ORIGINAL ARTICLE

Antimicrobial-resistant *Escherichia coli* in public beach waters in Quebec

Patricia Turgeon DVM PhD[1], Pascal Michel DVM MPVM PhD[1,2], Patrick Levallois MD FRCPC[3,4], Pierre Chevalier PhD[3], Danielle Daignault DVM DES Dipl ACVM[2], Bryanne Crago BSc[5,6], Rebecca Irwin DVM MSc[2], Scott A McEwen DVM DVS[7], Norman F Neumann[8,9], Marie Louie MD FRCPC[5,6]

P Turgeon, P Michel, P Levallois, et al. Antimicrobial-resistant *Escherichia coli* in public beach waters in Quebec. Can J Infect Dis Med Microbiol 2012;23(2):e20-e25.

INTRODUCTION: Human exposure to antimicrobial-resistant bacteria may result in the transfer of resistance to commensal or pathogenic microbes present in the gastrointestinal tract, which may lead to severe health consequences and difficulties in treatment of future bacterial infections. It was hypothesized that the recreational waters from beaches represent a source of antimicrobial-resistant *Escherichia coli* for people engaging in water activities.

OBJECTIVE: To describe the occurrence of antimicrobial-resistant *E coli* in the recreational waters of beaches in southern Quebec.

METHODS: Sampling occurred over two summers; in 2004, 674 water samples were taken from 201 beaches, and in 2005, 628 water samples were taken from 177 beaches. The minimum inhibitory concentrations of the antimicrobial-resistant *E coli* isolates against a panel of 16 antimicrobials were determined using microbroth dilution.

RESULTS: For 2004 and 2005, respectively, 28% and 38% of beaches sampled had at least one water sample contaminated by *E coli* resistant to one or more antimicrobials, and more than 10% of the resistant isolates were resistant to at least one antimicrobial of clinical importance for human medicine. The three antimicrobials with the highest frequency of resistance were tetracycline, ampicillin and sulfamethoxazole.

DISCUSSION: The recreational waters of these beaches represent a potential source of antimicrobial-resistant bacteria for people engaging in water activities. Investigations relating the significance of these findings to public health should be pursued.

Key Words: *Antimicrobial resistance*, E coli, *Environment*, *Public Health*, *Recreational waters*

L'*Escherichia coli* résistant aux antimicrobiens dans l'eau des plages publiques du Québec

INTRODUCTION : L'exposition humaine à des bactéries résistant aux antimicrobiens peut provoquer le transfert de la résistance à des microbes commensaux ou pathogènes présents dans le tube digestif, ce qui peut avoir de graves conséquences sur la santé et compliquer le traitement de futures infections bactériennes. On a soulevé l'hypothèse que les eaux de baignade des plages représentent une source d'infection à l'*Escherichia coli* résistant aux antimicrobiens pour les personnes qui s'adonnent à des activités aquatiques. La présente étude visait principalement à décrire l'occurrence d'*E coli* résistant aux antimicrobiens dans les eaux de baignade du sud du Québec.

MÉTHODOLOGIE : Les chercheurs ont procédé à l'échantillonnage sur deux étés. En 2004, ils ont prélevé 674 échantillons d'eau sur 201 plages, et en 2005, 628 échantillons d'eau sur 177 plages. Ils ont établi les concentrations inhibitrices minimales des isolats d'*E coli* résistant aux antimicrobiens par rapport à un groupe de 16 antimicrobiens au moyen d'une dilution en bouillon.

RÉSULTATS : En 2004 et en 2005, respectivement, 28 % et 38 % des plages échantillonnées comptaient au moins un échantillon d'eau contaminée par l'*E coli* résistant à au moins un antimicrobien, et plus de 10 % de ces isolats résistaient à un moins un antimicrobien d'importance clinique en médecine humaine. La tétracycline, l'ampicilline et le sulfaméthoxazole étaient les trois antimicrobiens les plus touchés par la résistance.

EXPOSÉ : Les eaux de baignade de ces plages représentent une source potentielle de bactéries résistant aux antimicrobiens pour les personnes qui s'adonnent à des activités aquatiques. Il faudrait poursuivre les recherches sur la signification de ces observations en matière de santé publique.

Human exposure to antimicrobial-resistant bacteria may result in the transfer of antimicrobial resistance to commensal or pathogenic microbes present in the gastrointestinal tract and may lead to severe health consequences. An increase in antimicrobial resistance can lead to greater morbidity and mortality due to bacterial infections, limited therapy alternatives and delayed administration of effective therapies. Bacterial resistance capacity can be intrinsic or can be the result of mutation or transfer of resistant genes from other bacteria (1). Microorganisms can be resistant to one or more antimicrobial agents and can come from different sources, including the food chain and the environment (2,3). There are multiple environmental sources, which can include water exposure through drinking or recreational activities. Therefore, aquatic ecosystems may represent a reservoir for antimicrobial-resistant bacteria and a potential medium for the spread and evolution of antimicrobial resistance (4,5).

