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A Longitudinal 6-Year Study of the Molecular Epidemiology of Clinical *Campylobacter* Isolates in Oxfordshire, United Kingdom

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Temporal and seasonal trends in *Campylobacter* genotypes causing human gastroenteritis were investigated in a 6-year study of 3,300 recent isolates from Oxfordshire, United Kingdom. Genotypes (sequence types [ST]) were defined using multilocus sequence typing and assigned to a clonal complex (a cluster of related strains that share four or more identical alleles with a previously defined central genotype). A previously undescribed clonal complex (ST-464) was identified which, together with ST-42, ST-45, and ST-52 complexes, showed increasing incidence. Concurrently, the incidence of ST-574, ST-607, and ST-658 complexes declined. The relative frequencies of three clonal complexes (ST-45, ST-283, and ST-42) peaked during summer and those of two (ST-353 and ST-403) peaked during winter. Nine clonal complexes (ST-22, ST-45, ST-48, ST-61, ST-257, ST-283, ST-403, ST-658, and ST-677) were significantly associated with ciprofloxacin sensitivity ($P < 0.05$). Seven clonal complexes (ST-49, ST-206, ST-354, ST-446, ST-460, ST-464, and ST-607) were associated with ciprofloxacin resistance ($P < 0.05$). Clonal complexes exhibited changing incidence and differences in seasonality and antibiotic resistance phenotype. These data also demonstrated that detailed surveillance at a single site captures information which reflects that observed nationally.

Campylobacter jejuni and *Campylobacter coli* are the most frequent causes of acute bacterial gastroenteritis in humans, representing an unrelenting worldwide public health problem. *C. jejuni* accounts for over 90% of cases, with the majority of the remainder caused by *C. coli* (14). The annual incidence of diagnosed human infections in 2008 was 92 per 100,000 individuals (16) in England and Wales, compared to 13 per 100,000 in the United States (4). However, estimates of underreporting indicate that the actual incidences are 7- and 38-fold higher, respectively (4, 47). Incidence varies with age, being highest among those under five years of age (16), and overall, males are more frequently affected, with a male-to-female sex ratio of 1.2 to 1 (28). Many wild and farmed avian and mammalian species carry campylobacters as commensal members of the gastrointestinal microbiota, with human infection resulting from either direct contact with contaminated feces or indirect transmission via contaminated food.

Multilocus sequence typing (MLST) studies have determined *C. jejuni* and *C. coli* population structures to be essentially nonclonal but comprising a large number of genetically related isolates, or clonal complexes (8, 10). Outbreak investigation (39), case control (35), and "natural" experiments (46, 50) have implicated contaminated poultry meat as a major cause of clinical infection. These findings have been strongly supported by source attribution models that have employed MLST data to estimate the contribution made by different potential infection sources to the burden of human disease (41, 42). While emphasizing the importance of contaminated poultry in this regard, these analyses also support a role for bovine, ovine, and other sources (7, 41, 42).

Reports from various temperate countries have shown that the incidence of human campylobacteriosis is at its highest consistently during the same week each year, although this time point shows variation among countries (26, 28, 38). This pronounced seasonality may be coincident with increased *Campylobacter* prevalence in reservoirs for infection and seasonal changes in human behavior that affect exposure (38). Variation in the prevalence of *Campylobacter* in various reservoirs has been found in some studies to correspond to the seasonal pattern of human campylobacteriosis (44), but this finding has not been universal (18). Seasonal variation in the incidence of human infection caused by certain clonal complexes has been described, notably increased incidences of sequence type 45 (ST-45) and ST-283 complex during the early summer (29, 43).

As human *Campylobacter* infection is generally self-limiting, antimicrobial therapy is not routinely recommended in the United Kingdom, but where symptoms are severe or prolonged, ciprofloxacin and clarithromycin are the treatments of choice (1). In Oxfordshire, United Kingdom, the proportion of clinical ciprofloxacin-resistant *Campylobacter* isolates increased from 3% in 1991 to 37.5% in 2008 (5). Resistance levels of 30 to 50% have been reported in Cambodia (2006) (30), 23.0% in the United States (2009) (49), and 71.4% in rural India (2002) (20), and more recently the level was 97.9% in China (17). An association between poultry consumption and acquisition of ciprofloxacin-resistant *Campylobacter* has been described, and some studies have detected possible associations between clonal complex and antibiotic resistance (15, 24). In Australia, where these drugs have not

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been approved for use in food-producing animals, levels of fluoroquinolone resistance remain low (48).

