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Characterization of *Escherichia coli* populations from gulls, landfill trash, and wastewater using ribotyping

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ABSTRACT: Due to their opportunistic and gregarious nature, gulls may be important reservoirs and vectors for anthropogenically derived fecal pathogens in coastal areas. We used ribotyping, a genotypic bacterial source tracking method, to compare populations of *Escherichia coli* among herring gulls *Larus argentatus*, great black-backed gulls *L. marinus*, wastewater, and landfill trash in New Hampshire and Maine, USA. Concentrations of *E. coli* in gull feces varied widely among individuals, but were generally high (6.0×10^1 to 2.5×10^9 g^{-1} wet weight). Of 39 *E. coli* isolates from *L. argentatus*, 67% had banding patterns that were ≥90% similar to those from wastewater and trash, whereas only 39% of 36 *L. marinus* isolates exhibited ≥90% similarity to these sources. Strains of *E. coli* from gulls matched (≥90% similarity) more strains from wastewater (39% matching) than from trash (15% matching). *E. coli* isolates from *L. marinus* feces exhibited a greater diversity of banding patterns than did isolates from *L. argentatus.* There were more unique *E. coli* banding patterns in trash samples than in wastewater, and higher diversity indices in the former compared to the latter. These findings suggest that both species of gulls, especially *L. argentatus*, obtain fecal bacteria from wastewater and landfill trash, which they may transport to recreational beaches and waters. Our results also indicate that *E. coli* populations may vary widely between gull species, and between the anthropogenic habitats that they frequent, i.e. landfills and wastewater treatment facilities.

KEY WORDS: Microbial source tracking · *Escherichia coli* · Ribotyping · *Larus argentatus* · *Larus marinus* · Gull · Wastewater · Landfill

INTRODUCTION

Coastal ecosystems have become increasingly important as sources of emerging infectious diseases arising from climate change (Hunter 2003), anthropogenic subsidies and landscape alteration (Skelly et al. 2006). As a result of burgeoning human populations along coasts, the volume of liquid and solid wastes being generated and disposed of in these areas has dramatically increased. Wildlife may play a critical role as reservoirs for pathogenic enteric bacteria and zoonotic diseases. Numerous wild bird species (e.g. crows and gulls) are attracted to untreated sewage, garbage dumps, manure, and other sources of enteric pathogens. As a consequence, a number of enteric bacteria such as *Escherichia coli*, *Salmonella* spp., *Campylobacter jejuni*, *C. coli*, *C. lari* and *Helicobacter canadensis* (Fenlon 1983, Kapperud & Rosef 1983, Whelan et al. 1988, Quessy & Messier 1992, Hatch 1996, Moore et al. 2002, Fogarty et al. 2003, Waldenström et al. 2007) have been isolated from intestinal samples of wild birds. Although potentially pathogenic fecal bacteria have been isolated from some species, recent reviews (Reed et al. 2003, Dixon 2007) suggest that the role wild birds play in human diseases is largely understudied, and that much work remains to determine the role of wild birds on zoonotic transmission of enteropathogens.

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Gulls may be particularly important reservoirs and vectors for anthropogenically derived enteric bacteria because of their opportunistic and gregarious nature. Gulls often commute between offshore breeding islands and anthropogenic sources of food (e.g. landfills) in order to feed mates and/or chicks (Belant et al. 1995, Bertellotti & Yorio 1999, Duhem et al. 2008). During the non-breeding season, gulls loaf at public beaches along coasts, wastewater treatment plants, open landfills, agricultural areas, and around inland bodies of freshwater. Gulls can transfer fecal bacteria to drinking water (Benton et al. 1983); at some sites concerns about their deleterious effect on quality of drinking water has resulted in culling programs (Jones et al. 1978). Gulls are also associated with increased prevalence of fecal coliforms in recreational waters (Lévesque et al. 1993) and at recreational beaches (Fogarty et al. 2003, Whitman & Nevers 2003).

Because fecal contamination can come from many different sources including wild birds, domestic animals, and sewage, the ability to identify specific sources has contributed greatly to management of recreational and drinking waters (McLellan & Salmore 2003, USEPA 2005). Ribotyping, a genotypic microbial source determination method, has been successfully used to identify sources of *Escherichia coli* contamination in shellfish beds and in recreational waters (Carson et al. 2001, Jones et al. 2006). To optimize the utility of *E. coli* ribotyping for pollution source tracking it is important to consider that *E. coli* strains, and thus species-specific ribopatterns, can change within sources over time (Meays et al. 2006), geographical distance (Hartel et al. 2002), and environmental conditions (Topp et al. 2003). Previous studies have indicated that the use of small, local source databases result in more effective and economic identification of the most significant sources compared to approaches that involve use of large regional databases and extensive sampling (Jones 2007).

In this study, we used ribotyping to investigate the role of herring gulls *Larus argentatus* and great black-backed gulls *L. marinus* as potential reservoirs of fecal bacteria obtained from anthropogenic sources. These 2 species of gull breed in large colonies on islands throughout the northeast US Atlantic coast. There is extensive dietary overlap between the 2 species (Pierotti & Good 1994, Good 1998, Rome & Ellis 2004) although some studies suggest that *L. argentatus* rely more heavily on landfills as a food source than do *L. marinus* (Cavanagh 1992, Wells 1994). Both species roost at wastewater treatment facilities, in and around treatment lagoons (authors' pers. obs.). In spite of their conspicuous presence at these anthropogenic habitats along the Atlantic coast of the USA, very few studies have examined the potential for gulls to serve as reservoirs and vectors of human-derived pathogens in this region.

Our study took place at the Isles of Shoals located off the coast of New Hampshire, US. Previous studies indicate that a large proportion of the diet of gulls breeding on Appledore Island, Maine, in the Isles of Shoals, consists of garbage (Ellis et al. 2005). Studies of banded gulls from Appledore Island have also shown that individuals visit the large open landfill and municipal waste water treatment facility in nearby Rochester, New Hampshire (J. C. Ellis unpubl. data). The main goals of this study were to compare *Escherichia coli* subtypes in the feces of *Larus marinus* and *L. argentatus* nesting on Appledore Island, and to compare the subtypes found in gull feces to those from wastewater and garbage obtained at the landfill and sewage treatment plant in Rochester.

MATERIALS AND METHODS

Study location and meteorological conditions. We sampled feces from gulls nesting on Appledore Island, Maine in the Isles of Shoals, a 9 island archipelago, located approximately 10 km off the coast of Portsmouth, New Hampshire. The Isles of Shoals is home to some of the largest colonies of both gull species in the northeast US. We took samples of water and trash from the Turnkey Landfill and Municipal Waste Water Treatment Plant in Rochester, New Hampshire, approximately 30 km from the coast (Fig. 1). The water quality at the Isles of Shoals is such that the shellfish harvesting classification is 'approved', except for the area around the wastewater treatment plant outfall on Star Island (New Hampshire Fish and Game Department unpubl. data). Thus, there are no significant sources of anthropogenic fecal microbial contamination near the islands. All samples were collected during the spring and summer of 2007. Because rainfall can afffect the microbiological quality of water and sewage, the precipitation amounts on and 48 h prior to the sampling dates were recorded: 0.74 cm for 28 and 29 May, 0.46 cm for 18 July and 0.43 cm for 25 July (www.weather.unh.edu/).

