazole, a drug primarily used to treat anaerobic infections in humans and animals, is highly variable [32].

In a survey by Stanley and Jones [33], a high frequency of metronidazole resistance among *C. jejuni* strains from birds, including starlings and gulls, in the United Kingdom was found, and it was noted that a host-phenotype relationship does indeed appear to exist and that metronidazole resistance may be a useful epidemiological marker. The absence of metronidazole resistance among *C. jejuni* from DCC in Atlantic Canada is interesting to note, as the phenotypes of *C. jejuni* found in these birds may be different from those found in other avian species. Whether the absence of resistance to this drug can serve as an epidemiological marker to differentiate *C. jejuni* originating from DCC from isolates in other wild birds is worth investigating.

There are few studies on antimicrobial drug resistance of commensal *E. coli* from wild birds. Commensal intestinal *E. coli* can serve as indicators of resistance pool, and since resistance genes can be transferred to pathogenic bacteria, there is a potential danger for dissemination of these genes following water contamination. The resistance rate among intestinal isolates from DCC in this study was very low, 95% of 187 isolates being susceptible to all 12 antibiotics tested. Resistance percentages of these isolates for AP, GN, and TE were 1.9, 0.5, and 3.7% respectively. Clinical isolates of *E. coli* from domestic poultry are frequently resistant to these three antibiotics; rates in Québec during 1993–1999 were 42–62% for AP, 49–66% for GN, and 84–89% for TE [34]. Resistance to TE and S among poultry isolates has also been high (> 75%) in the United States [35], and the use of antibiotics, especially TE and AP, in poultry has resulted in high resistance rates among *E. coli* isolates from these birds [36]. Therefore, it appears that DCC from this region are unlikely to contribute to a drug resistance pool among *Enterobacteriaceae* in water sources and the environment, although testing for resistance genes may be required for confirmation. If the pattern of antibiotic resistance among commensal intestinal *E. coli* isolated from poultry in this region is comparable to that in other regions of North America, our findings also indicate that DCC are not infected with *E. coli* from agricultural sources. Nonetheless, monitoring should continue over the years to determine changes in resistance patterns.

The presence of Gram-positive bacteria such as staphylococci in the pharynx is probably of no significance in healthy birds, although generalized infections have been reported in waterfowl subsequent to injury, lead poisoning or conditions associated with captivity [37]. *Pasteurella multocida*, the cause of avian cholera, was not present among Gram-negative isolates from the pharyngeal swabs. This bacterium is known to cause disease in a wide variety of birds, including cormorants [38]. Most of the Gram-negative pharyngeal isolates in our DCC were not identified to genus or species level. *W. virosa* isolates had a satisfactory identification with 80% acceptability. *Weeksellia* species may be commensals that are found on mucosal surfaces of humans and warm-blooded animals, with potential pathogenicity to at least humans [39,40].

Attempts to recover *E. rhusiopathiae*, a potential avian pathogen, from cloacal swabs of DCC were unsuccessful. According to Corstvet and Holmberg [41], culture of cecal tonsils, requiring euthanasia of the birds, is more appropriate for detection of this organism in asymptomatic carriers. It is reasonable to assume that DCC can harbor this organism subclinically.

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