Here, MLST was used (8, 10) to study genotypes causing *Campylobacter* infection in a defined human population (approximately 640,000 individuals in 2009, corresponding to approximately 1% of the total United Kingdom population) living in the catchment area of the Clinical Microbiology Laboratory, John Radcliffe Hospital, Oxfordshire, United Kingdom. These data permitted the investigation of clonal complex seasonality and ciprofloxacin resistance. Additionally, the data (available at http://pubmlst.org/perl/bigsdb/bigsdb.pl?db=pubmlst_campylobacter_isolates) provided a baseline to inform on the impact of proposed interventions intended to reduce *Campylobacter* infection rates in England and Wales and provided a comparator for other human and veterinary *Campylobacter* epidemiological studies.

MATERIALS AND METHODS

Isolates. The *Campylobacter* isolates included in this study were cultured from human stool samples submitted to the Clinical Microbiology Laboratory, Oxford University Hospitals NHS Trust, Oxford, United Kingdom, between 15 September 2003 and 14 September 2009. The specimens were from both hospital and community subjects. The size of the population served is approximately 640,000, representing around 1% of the United Kingdom population. Fecal samples were emulsified in cysteine selenite broth, plated onto *Campylobacter* blood-free selective agar (E & O Laboratories Limited, Bonnybridge, United Kingdom), and incubated at 42°C for 48 h under microaerophilic conditions. Bacterial colonies identified as oxidase-positive, catalase-producing, curved Gram-negative rods, with typical colonial morphology when subcultured to blood agar, were reported as *Campylobacter* species.

For long-term storage, isolates were emulsified in Luria broth containing 5% glycerol and stored at −80°C. The susceptibilities of the *Campylobacter* isolates to ciprofloxacin (1 μg) and erythromycin (5 μg) were determined using a modified Stokes disk diffusion method (Becton, Dickinson, Oxford, United Kingdom) (2). For isolates with reduced zones, the MIC was determined by Etest (bioMérieux Clinical Diagnostics). The method was subject to internal and external quality assurance (National External Quality Assessment Service [NEQAS]) with satisfactory performance. The isolation date was recorded for each isolate, together with corresponding anonymized details of patient age, sex, and recent travel outside the United Kingdom.

Multilocus sequence typing. Bacterial DNA samples were produced from cultured *Campylobacter* cells suspended in molecular biology-grade water (125 ml; Sigma-Aldrich Company Ltd., Dorset, United Kingdom). The suspension was vortex mixed briefly and heated at 100°C for 10 min, debris was removed by centrifugation at 13,000 × *g* for 10 min, and the supernatant, containing chromosomal DNA, was removed and stored at −20°C. When further DNA template was required, isolates were recultured on Columbia blood agar plates (Oxoid Ltd., Basingstoke, United Kingdom) and incubated at 42°C for 48 h under microaerophilic conditions.

Multilocus sequence typing was performed as described previously (8, 10, 32). Briefly, PCR amplification of the seven housekeeping loci was performed using either oligonucleotide primers designed to amplify the relevant loci from both *C. jejuni* and *C. coli* isolates (32) or those described previously (8, 10). Amplification products were purified by precipitation with 20% polyethylene glycol-2.5 M NaCl (11), and nucleotide sequencing was performed at least once on each DNA strand using the PCR primers and BigDye ready reaction mix (version 3; Applied Biosystems, Foster City, CA). Previously described and newly identified alleles, sequence types (ST), and clonal complexes were assigned by querying the MLST database located at http://pubmlst.org/campylobacter/ (21), which held details of 88,859 sequences, 5,705 MLST profiles, and 13,069 isolates as of 9 January 2012. Potential central genotypes of emergent or previously undescribed clonal complexes were identified by their frequency within the data set and the pubMLST database. Candidate central genotype allelic profiles were then tested for the presence of single-, double-, and triple-locus variants in the pubMLST database, and clonal complex members sharing four or more identical alleles with the putative central genotype were identified with the BURST algorithm, available at the website detailed above.

Statistical analyses. Frequency distributions of continuous or categorical variables within populations were compared by either the *t* test, Pearson's chi-square statistic, or, where sample sizes were small, Fisher's exact test. These and logistic regression analyses of secular and seasonal trends of clonal complexes were carried out using StataIC 10 (StataCorpLP, Texas). Genetic differentiation between populations was assessed using *Fst* pairwise comparisons of concatenated sequences for the seven MLST alleles with ARLEQUIN software version 3 (12). The diversity of STs within and between clonal complexes among populations was assessed using a modified version of Simpson's index of diversity (19), based on the probability of two unrelated strains being of the same type.