Collection of gull feces. Fecal samples were collected from live-caught gulls at Appledore Island. Adult *Larus argentatus* and *L. marinus* were captured during egg incubation using a walk-in nest trap (a chicken wire cage with an opening on the bottom and an entrance on one side). The trap was placed on top of the eggs and nest and the incubating bird entered the trap through the side entrance. Once the gull settled on its eggs, we quickly approached the trap, gently removed the bird then placed it into a cloth cone for restraint and to prevent injury. Each bird was banded

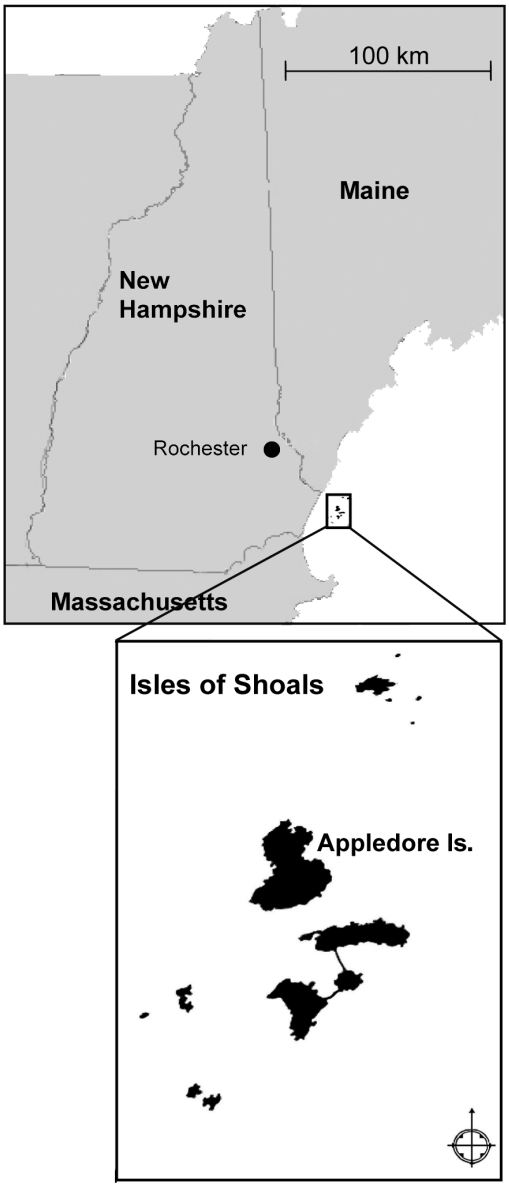

Fig. 1. Study sites. Fecal samples were collected from gulls nesting on Appledore Island, Maine, in the Isles of Shoals (New Hampshire, Maine). Samples of trash and wastewater were collected from the landfill and wastewater treatment facilities in Rochester, New Hampshire

and measured, and a cloacal swab was conducted to obtain samples of bacteria. A fresh sample of feces was also collected from each bird by placing it into a plastic box for <1 min just prior to releasing it; most birds responded to box placement by voiding their cloacas almost immediately. The liner at the bottom of the box was replaced between each bird, so as to avoid contamination. Fecal samples were collected from 12 individuals each of *L. argentatus* and *L. marinus*. Fecal samples and swabs were refrigerated during transport to the laboratory for processing. Samples were collected on 28 and 29 May 2007; all samples were processed on 30 May.

Collection of samples from the landfill and wastewater treatment plant. Samples of trash were collected from the actively worked area on top of the Turnkey Landfill in Rochester, New Hampshire on 18 July. Thousands of gulls forage at this landfill throughout the year. Five samples of trash from different sites on the open landfill were collected randomly and placed in sterile 500 ml Whirl-pak bags. We also collected one sample of untreated leachate, which is the liquid waste that drains to the bottom of the landfill and is collected in pipes then treated at the landfill leachate processing facility. All samples of trash were immediately taken to the laboratory for processing.

Samples of wastewater were collected on 25 July from the aeration lagoons at the Rochester Waste Water Treatment Facility. We used a collecting bucket attached to a 3 m pole to collect water samples; all samples were poured from the bucket into sterile 500 ml WhirlPak bags. One sample was collected from each of the open treatment lagoons where treatment plant staff had observed loafing gulls. These lagoons included an equalization basin (Lagoon 1), one in which the wastewater was awaiting secondary treatment (Lagoon 2), and a sludge aeration basin (Lagoon 5). Water samples were transported immediately to the laboratory for processing.

Detection of fecal coliforms and *Esherichia coli*. Sample volumes of wastewater or diluted solid samples ranging from 100 ml to 2.5 ml from decimally diluted (to 10^{-8}) samples were filtered to yield at least 20 *Escherichia coli* colonies on mTEC agar plates. Plates were inverted and incubated at 44.5 ± 0.2°C for 24 h (USEPA 1986). Fecal coliforms were enumerated after incubation by counting the number of yellow colonies. *E. coli* was enumerated by counting the number of yellow colonies present on the plate following incubation of the colonies on a urea substrate (Rippey et al. 1987). Ten isolates from each sample were selected for further testing, streaked onto Tryptic Soy Agar (TSA) plates, and incubated at 35°C for 24 h.

Cloacal swabs taken from gulls at Appledore Island were streaked directly to mTEC agar plates for isolation while the fresh fecal samples were set up in a dilution series in buffered peptone water (BPW), filtered through a 0.45 µm pore size membrane filter, and the filter rolled onto mTEC agar plates for incubation. To adddress concerns about possible contamination of fresh fecal samples collected from gulls placed into the plastic box, cloacal swabs were prioritized for use in isolation of *E. coli*. If there was too little or no growth, isolates were picked from the filtered fecal dilution mTEC plates.

Samples of trash collected from the landfill included pieces of rubber, plastic, soil, leaves, egg shells, cardboard, hair, paper, wood chips, foam, vegetables, and chicken meat. Samples were broken up by hand into small pieces, mixed, ¼ of the sample was broken into smaller bits, and ~1 g wet weight (WW) was placed into a tube of BPW and decimally diluted. Aliquots of these dilutions were filtered and placed on mTEC plates for incubation. A second ~1 g WW of each sample was weighed and then dried overnight at 105°C to obtain the dry weight and percent moisture. Finally, representative particle types from each trash sample were scraped with a sterile loop and streaked directly onto mTEC plates. All wastewater samples were set up in dilution series out to at least 10^{-6} and filtered onto mTEC plates.