To our knowledge, few studies have described the exposure to antimicrobial-resistant bacteria through recreational waters for people engaged in water/bathing activities. The present study was part of the Canada-wide research initiative entitled "Prospective Multi-Province Surveillance for Antimicrobial-Resistant *Escherichia coli* in Drinking and Recreational Source Waters: Impact on Humans and the Environment" (Antimicrobial Resistant Organism – ARO Water Study). The main objective of the present study was to determine the occurrence of antimicrobial-resistant microorganisms in recreational waters of beaches in southern Quebec. We hypothesized that recreational waters from beaches represent a source of antimicrobial-resistant *Escherichia coli* for people engaging in water activities. *E coli* is used as a biological indicator for fecal contamination in water sources. These bacteria are known to be a reservoir of resistance genes, and horizontal gene transfer between different strains of *E coli* and other intestinal organisms has been demonstrated (6,7).

[1]*Faculté de médecine vétérinaire, Université de Montréal, Saint-Hyacinthe, Quebec;* [2]*Laboratory for Foodborne Zoonoses, Public Health Agency of Canada, Guelph, Ontario;* [3]*Institut national de santé publique du Québec;* [4]*Centre Hospitalier Universitaire de Québec, Université Laval, Quebec, Quebec;* [5]*Faculty of Medicine, Microbiology and Infectious Diseases, University of Calgary;* [6]*Alberta Provincial Laboratory for Public Health (Microbiology), Calgary, Alberta;* [7]*Ontario Veterinary College, University of Guelph, Guelph, Ontario;* [8]*School of Public Health, University Terrace;* [9]*Alberta Provincial Laboratory for Public Health, Edmonton, Alberta*

Correspondence: Dr Patricia Turgeon, Faculté de médecine vétérinaire, Université de Montréal, CP 5000, Saint-Hyacinthe, Quebec J2S 7C6. Telephone 450-773-8521, fax 450-778-8129, e-mail patricia.turgeon@umontreal.ca

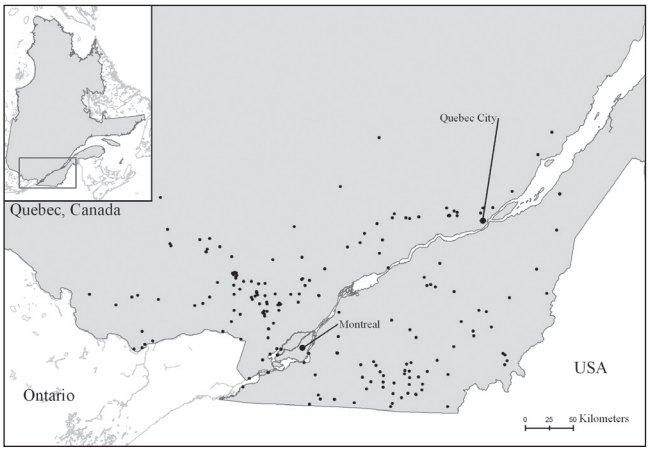

Figure 1) *Public beaches located in southern Quebec that were included in the present study (n=237)*

METHODS

The present prevalence study aimed to estimate the occurrence of antimicrobial-resistant *E coli* in public beaches located on lakes in southern Quebec. The beaches included in the present study participated in the provincial public beach surveillance program during the summers of 2004 and 2005 (Figure 1).

Sampling

Water samples were obtained from the provincial public beach surveillance program during the summers of 2004 and 2005. In this program, beaches were sampled continuously throughout the bathing period, usually from mid-June to the first week of September, and the sampling frequency for each beach was determined by the results of water quality testing from the preceding year. For this surveillance program, beaches were classified in four sanitary groups according to the fecal coliform concentration found in the sampled water. A group A beach has zero colony-forming units (CFU)/100 mL to 20 CFU/100 mL and is tagged as 'excellent quality'. Similarly, a group B beach ('good') has 21 CFU/100 mL to 100 CFU/100 mL, group C ('poor') has 101 CFU/100 mL to 200 CFU/100 mL and group D ('polluted') has more than 200 CFU/100 mL. Beaches in group A are sampled at least once every two years, with the possibility of being sampled annually or more frequently. Beaches in class B are sampled at least three times a year and beaches in classes C and D and new beaches are sampled at least five times a year. For each sampling session, or harvesting, the number of samples collected is a function of the beach length. The number of samples taken ranges from six, for a beach ≤60 m, to 30 for a beach ≥721 m. Each sample is taken inside the bathing area (delimited by floating cables) and the distance between each sampling location is set at equal lengths across a given beach. Water samples are taken at a 15 cm depth, and the sampling pattern is adapted to the beach layout (linear or circular) to make sure that the spatial distribution of the contamination is well assessed. For a linear beach with a bathing area depth of >1.2 m, sampling is performed using a 'W' pattern, while for a linear beach having a bathing area with a water depth <1.2 m, a linear sampling pattern is used. For a circular beach, a linear sampling pattern is also performed for the entire circumference of the bathing area with no consideration of water depth. All water samples are collected using a 250 mL sterile polypropylene bottle containing sodium thiosulfate, although beaches using chlorination (n=9) were not included in the present study. The bottle was submerged in water and the cap was removed only at the sampling time and replaced immediately after collection. Samples were preserved at 4°C and transported to the laboratory within 24 h. A detailed description of this beach surveillance program is available (8).