TABLE 1 Summary of isolates genotyped over the 6-year study period

Yr of study	No. (%) of isolates		
	Unique	Assigned to sequence type	Assigned to clonal complex
September 2003–September 2004	584	575 (98.5)	530
September 2004–September 2005	637	531 (83.4)	495
September 2005–September 2006	680	537 (79.0)	496
September 2006–September 2007	841	613 (72.9)	560
September 2007–September 2008	778	468 (60.2)	408
September 2008–September 2009	771	576 (74.7)	503

RESULTS

Isolates. A total of 4,291 *Campylobacter* isolates, each representing a unique infection, were cultured between September 2003 and September 2009. Isolates were genotyped by MLST, and the ST was determined for 3,300 (76.9%) (Table 1). The STs were used to identify isolates to the species level (41): 91.6% were *C. jejuni* and 8.4% *C. coli*. The remaining 991 isolates were partially typed or untyped due to loss of viability during storage. Comparison of patient demographic data (sex, age, and month of isolation) and isolate antibiotic resistance data (ciprofloxacin and erythromycin) for the total isolates (4,291) versus isolates with complete allelic profiles (3,300) confirmed that the genotyped cases were representative of the collection. Subsequent analyses include only the 3,300 cases for which the ST of the *Campylobacter* isolates was determined.

Age, sex, and seasonal distribution of cases. The age distribution of the 3,300 cases stratified by year of age was examined. The highest rates of illness occurred in children under two and young adults (Fig. 1A). The sex distribution of cases within the John Radcliffe Hospital Microbiology Laboratory catchment area was examined by 5-year age group. The proportion of illness in males was greater in all groups except those aged 75 to 79 years and individuals over 85 years of age, where numbers of cases in females were higher, and those aged 15 to 19, where no sex bias was observed (Fig. 1B). The seasonal distribution of cases showed a marked summer peak (Fig. 2).

MLST and clonal complexes. The 3,300 isolates comprised 368 *C. jejuni* and 70 *C. coli* STs, each of which was assigned to a