A series of biochemical tests were performed on the isolates in order to confirm their identity as *Escherichia coli*. After incubation the isolates on TSA plates were inoculated into (1) urea solution to test for urease activity; (2) Trypticase broth to test for indole production; and (3) EC-MUG (*E. coli* 4-methylumbelliferyl-β-D-glucuronide) medium to test for lactose fermentation and β-glucuronidase activity, respectively. Each isolate was also streaked onto Simmons Citrate agar to test for citrate utilization. All test cultures were incubated at 35.0 ± 0.5°C for 24 h. *E. coli* shows the following characteristics when undergoing these biochemical tests: urea negative, citrate negative, indole positive, EC positive, and MUG positive. Results of the biochemical tests were recorded and those that passed all 4 biochemical tests were assumed to be *E. coli.*

Ribotyping *Escherichia coli* isolates. Five isolates from 8 individual *Larus argentatus* and 8 *L. marinus* samples were chosen at random; between 36 and 39 isolates per species were ribotyped, yielding a total of 75 acceptable ribopatterns. Twenty *E. coli* isolates from the landfill trash and 22 from wastewater were also ribotyped.

The procedures used for ribotyping *Escherichia coli* isolates for this study are largely based on those of Parveen et al. (1999) and Jones et al. (2006). Cultures on TSA were incubated overnight at 35°C. A RiboPrinter® was used to process *E. coli* isolates for ribotype determinations. After preparation of the samples, the automated process involved lysing cells and cutting the released DNA into fragments via the restriction enzyme *Eco*R1. These fragments were separated by size through gel electrophoresis and then transferred to a membrane, where they were hybridized with a DNA probe and mixed with a chemiluminescent agent. The DNA probe targeted 5S, 16S, and 23S ribosomal RNA genes. A digitizing camera captured the light emission as image data, and these images were transferred from the RiboPrinter into GelComparII (Applied-Maths analytical software). The DNA bands in lanes containing the molecular weight standard were labeled and used for optimizing gel pattern images. The image densiometry data were processed for identification of DNA bands. All presumptive *E. coli* isolate ribopatterns were analyzed on the RiboPrinter using the Microbial Characterization System. All isolates included in this study had greater than 80% similarity (based on manufacturer protocols) to the *E. coli* ribopatterns in the manufacturer's microbial database (consisting of 6448 pattern entries for a wide range of bacterial species). The banding patterns (i.e. ribotypes) for these isolates were used to calculate similarity indices.

Ribotype similarity was analyzed using GelComparII software. Similarity dendrograms were constructed using the Dice coefficient algorithm with maximum similarity (Dice 1945). The software optimization setting was 0.15 and the position tolerance setting was 0.1. The similarity of replicate analyses of an *Escherichia coli* control strain (ATCC #51739) gave acceptable (>90%) matching of resulting ribopatterns compared to the cumulative previous analyses of this strain.

The source species profile with the highest similarity coefficient was accepted as an indication of the possible source species for the sample isolates. The predetermined threshold similarity index that was considered to be a minimum value for identifying source species was 90%. Generally, banding patterns can differ by no more than one band to give a Dice's similarity coefficient of ≥90% (Dice 1945, Kelsey et al. 2008). If the value calculated for an isolate was below the threshold similarity index, the sample isolate was considered to be of unknown origin. Hard copies of ribotype patterns and similarity coefficients for the unknown and most closely related source species were printed for verification of statistical analyses and further interpretation.

Cluster analyses were performed to determine the relationships among isolates from trash and wastewater samples and the 2 gull species, to identify banding patterns that were identical for different isolates, and to construct relational dendrograms. The ribopattern similarities for local source species isolates were analyzed separately for isolates from each sample, and then grouped according to species to determine the number of unique ribopatterns from a given sample and species. Cluster analyses were based on the unweighted pair group method by arithmetic averaging (UPGMA) or the neighbor-joining algorithms.

Diversity indices were used to assess the structure of *Escherichia coli* populations in each host (the 2 species of gulls) and source (trash and wastewater). The observed frequency and distribution of *E. coli* subtypes, determined by ribotype, were compared within and

between hosts and sources. Four different indices from the Hill family of diversity indices were used (following Anderson et al. 2006): (1) a richness estimator (S) was calculated as the number of different subtypes; (2) Shannon-Wiener diversity index (H') was calculated as $H' = -\Sigma p_i \ln(p_i)$ where p_i is (number of isolates with pattern [i])/total isolates; (3) Simpson's index (D) was calculated as $\Sigma[n_i(n_i - 1)]/[n(n - 1)]$ where n_i is the number of isolates with subtype [i] and n is the total number of isolates; and (4) Pielou's evenness (J') was calculated as H' $\ln S^{-1}$. Diversity measurements were calculated separately for each gull species, wastewater samples, and landfill samples. We used multiple measurements of diversity because each has its advantages and disadvantages. Richness captures the presence of rare subtypes by giving all subtypes equal value regardless of abundance; Simpson's index gives more weight to the most abundant, dominant subtypes over rare subtypes; and the Shannon-Wiener index falls in between, accounting for both frequency and abundance of each subtype (Hill et al. 2003). The bigger the value of the Simpson's index (D), the lower the diversity of the sample. Therefore, to avoid confusion, the results were expressed as Simpson's reciprocal index ($1/D$) so that a larger index indicates greater diversity (see Table 3).

Table 1. *Escherichia coli.* Numbers of colony forming units per g (cfu g^{-1} WW) in gull fecal samples collected from Appledore Island, Maine

Leg band ID	*E . coli* (cfu g^{-1} WW)
Herring gull *Larus argentatus*	
263	1.3×10^7
H23	1.9×10^2
H92	6.1×10^7
A33	1.4×10^5
C71	3.4×10^5
H17	3.9×10^7
H22	3.5×10^3
H93	2.5×10^9
E49	6.5×10^3
H18	8.6×10^7
H92	6.1×10^7
Geometric mean ± SD	$1.0 \times 10^6 \pm 7.7 \times 10^8$
Great black-backed gull *L. marinus*	
5H7	4.2×10^7
5H9	8.6×10^1
7E0	2.3×10^5
7E3	7.8×10^7
7E9	5.9×10^4
3E1	1.0×10^7
9C3	2.3×10^6
9H5	4.7×10^7
5H8	6.0×10^1
7E6	0
Geometric mean ± SD	$8.7 \times 10^4 \pm 2.8 \times 10^7$

RESULTS

Samples of gull feces, trash, and wastewater exhibited a wide range of *Escherichia coli* concentrations. The geometric mean concentration for feces of *Larus argentatus* was 1.0×10^6 cfu g^{-1} WW and ranged from 1.9×10^2 to 2.5×10^9 g^{-1} WW (Table 1). The geometric mean concentration in *L. marinus* fecal samples was 8.7×10^4 g^{-1} WW, with a range of 6.0×10^1 to 7.8×10^7 g^{-1} WW. The mean concentration of *E. coli* in *L. marinus* feces was somewhat lower than that in *L. argentatus* feces, but there was a great deal of variation among individuals of both species. The geometric mean concentration of *E. coli* in the 4 trash samples was 7.1×10^4 cfu g^{-1} DW and the mean concentration in the 3 wastewater samples was 3.2×10^3 cfu ml^{-1}.