Microbial analysis

As part of the same surveillance program, beach waters were quantified by membrane filtration and microbiologically tested for fecal coliforms on membrane fecal coliform (mFC) agar (Dalynn, Canada), following the procedure described by the *Centre d'expertise en analyse environnementale du Québec* (9). For each harvesting of a beach in the present study, up to five different morphotypical colonies suggestive of fecal coliforms were selected from five randomly chosen mFC agar plates. Selected colonies were cultured on a tryptic soy base (TSB) agar slant (Difco, USA) for 24 h at 44°C. Slants were then transferred on ice by priority airmail to the study laboratory in Calgary, Alberta, to ensure delivery in <24 h. Growth from the slant was frozen in skim milk upon receipt and processed within one year from the date of water sampling. Recovery of bacteria when samples were processed immediately versus after freezing for up to one year was not affected by prolonged storage (data not shown). To differentiate other fecal coliforms from *E coli*, bacteria archived in skim milk were cultured onto X-Gluc agar (Dalynn, Canada) for 18 h to 24 h at 35°C. Up to five blue/green colonies suggestive of *E coli* of different morphotypes were selected and inoculated into TSB broth and incubated at 35°C for 4 h to 6 h to promote growth.

An aliquot of 0.01 mL of TSB broth bacterial suspension was screened by an agar plate method for antimicrobial resistance using a MacConkey agar control plate without antimicrobials and six MacConkey plates each containing a different antimicrobial: ampicillin 8 µg/mL, gentamicin 8 µg/mL, nalidixic acid 4 µg/mL, streptomycin 32 µg/mL, sulfamethoxazole 156 µg/mL and tetracycline 4 µg/mL. Based on results from a pilot experiment, the concentration of antimicrobial used in the agar screen method was selected to be lower, if possible, than the human resistance breakpoint to be sufficiently sensitive for screening. The choice of these concentrations was made to improve the sensitivity of the test and to capture a maximum amount of antimicrobial-resistant isolates to be processed by the National Antimicrobial Resistance Monitoring System (NARMS) (Centers for Disease Control and Prevention, USA). These concentrations also contributed to preventing inadvertent background overgrowth by fecal coliforms other than *E coli*. For an isolate to be classified as 'resistant', the minimum inhibitory concentration (MIC) determined by NARMS had to be greater than or equal to the human breakpoint.

Antimicrobial screen plates were manually read to identify growth of morphologically presumptive *E coli* colonies. The pilot study determined that in a given water sample, multiple *E coli* colonies are most often clonal when typed by pulsed field gel electrophoresis regardless of the antimicrobial plate from which the isolates were selected, and results from the agar screen method were highly correlated with those generated by the microbroth dilution method (Table 1). Because the pilot study also determined that the highest frequency of resistance was detected on tetracycline media, if growth was seen on multiple antimicrobial screen plates, a single isolate was picked from the tetracycline plate and the selected isolate was assumed to be representative of the predominant *E coli* clone in the water sample. To ensure that the agar screening method did not miss any resistance, one presumptive *E coli* isolate was also selected from one in 10 (in 2004) and one in 20 (in 2005) water samples found to have no resistance by the agar screen method, and submitted for testing by microbroth dilution.

Presumptive *E coli* isolates screened as being resistant were confirmed as *E coli* by API 20E (Biomerieux, Canada). At NARMS, the MICs for the antimicrobial-resistant *E coli* isolates against a panel of 16 antimicrobials were determined using microbroth dilution: amikacin (concentration range 0.5 µg/mL to 4 µg/mL), amoxicillin/clavulanic acid (1/0.5 µg/mL to 32/16 µg/mL), ampicillin (1 µg/mL to 32 µg/mL), cefoxitin (0.5 µg/mL to 16 µg/mL), ceftiofur (0.12 µg/mL to 8 µg/mL), ceftriaxone (0.25 µg/mL to 64 µg/mL), cephalothin (2 µg/mL to 32 µg/mL), chloramphenicol (2 µg/mL to 32 µg/mL), ciprofloxacin (0.015 µg/mL to 4 µg/mL), gentamicin (0.25 µg/mL to 16 µg/mL), kanamycin (8 µg/mL to 64 µg/mL), nalidixic acid (0.5 µg/mL to 32 µg/mL), streptomycin (32 µg/mL to 64 µg/mL), sulfamethoxazole (16 µg/mL to 512 µg/mL) ,

TABLE 1
Pilot study assessing relatedness of *Escherichia coli* isolates in water samples isolated from different antimicrobial screen plates