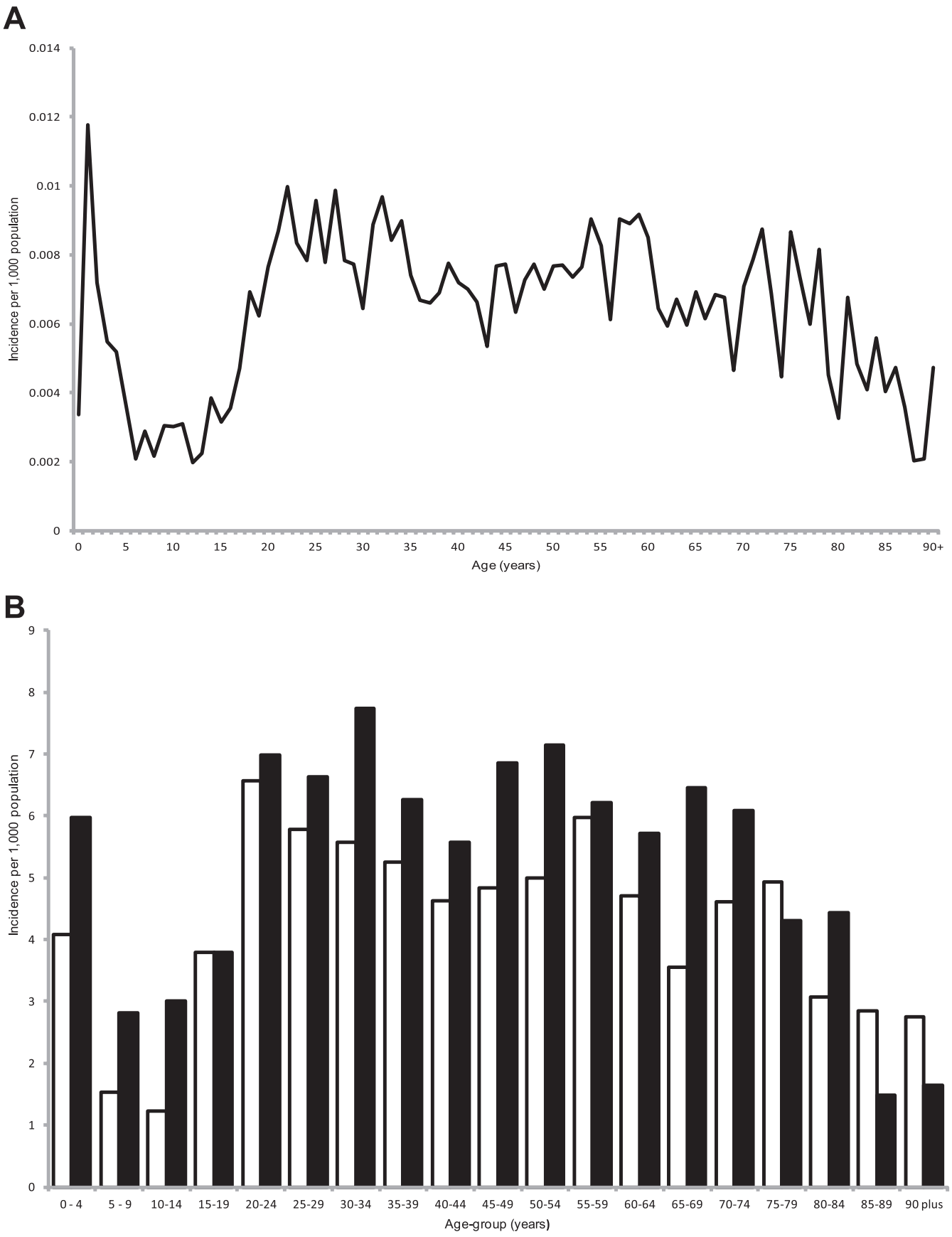

FIG 1 Age distribution of *Campylobacter* cases in the John Radcliffe Hospital catchment area, Oxfordshire, United Kingdom, between mid-September 2003 and mid-September 2009. (A) Incidence of infection per 1,000 individuals; (B) incidence among females (□) and males (■) per 1,000 age-related individuals.

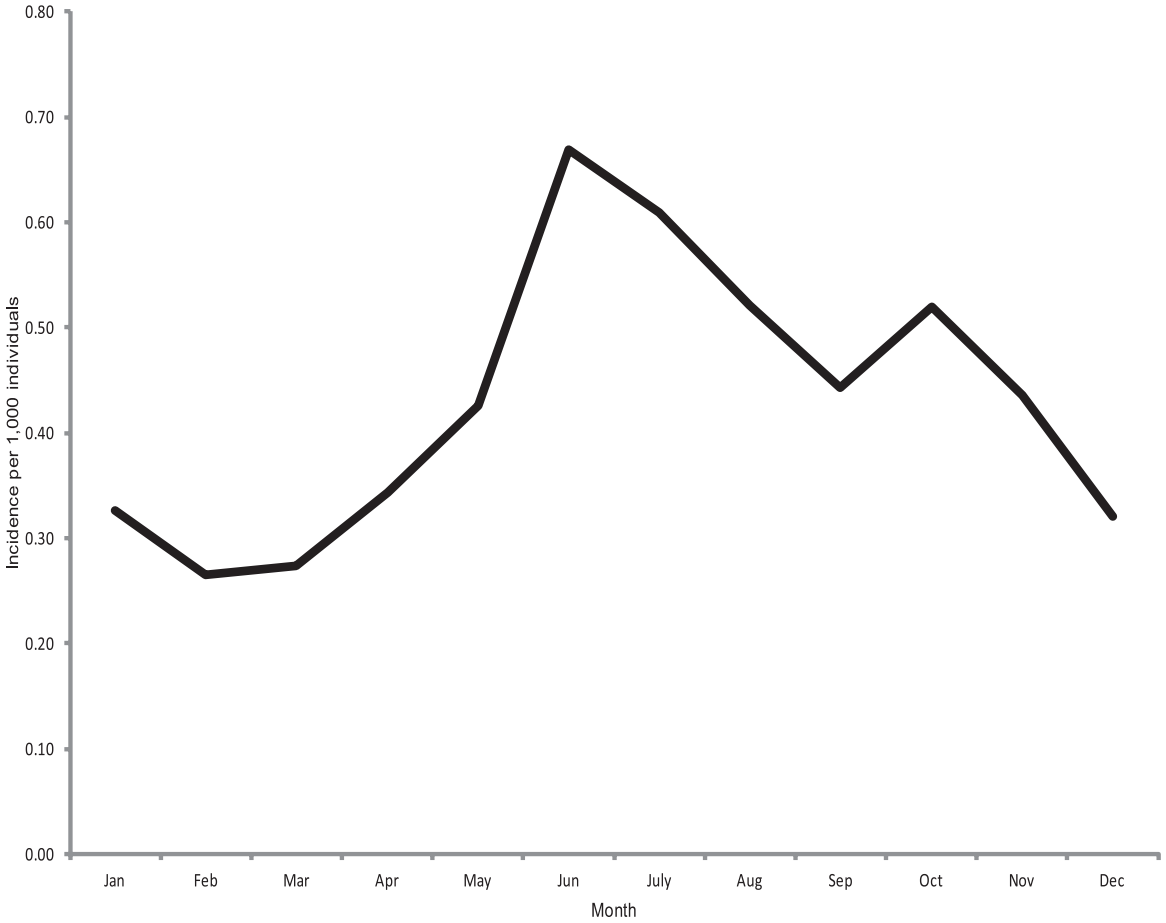

FIG 2 Seasonality of human *Campylobacter* genotyped isolates from the John Radcliffe Hospital catchment area, Oxfordshire, United Kingdom, between 2003 and 2009.

clonal complex using http://pubmlst.org/campylobacter. A total of 157 (26.8%) STs could not be assigned to a previously defined clonal complex; 137 were *C. jejuni*, and 20 were *C. coli*. These unassigned STs were investigated for the presence of undescribed central genotypes, leading to the identification of the *C. jejuni* ST-464 complex, containing eight STs and 71 isolates. In total, 35 *C. jejuni* clonal complexes and 1 *C. coli* clonal complex (ST-828 complex) were present within the data set, with 149 (25.4%) STs remaining unassigned. Six clonal complexes (ST-21, ST-257, ST-828, ST-45, ST-48, and ST-443 complexes) represented 59.8% of all isolates, ranging from 22.8% to 5.3%, respectively (Fig. 3). In total, *C. coli* belonging to the ST-828 complex or unassigned to an ST comprised 8.4% of isolates.