Escherichia coli isolates from gulls, trash, and wastewater were characterized using ribotyping. There were 75 isolates ribotyped from gulls; 39 from *Larus argentatus* and 36 from *L. marinus*. We obtained 22 isolates from the wastewater samples and 20 from trash. Of the 75 total *E coli* isolates from gulls, 40 (53%) had banding

Table 2. *Escherichia coli.* Numbers of isolates found in fecal samples collected from individually marked (using leg bands) *Larus argentatus* and *L. marinus* nesting on Appledore Island, Maine. The banding pattern, derived from ribotyping, of each isolate from gulls was compared to the banding patterns for *E. coli* isolated from wastewater and trash collected from the municipal waste water treatment facility and the Turnkey Landfill in Rochester, New Hampshire, respectively. If the banding patterns of a pair of isolates exhibited similarity indices of ≥90%, they were assumed to be the same strain of *E. coli*. The numbers of *E. coli* isolates from each individual gull that matched strains from wastewater or trash are indicated in the table

Leg band ID	Isolates	Wastewater	Trash
Herring gull *Larus argentatus*			
263	5	4	
H23	5	1	1
H92	5	3	2
A33	5	4	
C71	5		
H17	5	2	3
H22	5	4	
H93	4	1	1
Total	39	19	7
Great black-backed gull *L. marinus*			
5H7	5		
5H9	4	4	
7E0	5	2	1
7E3	5		
7E9	3		
3E1	4	1	
9C3	5	1	2
9H5	5	2	1
Total	36	10	4

patterns that were ≥90% similar to banding patterns from the 22 wastewater isolates and the 20 isolates from trash (Table 2). Of the 39 isolates from *L. argentatus*, 26 (67%) had patterns that were ≥90% similar to those in wastewater and trash, whereas only 14 (39%) of the 36 *L. marinus* isolates exhibited ≥90% similarity to these sources. There were more similarities between isolates from gulls and isolates from wastewater than there were between gull isolates and trash; 39% of the isolates from gulls had banding patterns that were ≥90% similar to isolates from wastewater, whereas only 15% were ≥90% similar to isolates from trash.

There were differences in the numbers and clustering of banding patterns in the dendrograms generated for wastewater and landfill isolates (Fig. 2). At >70% similarity, all but 3 wastewater isolates were part of 1 large cluster. In contrast, there were 4 clusters and 1 unique isolate at the same similarity threshold for the trash samples, indicating that the isolates from trash samples were more heterogeneous than those from

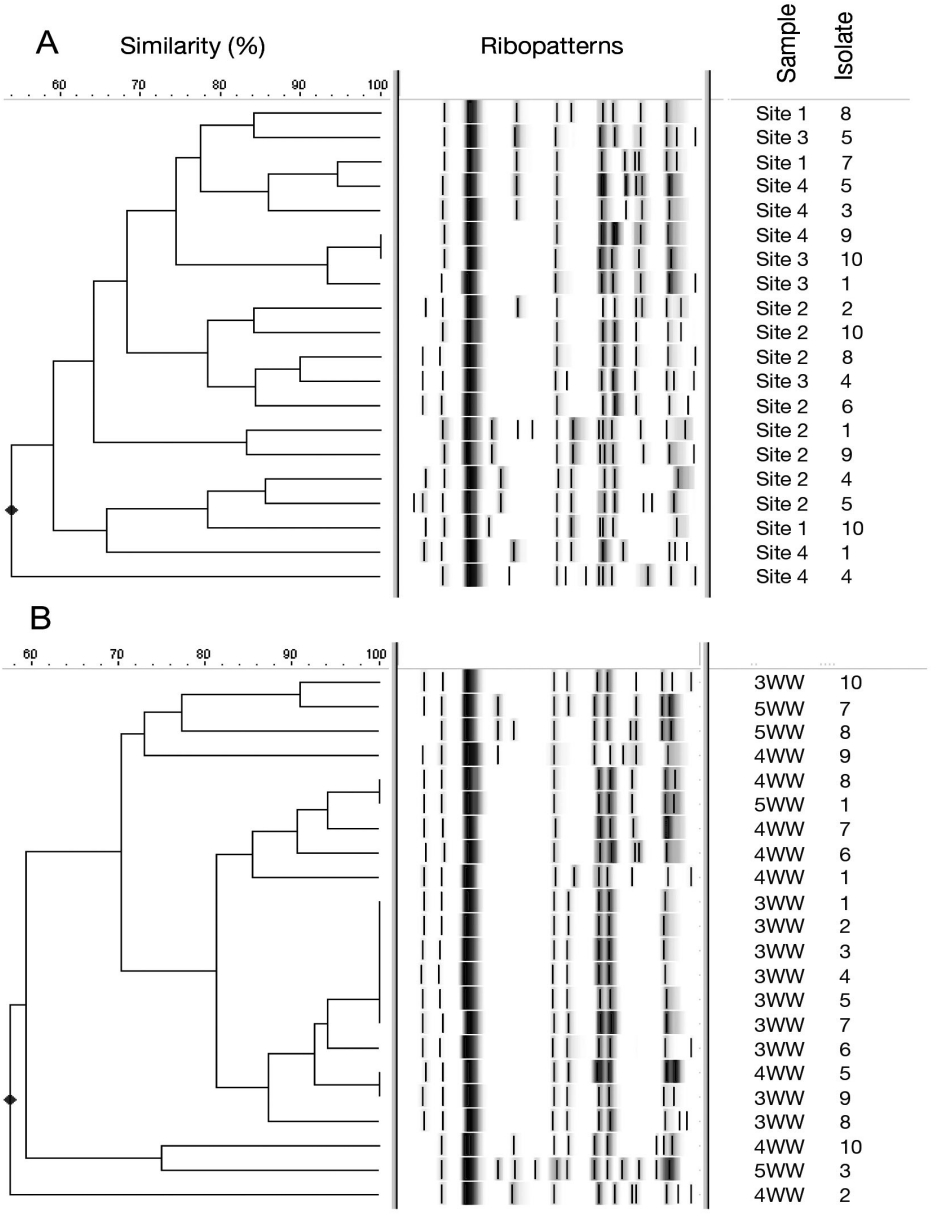

Fig. 2. *Escherichia coli*. (A) Dendrogram (unweighted pair group method by arithmetic averaging clustering based on Dice correlation coefficient) of 22 ribopatterns for isolates obtained from wastewater from treatment lagoons at the municipal treatment plant in Rochester, New Hampshire. (B) Dendrogram of 20 ribopatterns for isolates obtained from trash collected at the Turnkey Landfill in Rochester, New Hampshire. Black lines overlaid to emphasise banding patterns

wastewater. Visual inspection of the dendrogram of *Larus argentatus* isolates showed that at >75% similarity, there was one large cluster, which included over 50% of the isolates (Fig. 3). At the same similarity threshold, there were 5 clusters and 2 unique isolates from the *L. marinus* samples (Fig. 4), indicating greater dissimilarity between isolates in this species compared to *L. argentatus*.