Water sample	*E coli* isolates	Antimicrobial screen plate from which *E coli* isolate was obtained*	PFGE result†
1	ARO-1	Streptomycin	ARO-1 and ARO-8 had >95% similarity to each other. ARO-15 only 70% similarity to other two isolates
	ARO-8	Sulfamethoxazole	
	ARO-15	Tetracycline	
2	ARO-2	Cephalothin	>95% similarity
	ARO-9	Tetracycline	
3	ARO-16	Tetracycline	95% similarity among all three isolates
	ARO-3	Streptomycin	
	ARO-10	Sulfamethoxazole	
4	ARO-4	Cephalothin	95% similarity among all three isolates
	ARO-11	Sulfamethoxazole	
	ARO17	Tetracycline	
5	ARO-5	Streptomycin	95% similarity among all three isolates
	ARO-12	Sulfamethoxazole	
	ARO-18	Tetracycline	
6	ARO-6	Streptomycin	ARO-6 and ARO-13 had 95% similarity to each other. ARO -19 only 70% similarity to other isolates
	ARO-13	Sulfamethoxazole	
	ARO-19	Tetracycline	
7	ARO-7	Gentamicin	ARO-14 and ARO-20 had 95% similarity to each other. ARO -7 only 70% similarity to other isolates
	ARO-14	Sulfamethoxazole	
	ARO-20	Tetracycline	

*E coli *from individual water samples were grown on different antimicrobial screen plates listed in the Methods, with one* E coli *isolate from each screen plate from which microbial growth was observed selected and typed by pulsed-field gel electrophoresis (PFGE). †PFGE was performed at the Provincial Laboratory for Public Health according to standard operating procedures. Only XbaI digests were used for comparing similarity in this pilot study. Cluster analysis, based on the Dice coefficient, and using an unweighted pair group method with a 1.5% positional tolerance, was used to calculate per cent similarity (BioNumerics, Applied Maths NV, Belgium). Isolates displaying 95% similarity were considered to be closely related. ARO Antimicrobial resistant organism*

tetracycline (4 µg/mL to 32 µg/mL) and trimethoprim/sulfamethoxazole (0.12/2.38 µg/mL to 4/76 µg/mL) (10). The microbroth dilution method was performed using an automated system (Sensititre Automated Microbiology System, Trek Diagnostic Systems Ltd, United Kingdom) that is a commercially available broth dilution technique that makes use of dehydrated antimicrobials in the wells of microtitre plates. Results were interpreted using the resistance breakpoints relevant to human health as outlined by the Clinical and Laboratory Standards Institute guidelines (11). An isolate was considered resistant if it had an MIC value above the human breakpoint for at least one antimicrobial.

Statistical analysis

Fisher's exact test was performed to determine whether the proportions of resistant *E coli* isolates and the proportion of beaches with at least one resistant *E coli* isolate were different between 2004 and 2005. Differences among the proportion of *E coli* isolates resistant to the three antimicrobials most represented were assessed with a McNemar test. To determine whether the level of resistance differed across the various beach sanitary groups, a χ^2 test was performed. All statistical procedures were performed using SAS version 9.1 (SAS Institute Inc, USA).

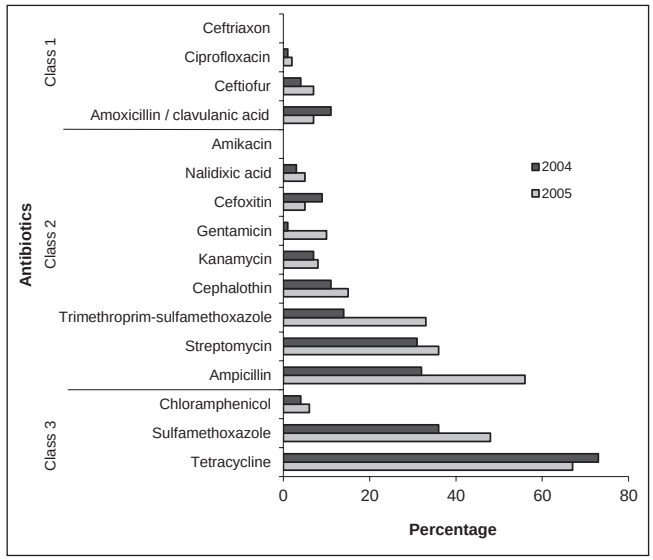

Figure 2) *Antimicrobial resistance distribution for the 16 antimicrobials tested for all the resistant samples from the summers of 2004 (n=89) and 2005 (n=101).*

RESULTS

According to the provincial surveillance program, sampling for both summers happened from mid-June to mid-August and was continuous throughout these periods. In 2004, 201 beaches were included in the study and were sampled, on average, three times in the summer (range: one to eight), with a mean of eight samples per harvesting (range: six to 12). In 2005, 177 beaches were included in the study and were sampled, on average, three times in the summer (range: one to six), with a mean of eight samples per harvesting (range: six to 18). A total of 674 *E coli* isolates from the 201 beaches in 2004, and 628 *E coli* isolates from the 177 beaches in 2005 were analyzed for antimicrobial resistance by microbroth dilution, with a mean of three isolates per beach for each summer. In total, 237 unique beaches were sampled during the study period over the two summers and 141 beaches were sampled in both 2004 and 2005. The smallest beach was 10 m long, the longest was 400 m, and the majority (60%) were <60 m. Figure 2 shows the distribution of antimicrobial resistance to the 16 antimicrobials tested for all resistant *E coli* isolates for both summers.