Temporal trends in clonal complexes. The temporal distribution of the 20 most abundant clonal complexes was analyzed using logistic regression analysis, which identified a year-on-year increase in the number of isolates assigned to ST-42, ST-45, ST-464, and ST-52 clonal complexes, whereas isolates belonging to ST-574, ST-607, and ST-658 complexes decreased in incidence over the study period. The overall trends were summarized as odds ratios representing the average increase or decrease in probability of an isolate belonging to that clonal complex per year of the study (Table 2). Although these seven clonal complexes do show an overall linear trend across the 6-year period, none are monotonic and there are substantial year-to-year variations, which in some cases (ST-42, ST-45, and ST-607 complexes) equal or exceed the overall change from year 1 to year 6.

Seasonality of clonal complexes. The data were examined for seasonal variation in the clonal complexes that occurred during the summer peak in incidence (June to September), compared to those that occurred during the rest of the year, using Pearson's chi-square test. The composition of clonal complexes that occurred between June and September was significantly different from that which occurred during the rest of the year (P value with the chi-square test [χ^2 P] < 0.001).

A logistic regression model was then used to identify significant seasonal trends for individual clonal complexes. Isolates belonging to ST-283, ST-42, and ST-45 complexes peaked as a proportion of the total during June and July ($P = 0.013$, $P = 0.018$, and $P < 0.001$, respectively) (Fig. 4). ST-353 and ST-403 complexes ($P < 0.005$ and $P = 0.021$, respectively) were proportionally most prevalent during November. Variation that deviated from overall harmonic seasonal patterns was detected for the ST-257 complex (χ^2 $P = 0.008$), which was lower than expected in July and November, and for the ST-354 complex (χ^2 $P < 0.001$), which peaked in November (data not shown).

Antibiotic resistance and clonal complex. Following primary culture, 3,262 isolates (98.9%) were examined by disk diffusion