Calculations of diversity indices corroborated the patterns observed through visual inspection of the dendrograms. Wastewater *Escherichia coli* isolates exhibited lower diversity indices than landfill trash isolates (Table 3). Similarly, wastewater isolates showed greater evenness than did isolates from trash samples. Although there were similar numbers of isolates from trash and wastewater samples, there were twice as many unique banding patterns in the trash samples. Among gulls, *Larus marinus* isolates exhibited a greater diversity of *E. coli* banding patterns than did *L. argentatus* (Table 3). Overall, the greatest diversity of strains was observed in samples from *L. marinus* and samples of landfill trash.

The fraction of unique (similarity <100%) banding patterns was 0.88 for wastewater and 0.95 for trash iso-

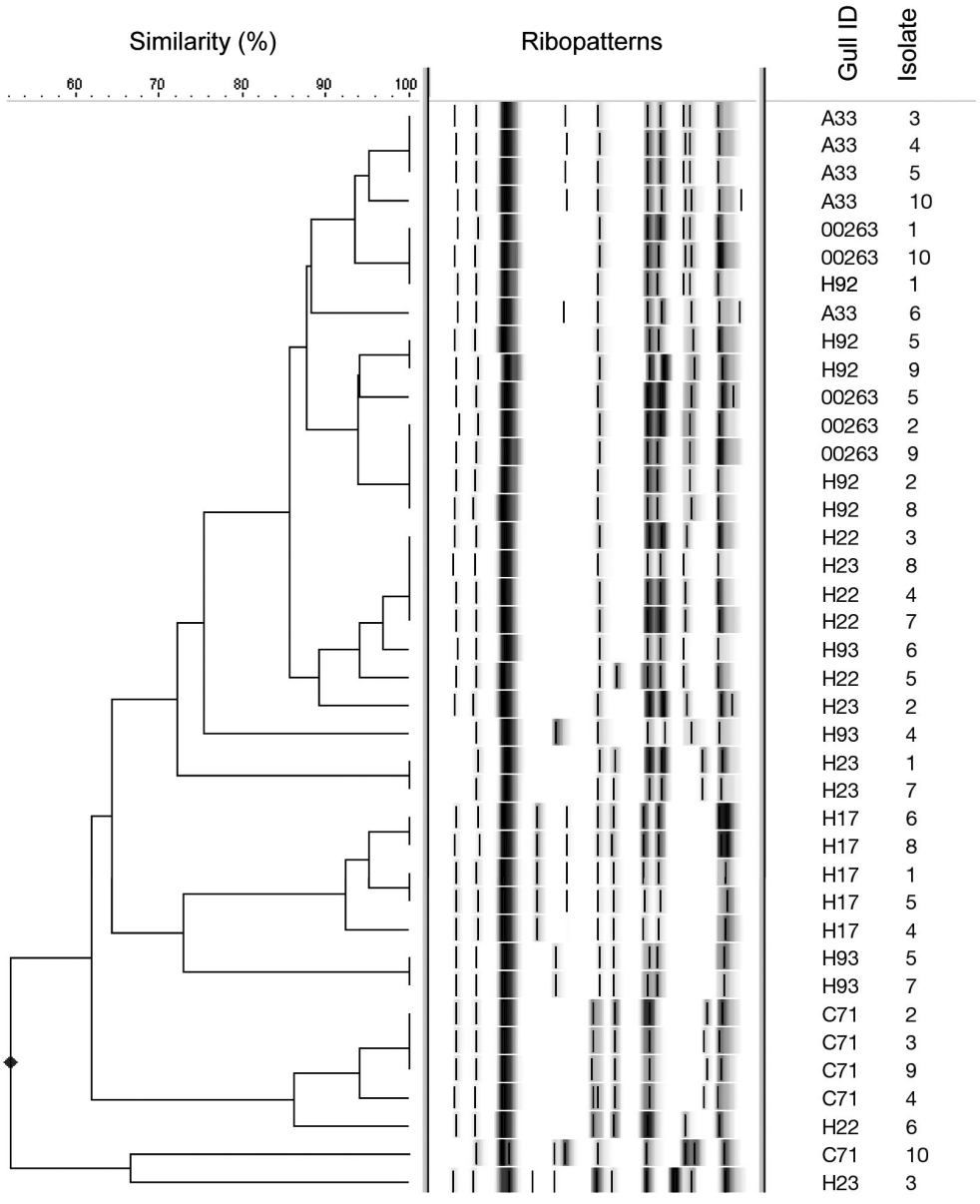

Fig. 3. *Escherichia coli.* Dendrogram of 39 ribopatterns for isolates obtained from feces collected from herring gulls *Larus argentatus* breeding on Appledore Island, Maine. Black lines overlaid to emphasise banding patterns