The three antimicrobials most represented were tetracycline, ampicillin and sulfamethoxazole. In 2004, the proportion of *E coli* isolates resistant to tetracycline was significantly greater than the proportion of *E coli* isolates resistant to ampicillin (McNemar test; P<0.001) and sulfamethoxazole (McNemar test; P<0.001). Comparable results were obtained from the data for 2005, with the proportion of *E coli* isolates resistant to tetracycline being significantly greater then the proportion of *E coli* isolates resistant to ampicillin (McNemar test; P=0.042) and sulfamethoxazole (McNemar test; P=0.004). The differences between the proportion of *E coli* isolates resistant to ampicillin and sulfamethoxazole were not statistically different in 2004 (McNemar test; P=0.527) or 2005 (McNemar test; P=0.206). In 2004, 89 (13.2%) isolates were resistant to at least one antimicrobial ('general resistance') and of these, 56 (62.9%) were resistant to at least two antimicrobials and 41 (46.1%) were resistant to at least three antimicrobials. According to the 2006 categorization of antimicrobial drugs, based on importance in human medicine by Health Canada, 12 (13.5%) of the 89 isolates with a general resistance were resistant to at least one antibiotic in class I (very high importance), 59 (66.3%) in class II (high importance) and 79 (88.8%) in class III (medium importance). In the summer of 2005, 101 (16.1%) isolates showed a general resistance and of these, 67 (66.3%) were resistant to at least two antimicrobials and 57 (56.4%) were resistant to at least three antimicrobials. For 2005, 11 (10.9%) of the resistant isolates were resistant to antimicrobials belonging to class I and 79 (78.2%)

TABLE 2
Antimicrobial resistance distribution according to antimicrobial and beach sanitary group for 2004 (n=201 beaches) and 2005 (n=177 beaches)

	2004					2005				
Antimicrobial	**A**	**B**	**C***	**D***	**Total**	**A**	**B**	**C***	**D***	**Total**
Class 1										
Ceftriaxon	0 (0)	0 (0)	0	0	0 (0)	0 (0)	0 (0)	0	0	0 (0)
Ciprofloxacin	1 (0.6)	0 (0)	0	0	1 (0.5)	0 (0)	1 (2)	0	0	1 (0.6)
Ceftiofur	3 (2)	0 (0)	0	0	3 (1)	5 (4)	1 (2)	0	0	6 (3)†
Amoxicillin/clavulanic acid	6 (4)	1 (3)	0	0	7 (3)	6 (5)	0 (0)	0	2	6 (3)
Class 2										
Amikacin	0 (0)	0 (0)	0	0	0 (0)	0 (0)	0 (0)	0	0	0 (0)
Nalidixic acid	3 (2)	0 (0)	0	0	3 (1)	1 (1)	1 (2)	0	1	3 (2)
Cefoxitin	4 (2)	1 (3)	0	0	5 (2)	4 (3)	0 (0)	0	0	4 (2)
Gentamicin	1 (0.6)	0 (0)	0	0	1 (0.5)	5 (4)	2 (5)	0	1	8 (5)
Kanamycin	3 (2)	1 (3)	0	2	6 (3)	2 (2)	3 (7)	0	3	8 (5)
Cephalothin	6 (4)	1 (3)	0	0	7 (3)	11 (9)	1 (2)	0	1	13 (7)
Trimethroprim-sulfamethoxazole	8 (5)	2 (7)	0	1	11 (5)	15 (12)	7 (17)	2	2	26 (15)
Streptomycin	15 (9)	4 (13)	1	4	24 (12)	14 (11)	8 (19)	2	4	28 (16)
Ampicillin	20 (12)	4 (13)	1	1	26 (13)	29 (23)	9 (22)	0	0	38 (23)
Class 3										
Chloramphenicol	2 (1)	1 (3)	0	0	3 (1)	2 (2)	1 (2)	0	1	4 (2)
Sulfamethoxazole	19 (12)	5 (2)	1	3	28 (14)	20 (16)	12 (29)	2	4	38 (21)
Tetracycline	32 (20)	10 (34)	1	5	48 (24)	26 (20)	14 (34)	2	4	46 (26)
Number of beaches per group, n	163	29	2	7	201	128	41	3	5	177