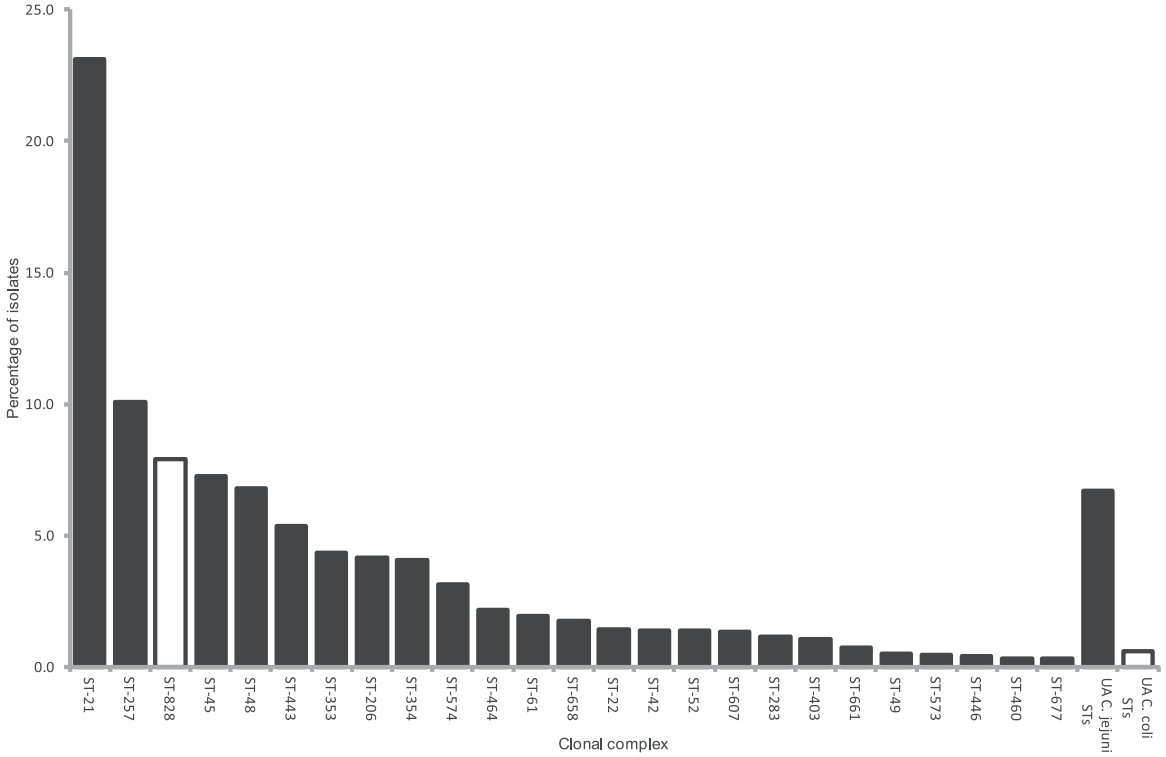

FIG 3 Relative proportion of clonal complexes represented more than 10 times in the data set in decreasing order of abundance. UA indicates sequence types that are not assigned to a clonal complex. Isolates belonging to *C. coli*, indicated by white bars, either were assigned to the ST-828 complex or belonged to sequence types unassigned to a clonal complex.

for sensitivity to ciprofloxacin (1 μg) and 3,265 (98.9%) isolates for sensitivity to erythromycin (5 μg). The proportion (percentage) of all ciprofloxacin-sensitive isolates (2,243; 69.8%) and all resistant isolates (972; 30.2%) associated with each of the 25 clonal complexes represented 10 times or more in the data set was determined. Isolates unassigned to a clonal complex were considered as a separate group for each species. Equivalent calculations were made for erythromycin-sensitive (3,167; 98.2%) and -resistant (57; 1.8%) isolates.

Nine clonal complexes (ST-22, ST-45, ST-48, ST-61, ST-257, ST-283, ST-403, ST-658, and ST-677) were significantly associated with ciprofloxacin sensitivity ($P < 0.05$) (Table 3), and seven clonal complexes (ST-49, ST-206, ST-354, ST-446, and ST-460, along with the newly described ST-464 and ST-607 complexes) were significantly associated with ciprofloxacin resistance, as were unassigned *C. jejuni* STs. One clonal complex (ST-257) was significantly associated with erythromycin-sensitive isolates, whereas the only significant association between either of the antibiotics and *C. coli* isolates was that between both ST-828 complex and unassigned *C. coli* STs with erythromycin resistance.

Patients reporting recent travel outside the United Kingdom ($n = 381$) were five times more likely to have a ciprofloxacin-resistant *Campylobacter* than those who had not (χ^2 $P < 0.001$). No difference in the proportion of erythromycin-resistant isolates was detected between travelers and nontravelers.

Patient demographics and genotype. The MLST and clonal complex data for each of the 3,300 isolates were examined to detect associations among infecting genotype and patient age, gen-

TABLE 2 Overall trends and odds ratios[a]

Clonal complex	% of isolates in complex						Odds ratio[b]	*P* value	CI[c]
	Yr 1	Yr 2	Yr 3	Yr 4	Yr 5	Yr 6			
ST-42	1.6	0.6	0.6	1.3	1.7	2.4	1.227	0.02	1.033–1.459
ST-45	6.8	6.6	8.5	4.9	7.9	8.7	1.087	0.03	1.008–1.172
ST-52	0.5	0.8	1.7	1.8	1.3	2.1	1.218	0.025	1.025–1.447
ST-464	0.9	0.4	1.7	1.3	3.2	5.6	1.612	<0.001	1.367–1.877
ST-574	5.2	3.2	2.4	3.3	1.7	2.4	0.846	0.004	0.754–0.948
ST-607	1.4	2.7	1.7	0.7	0.4	1.0	0.818	0.025	0.686–0.976
ST-658	2.6	2.3	1.7	1.8	0.9	1.2	0.824	0.012	0.708–0.959

[a] Overall trends and odds ratios representing the average increase or decrease in probability of an isolate belonging to that clonal complex per year of the study.
[b] Changing odds per year.
[c] 95% confidence interval.

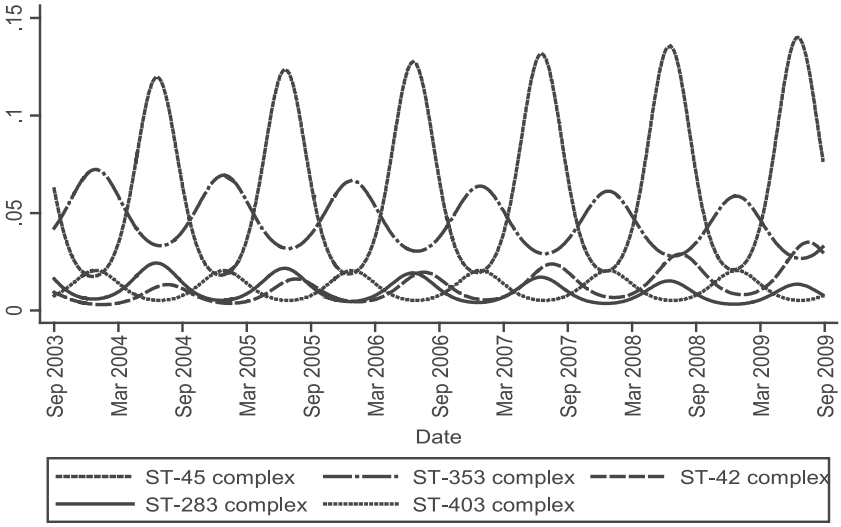

FIG 4 Seasonal and longer-term trends observed using a harmonic regression model of the data for clonal complexes whose frequency as a proportion of the total incidence were significantly higher during summer (ST-45, ST-283, and ST-42 complexes) or winter months (ST-353 and ST-403 complexes).