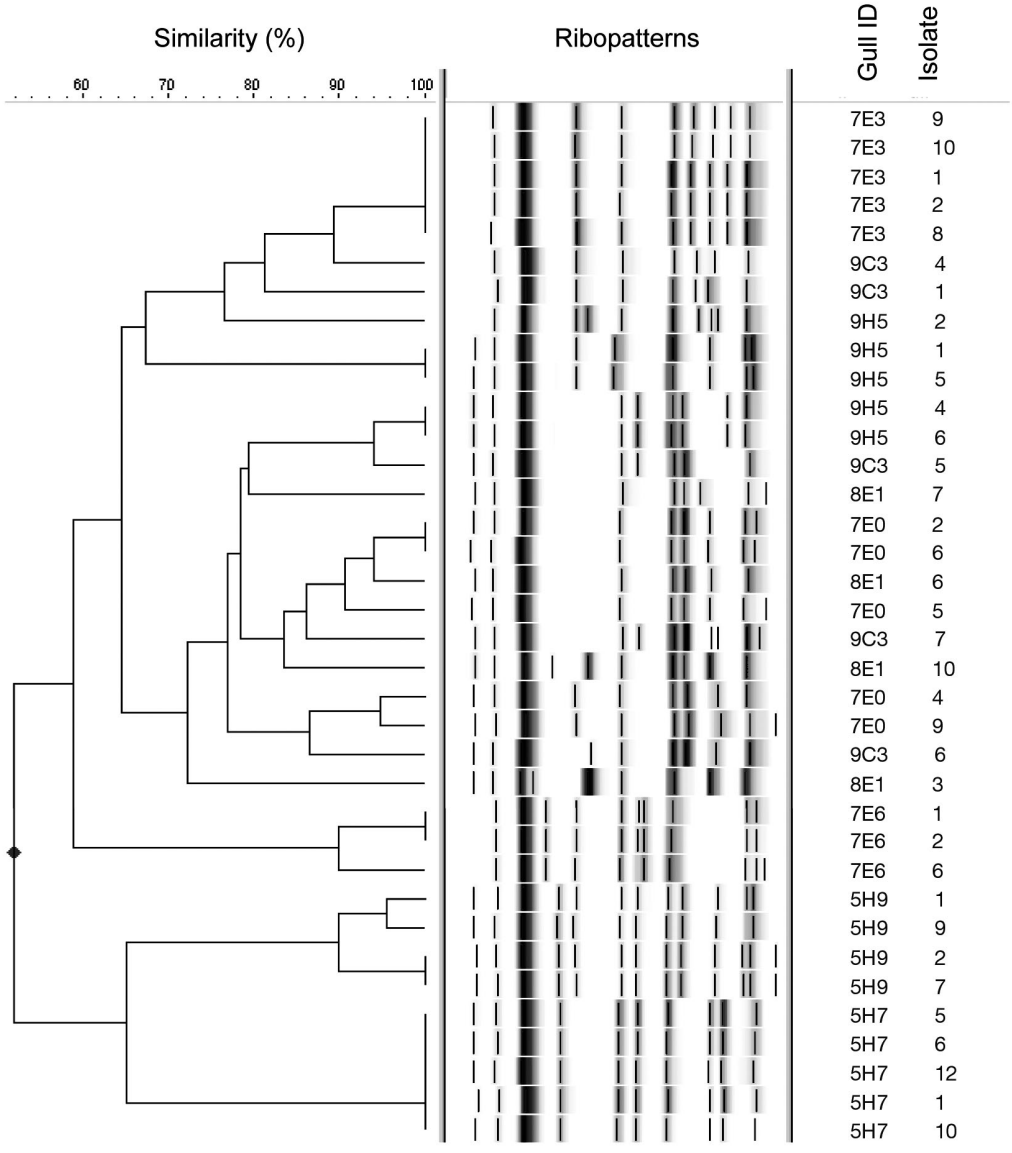

Fig. 4. *Escherichia coli.* Dendrogram of 36 ribopatterns for isolates obtained from feces collected from great black-backed gulls *Larus marinus* breeding on Appledore Island, Maine. Black lines overlaid to emphasise banding patterns

lates. Unique banding patterns constituted 64% of *Larus marinus* isolates and 56% for herring gulls. There was only one shared (100% similarity) pattern when all gull isolates were pooled. There were no patterns shared between wastewater and landfill source isolates when they were pooled for cluster analysis.

DISCUSSION

Our results indicate that *Larus argentatus* and *L. marinus* nesting at Appledore Island, Maine, share strains of *Escherichia coli* in common with both the Turnkey Landfill and Municipal Wastewater Treatment Plant in Rochester, New Hampshire. Evidence of shared strains with these environments indicates that gulls may acquire fecal bacteria from ingestion of trash from the landfill and while loafing and bathing in wastewater. These pathogens may then be transported and deposited to local shellfish growing areas and recreational beaches (Jones et al. 2006). We also found high concentrations of *E. coli* in gull feces, suggesting that gulls may be a significant source of fecal bacteria. Previous studies have also found large numbers of fecal bacteria in gull feces, but very few studies have enumerated *E. coli* in the feces; most quantify the number of fecal coliforms instead. The concentrations of *E. coli* found in our study (*Larus argentatus*: 1.9×10^2 to 2.5×10^9 g^{-1}) were similar

Table 3. *Escherichia coli.* Diversity measurements of subtypes isolated from gull feces, trash samples, and wastewater

Subtyping source	No. of isolates	Richness estimator (S)	Shannon index (*H'*)	Pielou's evenness (*J'*)	Simpson's reciprocal index (1/*D*)
Larus argentatus	39	13	2.8894	1.1265	9.9014
L. marinus	36	16	2.9439	1.0618	15.7500
Wastewater	22	10	2.6191	1.1375	5.3721
Landfill trash	20	16	2.9264	1.0555	47.4992

to those found in gull (*Larus* spp.) feces at the Great Lakes ($<1.0 \times 10^5$ to 1.9×10^9 g^{-1}; Fogarty et al. 2003). These concentrations are also similar to fecal coliform concentrations found in other studies (1.1×10^6 to 1.5×10^9 g^{-1}; Lévesque et al. 1993, Alderisio & DeLuca 1999).

In many previous studies, digestion of DNA with a single restriction enzyme has been successfully used to differentiate species-specific strains of targeted bacteria (Hartel et al. 1999, Parveen et al. 1999, Hartel 2002, Myoda et al. 2003). Others have used a 2-enzyme digest (Meays et al. 2006), or separate ribotyping analyses with 2 different enzymes (Harwood et al. 2003, Jenkins et al. 2003, Jones et al. 2006), to increase the potential diversity in ribopatterns. However, studies where DNA profiles from many isolates from an individual animal have been compared show saturations of diversity, or richness, at different numbers of profiles (Carson et al. 2001, Johnson et al. 2004, McLellan 2004, Anderson et al. 2006). Myoda et al. (2003) suggested DNA banding patterns produced by ribotyping and other genotypic methods are probably random with respect to what makes individual and host-specific strains of target bacteria unique, so the added cost of using a second enzyme may not be necessary. In addition, the number of isolates that were ribotyped and analyzed for this study were adequate to enable detection of differences in diversity between the 2 gull species and the 2 sources.

Direct comparisons of *Escherichia coli* populations in various host animals are rare in the literature, as most studies have focused on one host species (Anderson et al. 2006). No prior studies have specifically compared *E. coli* isolates between gull species or between more than one gull species and multiple environmental sources. Our study revealed differences between gull species in both the diversity of *E. coli* ribopatterns, and the number of *E. coli* strains shared with environmental sources. *Larus argentatus* shared more *E. coli* subtypes with landfill trash and wastewater than did *L. marinus*. This result may reflect a greater reliance on anthropogenic sources of food and roosting sites by *L. argentatus* compared to *L. marinus*, a finding that corroborates previous studies that recorded larger numbers of the former than the latter at landfills in New England (Wells 1994, Cavanagh 1992).

We found a greater diversity of *Escherichia coli* isolates in *Larus marinus* compared to *L. argentatus*. The cause of this difference remains to be determined, but may be related to differences in diet and feeding ecology between the 2 species. Although the diets of these 2 species of gull exhibit considerable overlap, *L. marinus* outcompetes *L. argentatus* for preferred invertebrate prey in rocky intertidal habitats (Rome & Ellis 2004), steals prey from *L. argentatus* at landfills (Verbeek 1979), outcompetes *L. argentatus* for fisheries discards (Furness et al. 1992), and preys on adult and juvenile *L. argentatus* on breeding colonies (Ellis & Good 2006). At Appledore Island, *L. marinus* tends to attack and eat larger terrestrial mammals (muskrats *Ondatra zibethica* and rats) than does *L. argentatus* (J. C. Ellis pers. obs.). Season- and age-specific foraging ecology is associated with variation in abundance of *Salmonella* in gull feces (Hatch 1996). Studies of other animals also indicate that intestinal microflora can be affected by small changes in diet (Netherwood et al. 1999, Souza et al. 1999). Alternatively, it is possible that the environment of the gastrointestinal tract differs between the 2 species of gulls, thereby affecting the structure of *E. coli* populations found in the feces. This hypothesis warrants further study.