*Data presented as n (%) unless otherwise indicated. *Percentage not calculated due to small denominators; †In 2005, among the 177 beaches sampled, six (3%) beaches had at least one* E coli *sample resistant to ceftiofur and five of these were in group A. A group A beach has zero colony-forming units (CFU)/100 mL to 20 CFU/100 mL and is tagged as 'excellent quality', group B beach ('good') has 21 CFU/100 mL to 100 CFU/100 mL, group C ('poor') has 101 CFU/100 mL to 200 CFU/100 mL and group D ('polluted') has over 200 CFU/100 mL*

TABLE 3
Distribution of general resistance among each beach group in 2004/2005

Group	Beaches with at least one *E coli* sample resistant,	Beaches with all *E coli* samples susceptible,	Total, n/n
A	39 (24)*/43 (34)	124 (76)/85 (66)	163/128
B	10 (34)/19 (46)	19 (66)/22 (54)	29/41
C	2 (100)/2 (75)	0 (0)/1 (25)	2/3
D	5 (71)/4 (80)	2 (29)/1 (20)	7/5
Total	56 (28)/68 (38)	145 (72)/109 (62)	201/177

*Data presented as n (%) unless otherwise indicated. *In 2004, among the 163 beaches in group A, 39 (24 %) had at least one* Escherichia coli *sample resistant. A group A beach has zero colony-forming units (CFU)/100 mL to 20 CFU/100 mL and is tagged as 'excellent quality', group B beach ('good') has 21 CFU/100 mL to 100 CFU/100 mL, group C ('poor') has 101 CFU/100 mL to 200 CFU/100 mL and group D ('polluted') has over 200 CFU/100 mL*

to both classes II and III. The difference between the proportions of isolates showing a general resistance in 2004 and 2005 was not statistically different (P=0.15 [Fisher's exact test]).

Among the 201 beaches sampled in 2004, 56 (27.9%) had at least one isolate classified resistant to at least one antimicrobial, 40 (19.9%) had at least one isolate resistant to two or more antimicrobials and 32 (15.9%) had at least one isolate resistant to three or more antimicrobials. Similar results were observed in 2005, in which 177 beaches were sampled, and 68 (38.4%) had at least one isolate classified resistant to at least one antimicrobial, 50 (28.2%) had at least one isolate resistant to two or more antimicrobials and 45 (25.4%) had at least one isolate resistant to three or more antimicrobials. Table 2 shows the antimicrobial resistance distribution according to beach sanitary group for both 2004 and 2005. Table 3 shows the distribution of general resistance for each beach group.

A χ^2 test was performed to determine whether the level of resistance differed across the various beach sanitary groups, which found no statistically significant difference in the percentage of beaches with at least one *E coli* isolate resistant to one or more antimicrobial(s) between the group 'A' (Excellent) and 'B' (good) beaches for the year 2004 (P=0.251) and 2005 (P=0.192). Statistical testing was not performed for groups 'C' and 'D' due to very low numbers of beaches falling into these categories during the two years of the study. In terms of consistency of antimicrobial resistance results for the same beach, 22 beaches had at least two *E coli* resistant isolates in 2004 and of these, 10 (45.4%) had all their isolates resistant to the same antimicrobials, and nine (40.9%) had all their isolates resistant to two or more antimicrobials. In 2005, 24 beaches had at least two *E coli* resistant isolates and of these, 12 (50%) had all their isolates resistant to the same antimicrobials and 11 (45.8%) had all of their isolates resistant to two or more antimicrobials. Of the 141 beaches sampled in both summers, 17 (12.1%) had at least one isolate in each summer with general resistance and 65 (46.1%) beaches from both years had all their isolates susceptible to the 16 antimicrobials tested. Among the 17 beaches with resistant *E coli* samples in 2004 and 2005, none had all their isolates with the same antimicrobial resistance profile and four (23.5%) had all their sample isolates resistant to two or more antimicrobials. Among the 141 beaches sampled in both summers, the difference between the proportions of beaches having at least one isolate resistant to at least one antimicrobial in 2004 and 2005 was not statistically different (P=0.076 [Fisher's exact test]).

DISCUSSION

Overall, these results are in agreement with another study showing that 14% of *E coli* isolates from Great Lakes recreational waters (Ontario) carried antimicrobial resistance genes and the most frequently found genes were those coding for resistance to tetracycline, ampicillin and streptomycin (12). Resistance to these three antimicrobials is also present in other sources of human exposure. Interestingly, tetracycline and ampicillin were the two types of antimicrobials against which resistance was detected in a high percentage of *E coli* isolated from pork and beef retailed meat products, according to figures presented in the 2004 and 2005 reports of the Canadian Integrated Program of Antimicrobial

Resistance Surveillance (2,13). Our results are also in agreement with a previous study investigating the prevalence of antimicrobial-resistant bacteria in drinking water of private wells in Ontario, which found that the highest frequencies of antimicrobial resistance were for the similar antimicrobials (tetracycline, sulfamethoxazole and streptomycin) (Nguon RS, unpublished master thesis, Université de Montréal).