der, and recent travel outside the United Kingdom. Seven 10-year age groups and an eighth, comprising individuals aged 70 or over, were used to segregate the STs. Simpson's index of diversity was calculated for each group (see Fig. S1 in the supplemental material). This revealed no difference in the genotypic diversity of isolates infecting each group (diversity index [DI], 0.973 to 0.980). Similarly, differences in nucleotide content as indexed by F_{ST} values calculated from concatenated nucleotide sequences of the MLST loci indicated that the maximum level of sequence differentiation between the STs of each 10-year age group was <1%.

Clonal complexes affecting males and females were similar (χ^2 $P = 0.338$), as were the levels of nucleotide sequence diversity for isolates infecting both genders (male DI, 0.975 to 0.979; female DI, 0.973 to 0.978). Recent travel outside the United Kingdom was reported by 11.5% of individuals, isolates from whom belonged to clonal complexes that were significantly different ($P < 0.001$) from those from subjects who had not recently been abroad.

DISCUSSION

Sequence-based characterization, and especially MLST, of *Campylobacter* isolates has become the principal tool for understanding the molecular epidemiology of this important pathogen (10). Such studies have established that although genetically diverse, *Campylobacter* populations are highly structured and that the clonal complex is an informative unit of analysis (9). A key insight has been that particular clonal complexes are associated with the colonization of certain host species, which has permitted the genetic attribution of cases of human disease to likely infection sources and in so doing has principally implicated retail chicken meat products in a variety of settings (34, 42, 52). Longitudinal surveillance of the genotypes of human clinical isolates therefore provides a means of monitoring changes in infection sources or the appearance of new disease-associated *Campylobacter* genotypes. The present 6-year study examined temporal and seasonal trends in the nature and properties of the *Campylobacter* clonal complexes responsible for human disease in a defined, centrally located United Kingdom region (Oxfordshire) which represents approximately 1% of United Kingdom residents.

Patterns of age-related illness and gender bias within the present study were largely as reported for England and Wales (16). In common with similar studies of human infection (31, 33, 41, 43), the *Campylobacter* genotypes responsible for human disease were highly diverse, with a predominance of genotypes similar to those seen previously. However, investigation of isolates having an ST unassigned to a clonal complex identified 62 isolates of ST-464. This ST was first submitted to the pubMLST *Campylobacter* isolate database from a human isolate cultured in Japan in 2001. A further 29 ST-464 isolates and 54 isolates representing 47 single-, double-, and triple-locus variants of this ST have since been submitted to the database from Canada, China, Europe, Japan, and Thailand (January 2012). Furthermore, ST-464 was isolated from chicken breast meat in the United States in 2002 (51) and ST-464 together with single-, double-, and triple-locus variants from either chicken or human stools in Northern China between 2003 and 2008 (53). On the basis of the above data, ST-464 was assigned as the central genotype of a novel clonal complex. Furthermore, these findings illustrate that the global diversity of *Campylobacter* isolates responsible for human disease remains incompletely understood.

There was evidence for temporal changes in the clonal complexes causing human disease over the 6-year study period: four clonal complexes (ST-42, ST-45, ST-52, and ST-464) increased in relative incidence, whereas three complexes (ST-574, ST-607, and ST-658) decreased (Table 2). The ST-42 complex has been reported to be mainly isolated from ruminants, ST-45 complex from chicken, wild bird, and environmental sources (6, 13, 41, 44), and ST-464 complex predominantly from chickens (21, 51, 53), while the ST-52 complex has a less well-defined host range. Both ST-574 and ST-607 complex isolates are largely chicken associated (21, 41). Therefore, changes in the relative incidence of different clonal complexes did not provide any evidence for changing importance of different reservoirs of human infection. The annual periodicity observed was typical of temperate regions, with a marked summer peak in human disease incidence between June and September (Fig. 3) (26, 38). The most prevalent clonal complexes overall were ST-21, ST-257, ST-828, ST-45, ST-48, and

TABLE 3 Clonal complexes represented by 10 or more isolates, demonstrating a nonrandom association with antibiotic sensitivity or resistance as determined by Pearson's χ^2 or Fisher's exact test[c]