The trash samples had a greater number of unique ribotypes and higher diversity measures than did the wastewater samples. Although pet feces and feces from direct deposition by animals roosting at lagoon sites may be present in wastewater at treatment facilities, bacterial inputs from human waste are likely the most significant source. These human-derived strains would have been subject to some degree of selection resulting from exposure to the wastewater treatment conditions. These selective forces may homogenize *Escherichia coli* populations in wastewater lagoons. In contrast, the landfill may have had a wider variety of inputs, and/or a greater prevalence of environmentally persistent strains (Ishii et al. 2006), and thus a more diverse *E. coli* population.

This study is one of the few to compare diversity of

Escherichia coli populations from different animal hosts and from different sources. Due to the relatively small numbers of isolates ribotyped from the gull species and environmental sources in our study, we almost certainly underestimated the diversity of *E. coli* populations in each of these sources. However, because our sample sizes between gull species and between the 2 sources were similar, the relative diversity estimates provided an informative comparative tool for this study.

In spite of similar *Escherichia coli* concentrations from wastewater and landfill trash, we found a greater number of *E. coli* strain matches between gull feces and wastewater than between gull feces and landfill isolates. This may be a function of differences in gull exposure to these 2 potential sources, the relative strength of bacterial contaminant levels, or differences in strains with varying capabilities to persist in the source environments and the gull intestines. The greater prevalence of wastewater isolates in gull feces is significant because of the greater likelihood of enteric pathogen incidence from human feces in wastewater compared to landfill trash, where the sources of *E. coli* are much more heterogeneous.

Adult gulls breeding at Appledore Island frequently occur at public beaches in coastal New Hampshire and Maine during the summer months where they may come into contact with people engaging in recreational activities at these beaches. Interestingly, some of the banded adults sampled in this study (leg band numbers C71, H22, 9H5) have been observed repeatedly at public beaches during the summer and fall (J. C. Ellis unpubl. data). Our findings suggest that *Larus argentatus* may be a more important reservoir and vector of anthropogenically derived fecal pathogens than *L. marinus*. Banded juvenile *L. argentatus* from Appledore Island have been resighted as far south as Florida (J. C. Ellis unpubl. data), suggesting that they are capable of dispersing fecal bacteria long distances. If the bacteria found in gull feces are pathogenic or antibiotic resistant (Bogomolni et al. 2008, this issue), then gulls may be an important source of zoonoses throughout the Atlantic coast.

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LITERATURE CITED

Alderisio KA, DeLuca N (1999) Seasonal enumeration of fecal coliform bacteria from the feces of ring-billed gulls (*Larus delawarensis*) and Canada geese (*Branta canadensis*). Appl Environ Microbiol 65:5628–5630

Anderson MA, Whitlock JE, Harwood VJ (2006) Diversity and distribution of *Escherichia coli* genotypes and antibiotic resistance phenotypes in feces of humans, cattle, and horses. Appl Environ Microbiol 72:6914–6922

Belant JL, Seamans TW, Gabrey SW, Dolber RA (1995) Abundance of gulls and other birds at landfills in northern Ohio. Am Midl Nat 134:30–40

Benton C, Khan F, Monaghan P, Richards WN, Shedden CB (1983) The contamination of a major water supply by gulls (*Larus* sp.) — a study of the problem and remedial action taken. Water Res 17:789–798

Bertellotti M, Yorio P (1999) Spatial and temporal patterns in the diet of the kelp gull in Patagonia. Condor 101:790–798

Bogomolni AL, Gast RJ, Ellis JC, Dennett M, Pugliares KR, Lentell BJ, Moore MJ (2008) Victims or vectors: a survey of vertebrate zoonoses from coastal waters of the Northwest Atlantic. Dis Aquat Org 81:13–38

Carson CA, Shear BL, Ellersieck MR, Asfaw A (2001) Identification of fecal *Escherichia coli* from humans and animals by ribotyping. Appl Environ Microbiol 67:1503–1507

Cavanagh PM (1992) Population dynamics, foraging ecology, and management of gulls on Monomoy NWR. PhD dissertation, University of Massachusetts, Amherst, MA

Dice LR (1945) Measures of the amount of ecologic association between species. Ecology 26:297–302

Dixon B (2007) Disseminators aloft? Microbe 2:416–417

Duhem C, Roche P, Vidal E, Tatoni T (2008) Effects of anthropogenic food resources on yellow-legged gull colony size on Mediterranean islands. Popul Ecol 50:91–100

Ellis JC, Good TP (2006) Nest attributes, aggression, and breeding success of gulls in single and mixed species subcolonies. Condor 108:211–219

Ellis JC, Chen W, O'Keefe B, Shulman MJ, Witman JD (2005) Predation by gulls on crabs in rocky intertidal and shallow subtidal zones of the Gulf of Maine. J Exp Mar Biol Ecol 324:31–43

Fenlon DR (1983) A comparison of *Salmonella* serotypes found in the feces of gulls feeding at a sewage works with serotypes present in the sewage. J Hygiene 91:47–52

Fogarty LR, Haack SK, Wolcott MJ, Whitman RL (2003) Abundance and characteristics of the recreational water quality indicator bacteria *Escherichia coli* and enterococci in gull faeces. J Appl Microbiol 94:865–878

Furness RW, Ensore K, Hudson AV (1992) The use of fisheries waste by gull populations around the British Isles. Ardea 80:105–113

Good TP (1998) Great black-backed gull (*Larus marinus*). In: Poole A, Gill F (eds) The birds of North America, Np. 330. Academy of Natural Sciences, Philadelphia, PA, and American Ornithologists' Union, Washington, DC

Hartel PG (2002) Ribotyping enterococci. Microbiological Source Tracking Workshop, 5 February 2002, Irvine, CA. NWRI Abstract Report NWRI-02-01:11–15

Hartel PG, Segars WI, Stern NJ, Steiner J, Buchan A (1999) Ribotyping to determine the host origin of *Escherichia coli* isolates in different water samples. In: Olsen DS, Potyondy JP (eds) Wildland hydrology. American Water Resource Association, Herndon, VA, p 377–382

Hartel PG, Sumner JD, Hill JL, Collins JV, Entry JA, Segars WI (2002) Geographic variability of *Escherichia coli* ribotypes from animals in Idaho and Georgia. J Environ Qual 31:1273–1278

Harwood VJ, Wiggins B, Hagedorn C, Ellender RD and others

(2003) Phenotypic library-based microbial source tracking methods: efficacy in the California collaborative study. J Water Health 1:153–166

Hatch JJ (1996) Threat to public health from gulls. Int J Environ Health Res 6:5–16