We also noted that over the two years investigated, an average of one beach out of three demonstrated the presence of *E coli* with general resistance (resistance to a least one antimicrobial) and that more than 10% of the resistant isolates were resistant to at least one antimicrobial in the very high importance category for human medicine. These findings are noteworthy in terms of public health, given that these antimicrobials are important for the treatment of serious bacterial infections in humans. There is a concern that emergence of resistance to these agents may result in limited, or lack of, alternative effective treatment options. In light of these preliminary findings, it also appears that public beaches in southern Quebec may represent a source of exposure to antimicrobial-resistant bacteria, which could constitute a health risk for people engaging in water activities at these locations. Although there are currently no published data confirming direct transfer of antimicrobial resistance genes from the environment to humans, some studies have shown that the in vitro and in vivo intestinal transfer of genetic material with resistance genes is possible among transient or commensal resident bacteria (7,9-16). Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *E coli* and *Salmonella* species isolates from food animals and humans have been reported (7); these plasmids can readily move between different organisms and can harbour multiple antimicrobial resistance genes. In a previous report, an acquired, plasmid-mediated, AmpC beta-lactamase gene, CMY-2 has also been identified in *E coli* from water samples (18). Similarities of the CMY-2-containing plasmids identified in *E coli* isolates of water origin, as well as in human and animal clinical isolates, highlight the potential for transmission of multidrug resistance phenotypes from water sources. Antimicrobial-resistant bacteria ingested with water during bathing activities could thus transfer their antimicrobial resistance genes to bacteria of the intestinal flora of bathers. Transferred genes can act as a reservoir of genetic material available to transient or colonized bacterial populations, including pathogens. Horizontal transfers like these can involve genetic material such as plasmids and transposons that carry more than one marker of resistance and have the potential to transfer many mechanisms of resistance against different antimicrobials in a single transfer (17). The presence of antimicrobial-resistant *E coli* may also indicate the presence of other antimicrobial-resistant bacteria, due to a transfer of antimicrobial resistance genes in the water environment (19-21). Overall, antimicrobial resistance in bacterial populations could have serious public health consequences, including prolonged disease duration and increased frequencies of septicemia, hospitalization and death (22).

One of the limitations of the present study was the choice of *E coli* as the indicator of the presence of antimicrobial-resistant bacteria. *E coli* are used as a biological indicator for fecal contamination in water sources and are also known to be a reservoir of resistance genes, but they are also naturally resistant to some antimicrobials, such as glycopeptides. For this reason, we did not test our water samples for this class of antibiotic. A combination of two indicators, such as *E coli* (Gram-negative) and *Enterococcus* species (Gram-positive) could have resulted in a more accurate estimate of the occurrence of antimicrobial resistance level in this setting, allowing us to test for a broader antimicrobial spectrum. The extent of resistance in beach water samples may have been underestimated given that we selected only one representative *E coli* isolate from each water sample to be screened for resistance. This isolate was only a representative of the composite of all the *E coli* from a water sample, and the antimicrobial resistance phenotype represented, at the least, the predominant clone within the sample. The intent of the present study was to establish whether water is a potential reservoir for antimicrobial resistance, and if so, to determine the spectrum of antimicrobial resistance phenotypes. Further studies would be needed to determine the ecology of antimicrobial-resistant *E coli* in this environment, and the dynamics of transmission and persistence over time. Another limiting element of the present study relates to the sampling frequency of beaches under investigation. Because the sampling frequency of a given beach in the provincial surveillance program is determined by the result of the previous year's water quality, beaches with a better quality (the most common in the present study) are only sampled a few times. This low power of detection may have affected the representativeness of beaches with excellent water quality and introduced a bias in the estimation of resistance prevalence, giving a lower prevalence of beaches with at least one water sample resistant to antimicrobial.

Beach contamination with fecal bacteria carrying antimicrobial resistance characteristics involves an interaction of complex phenomena, including factors related to human and animal population densities, medical and veterinary use of antimicrobials, meteorological events and landscape features. Public health risk related to human exposure to water containing antimicrobial-resistant bacteria is difficult to quantify. Ongoing initiatives and surveillance programs aimed at monitoring microbial water quality should include activities specific to the detection of resistance bacteria from recreational water to further assess the significance and the geographical extent of this concern in various populations.

ACKNOWLEDGEMENTS: This study was made possible by the valued contribution of the members of the ARO Research Group (alphabetical order): S Braithwaite, M Buzzelli, P Cantin, B Ciebin, B Coleman, C Guenette, F Jamieson, M Jerrett and M Mulvey. The private testing laboratories that also played an integral role in this study are as follows: AquaMac: M Guilbeault; BIOLAB, Joliette, Thetford Mines and Cap-de-la-Madeleine: I Barette, M Boily, K Guay; BioServies 1995 Inc: C Dalpe; MicroB: B Skora; Microbios Analytique Inc: C Coulombe; Laboratoire d'analyses SM Inc, Sherbrooke, Varennes: C Letoumeau, L Gagnon; Les Consultants VETCO Inc: V Germain. The authors also acknowledge Linda Chui for her contribution in the pilot study and her significant input in the data interpretation. The authors also acknowledge people involved in the provincial public beach surveillance program (Programme Environnement-plage), and more specifically, Alain Lavoie, Agnès Godin and Marc Gignac. This study was funded by the Canadian Health Research Institutes (SFW 66539), the Public Health Agency of Canada and the Université de Montréal.