Hill TCJ, Walsh KA, Harris JA, Moffett BF (2003) Using ecological diversity measures with bacterial communities. FEMS Microbiol Ecol 43:1–11

Hunter PR (2003) Climate change and waterborne and vector-borne disease. J Appl Microbiol 94:37S–46S

Ishii S, Winfried B, Randall K, Hicks E, Sadowsky MJ (2006) Presence and growth of naturalized *Escherichia coli* in temperate soils from Lake Superior watersheds. Appl Environ Microbiol 72:612–621

Jenkins MB, Hartel PG, Olexa TJ, Stuedemann JA (2003) Putative temporal variability of *Escherichia coli* ribotypes from yearling steers. J Environ Qual 32:305–309

Johnson LK, Brown MB, Carruthers EA, Ferguson JA, Dombek PE, Sadowsky MJ (2004) Sample size, library composition, and genotypic diversity influence accuracy of determining sources of fecal pollution among natural populations of *Escherichia coli* from different animals. Appl Environ Microbiol 70:4478–4485

Jones F, Smith P, Watson DC (1978) Pollution of a water supply catchment by breeding gulls and the potential environmental health implications. J Inst Water Eng Sci 32:469–482

Jones SH (2007) Optimized use of *Escherichia coli* ribotyping for identifying pollution sources in New Hampshire's coastal waters. New Hampshire Department of Environmental Services, Concord, NH

Jones SH, O'Hara B, Bryant T (2006) Source species identification of *Escherichia coli* in oysters, *Crassostrea virginica*, and overlying waters. In: Henshilwood K, Deegan B, McMahon T, Cusack C and others (eds) Molluscan shellfish safety. Proc 5th Int Conf Molluscan Shellfish Safety, Galway, Ireland, 14–18 June 2004. The Marine Institute, Rinville, Oranmore, Galway, p 107–114

Kapperud G, Rosef O (1983) Avian wildlife reservoir of *Campylobacter fetus* subsp. *jejuni*, *Yersinia* spp., and *Salmonella* spp. in Norway. Appl Environ Microbiol 45:375–380

Kelsey RH, Webster LF, Kenny DJ (2008) Spatial and temporal variability of ribotyping results at a small watershed in South Carolina. Water Res 42(8–9): 2220–2228

Lévesque B, Brousseau P, Berner F, Dewailly E, Joly J (1993) Study of the bacterial content of ring-billed gull (*Larus delawarensis*) on the microbial quality of recreational water. Appl Environ Microbiol 59:1228–1230

McLellan SL (2004) Genetic diversity of *Escherichia coli* isolated from urban rivers and beach water. Appl Environ Microbiol 70:4658–4665

McLellan SL, Salmore AK (2003) Evidence for localized bacterial loading as the cause of chronic beach closings in a freshwater marina. Water Res 37:2700–2708

Meays CL, Broersma K, Nordin R, Mazumder A, Samadpour M (2006) Spatial and annual variability in concentrations and sources of *Escherichia coli* in multiple watersheds. Environ Sci Technol 40:5289–5296

Moore JE, Gilpin D, Crothers E, Canney A, Kaneko A, Matsuda M (2002) Occurrence of *Campylobacter* spp. and *Cryptosporidium* spp. in seagulls (*Larus* spp.). Vector Borne Zoonotic Dis 2:111–114

Myoda SP, Carson CA, Fuhrmann JJ, Hahm BK and others (2003) Comparing genotypic bacterial source tracking methods that require a host origin database. J Water Health 1:167–180

Netherwood T, Gilbert HJ, Parker DS, O'Donnel AG (1999) Probiotics shown to change bacterial community structure in the avian gastrointestinal tract. Appl Environ Microbiol 65:5134–5138

Parveen S, Portier KM, Robinson K, Edmiston L, Tamplin ML (1999) Discriminant analysis of ribotype profiles of *Escherichia coli* for differentiating human and nonhuman sources of fecal pollution. Appl Environ Microbiol 65: 3142–3147

Pierotti RJ, Good TP (1994) Herring gull (*Larus argentatus*). In: Poole A, Gill F (eds) The birds of North America, No. 124. Academy of Natural Sciences, Philadelphia, PA, and American Ornithologists' Union, Washington, DC

Quessy S, Messier S (1992) Prevalence of *Salmonella* spp., *Campylobacter* spp. and *Listeria* spp. in ring-billed gulls (*Larus delawarensis*). J Wildl Dis 28:526–531

Reed KD, Meece JK, Henkel JS, Shukla SK (2003) Birds, migration and emerging zoonoses: West Nile virus, Lyme disease, Influenza A and enteropathogens. Clin Med Res 1:5–12

Rippey SR, Adams WN, Watkins DW (1987) Enumeration of fecal coliforms and *E. coli* in marine and estuarine waters: an alternative to the APHA-MPN approach. J Water Pollut Control Fed 59:795–798

Rome MS, Ellis JC (2004) Foraging ecology and interactions between herring gulls and great black-backed gulls in New England. Waterbirds 27:200–210

Skelly DK, Bolden SR, Holland MP, Freidenburg LK, Freidenfelds NA, Malcolm TR (2006) Urbanization and disease in amphibians. In: Collinge SK, Ray C (eds) Disease ecology. Oxford University Press, New York, p 153–167

Souza V, Rocha M, Valera A, Equirte LE (1999) Genetic structure of natural populations of *Escherichia coli* in wild hosts on different continents. Appl Environ Microbiol 65: 3373–3385

Topp E, Welsh M, Tien YC, Dang A, Lazarovitis G, Cohn K, Zhu H (2003) Strain-dependent variability in growth and survival of *Escherichia coli* in agricultural soil. FEMS Microbiol Ecol 44:303–308

USEPA (US Environmental Protection Agency) (1986) Test methods for *Escherichia coli* and enterococci by the membrane filtration procedure. EPA 600/4-85/076. Environmental Monitoring and Support Laboratory, USEPA, Cincinnati, OH

USEPA (US Environmental Protection Agency) (2005) Microbial source tracking guide document, EPA/600-R-05-064. Office of Research and Development, USEPA, Cincinnati, OH

Verbeek NAM (1979) Some aspects of the breeding biology and behavior of the great black-backed gull *Larus marinus*. Wilson Bull 91:575–582

Waldenström J, On SLW, Ottvall R, Hasselquist D, Harrington CS, Olsen B (2003) Avian reservoirs and zoonotic potential of the emerging pathogen *Helicobacter canadensis.* Appl Environ Microbiol 69:7523–7526

Wells JV (1994) Correlates of the distribution and abundance of wintering gulls in Maine. J Field Ornithol 65:283–294

Whelan CD, Monaghan P, Girdwood RWA, Fricker CR (1988) The significance of wild birds (*Larus* sp.) in the epidemiology of *Campylobacter* infections in humans. Epidemiol Infec 101:259–267

Whitman RL, Nevers MB (2003) Foreshore sand as a source of *Escherichia coli* in nearshore water of a Lake Michigan beach. Appl Environ Microbiol 69:5555–5562

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