REFERENCES

1. Tenover FC. Mechanisms of antimicrobial resistance in bacteria. Am J Med 2006;119:S3-10; discussion S62-70.
2. Public Health Agency of Canada. Canadian Integrated Program of Antimicrobial Resistance Surveillance (CIPARS) 2005. Government of Canada, 2007.<http://publications.gc.ca/site/eng/312034/publication.html> (Accessed May 3, 2012).
3. Schmitt H, Stoob K, Hamscher G, et al. Tetracyclines and tetracycline resistance in agricultural soils: Microcosm and field studies. Microb Ecol 2006;51:267-76.
4. Ash RJ, Mauck B, Morgan M. Antibiotic resistance of Gram-negative bacteria in rivers, United States. Emerg Infect Dis 2002;8:713-6.
5. Biyela PT, Lin J, Bezuidenhout CC. The role of aquatic ecosystems as reservoirs of antibiotic resistant bacterial and antibiotic resistance genes. Water Sci Technol 2004;50:45-50.
6. Nikolich MP, Hong G, Shoemaker NB, et al. Evidence for natural horizontal transfer of tetQ between bacteria that normally colonize humans and bacteria that normally colonize livestock. Appl Environ Microbiol 1994;60:3255-60.
7. Winokur PL, Vonstein DL, Hoffman LJ, et al. Evidence for transfer of CMY-2 AmpC beta-lactamase plasmids between *Escherichia coli* and Salmonella isolates from food animals and humans. Antimicrob Agents Chemother 2001;45:2716-22.
8. Programme Environnement-plage. Ministère du développement durable, de l'environnement et des parcs du Québec. <www.mddep.gouv.qc.ca/programmes/env-plage/index.htm> (Accessed May 15, 2008).

9. CEAEQ. Recherche et dénombrement des coliformes fécaux (thermotolérants) et confirmation à l'espèce *Escherichia coli* : méthode par filtration sur membrane. MA. 700 – Fec.Ec 1.0. Centre d'expertise en analyse environnementale du Québec, 2005:20 p.
10. CDC. Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS): 2002 Human Isolates Final Report. Center for Disease Control and Prevention, 2004. <www.cdc.gov/narms/annual/2002/2002ANNUALREPORTFINAL.pdf> (Accessed May 3, 2012).
11. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing; thirteenth informational supplement, M100-S13.
12. Hamelin K, Bruant G, El-Shaarawi A, et al. A virulence and antimicrobial resistance DNA microarray detects a high frequency of virulence genes in Escherichia coli isolates from Great Lakes recreational waters. Appl Environ Microbiol 2006;72:4200-6.
13. Public Health Agency of Canada . Canadian Integrated Program of Antimicrobial Resistance Surveillance (CIPARS) 2004. Government of Canada, 2006. <http://publications.gc.ca/site/eng/288249/publication.html> (Accessed May 3, 2012).
14. Blake DP, Hillman K, Fenlon DR, et al. Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ileal conditions. J Appl Microbiol 2003;95:428-36.
15. Lester CH, Frimodt-Moller N, Hammerum AM. Conjugual transfer of aminoglycoside and macrolide resistance between *Enterococcus faecium* isolates in the intestine of streptomycin-treated mice. FEMS Microbiol Lett 2004;235:385-91.
16. Lester CH, Frimodt-Moller N, Sorensen TL, et al. In vivo transfer of the vanA resistance gene from an *Enterococcus faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestines of human volunteers. Antimicrob Agents Chemother 2006;50:596-9.
17. Acar J, Rostel B. Antimicrobial resistance: An overview. Rev Sci Tech (OIE) 2001;20:797-810.
18. Mataseje LF, Neumann N, Crago B, et al. Characterization of cefoxitin-resistant *Escherichia coli* isolated from recreational beaches and private drinking water in Canada between 2004 and 2006. Antimicrob Agents Chemother 2009;53:3126-30.
19. Jones OAH, Voulvoulis N, Lester JN. Potential impact of pharmaceuticals on environmental health. Bull World Health Organ 2003;81:768-9.
20. Agerso Y, Peterson A. The tetracycline resistance determinant Tet39 and the sulphonamide resistance gene sulII are common among resistant *Acinetobacter* spp. isolated from integrated fish farms in Thailand. J Antimicrob Chemother 2007;59:23-7.
21. Baquero F, Martinez J-L, Canton R. Antibiotics and antibiotic resistance in water environments. Curr Opin Biotechnol 2008; 19:260-5.
22. Barza M, Travers K. Excess infections due to antimicrobial resistance: The "attributable fraction". Clin Infect Dis 2002;34:S126-S30.