	Ciprofloxacin					Erythromycin				
	Sensitive		Resistant			Sensitive		Resistant		
Clonal complex	No. of isolates	%	No. of isolates	%	*P* value	No. of isolates	%	No. of isolates	%	*P* value
ST-21 complex	531	71.5	212	28.5	0.250	731	98.4	12	1.6	0.718
ST-22 complex	39	84.8	7	15.2	0.026	46	100	0	0	1[a]
ST-42 complex	35	77.8	10	22.2	0.274	45	100	0	0	1[a]
ST-45 complex	213	90.6	22	9.4	<0.001	229	97.5	6	2.6	0.366
ST-48 complex	182	82.7	38	17.3	<0.001	215	97.7	5	2.3	0.556
ST-49 complex	8	47.1	9	52.9	0.041	17	100	0	0	1[a]
ST-52 complex	29	64.4	16	35.6	0.434	45	100	0	0	1[a]
ST-61 complex	57	93.4	4	6.6	<0.001[a]	62	100	0	0	0.628[a]
ST-206 complex	80	59.7	54	40.3	0.010	133	99.3	1	0.8	0.731[a]
ST-257 complex	275	84.9	49	15.1	<0.001	323	99.7	1	0.3	0.041[a]
ST-283 complex	34	91.9	3	8.1	0.002[a]	37	100	0	0	1[a]
ST-353 complex	94	67.6	45	32.4	0.574	138	99.3	1	0.7	0.516[a]
ST-354 complex	28	21.4	103	78.6	<0.001	131	100	0	0	0.172[a]
ST-403 complex	31	91.2	3	8.8	0.004[a]	34	100	0	0	1[a]
ST-443 complex	122	70.1	52	29.9	0.918	170	97.1	5	2.9	0.261
ST-446 complex	3	21.4	11	78.6	<0.001[a]	13	92.9	1	7.1	0.221[a]
ST-460 complex	4	36.4	7	63.6	0.023[a]	11	100	0	0	1[a]
ST-464 complex	14	20.6	54	79.4	<0.001	67	98.5	1	1.5	1[a]
ST-573 complex	13	86.7	2	13.3	0.257[a]	15	100	0	0	1[a]
ST-574 complex	63	61.8	39	38.2	0.074	102	100	0	0	0.262[a]
ST-607 complex	18	42.9	24	57.1	<0.001	42	97.7	1	2.3	0.538[a]
ST-658 complex	48	82.8	10	17.2	0.030	58	100	0	0	0.626[a]
ST-661 complex	19	82.7	4	17.4	0.254[a]	23	100	0	0	1[a]
ST-677 complex	11	100	0	0.0	0.041[a]	11	100	0	0	1[a]
ST-828 complex[b]	170	65.9	88	34.1	0.158	245	95.0	13	5.0	<0.001
UA *C. jejuni* STs	112	53.5	97	46.4	<0.001	208	96.7	7	3.3	0.087
UA *C. coli* STs[b]	10	52.6	9	47.4	0.103	16	84.2	3	15.8	0.004[a]
Total	2,243		972			3,167		57		

[a] Values calculated using Fisher's exact test (values without this superscript were calculated using Pearson's chi-square test).
[b] *C. coli* isolates.
[c] Underlined values have statistically significant *P* values. Data were calculated with Pearson's chi-square test unless noted otherwise. UA, sequence types unassigned to a clonal complex.

ST-443 (Fig. 3), which were also among the most frequently isolated, and of comparable incidence with those in a similar-sized study of human campylobacteriosis in Scotland from 2005 until 2006 (41). These data therefore established that detailed surveillance at a single sentinel surveillance site captures information that reflects that observed nationally.

Evidence of seasonality contributing significantly to the summer peak in disease incidence was observed for three clonal complexes; ST-45 (June), ST-283 (July), and ST-42 (July). This pattern has been reported previously for ST-45 (22, 29, 44) and ST-283 complexes (29) but not for the ST-42 complex. The ST-45 and ST-283 complexes have both been identified as chicken associated (7, 21, 41), and the ST-45 complex has additionally been identified from a wide range of wild animal sources (27), but the ST-42 complex has been predominantly associated with ruminant hosts (6, 13, 21). While MLST data for isolates belonging to ST-45 and ST-283 complexes confirms a recent common ancestor (29), no similarity in genotype or host association was observed between these two complexes and isolates belonging to the ST-42 complex. The present study identified two clonal complexes (ST-353 and ST-403) that peak in relative incidence during the winter (November). The former is predominantly isolated from chickens, as well as cases of human infection, but the latter has a much wider host range which largely excludes poultry (21, 41). The basis for their shared seasonal pattern is therefore unclear and does not appear to be associated with a shared host. Seasonal differences are likely to reflect the *Campylobacter* genotypes that are present in the food chain or, possibly, exposure of the human population to different infection sources.

Rapidly increasing fluoroquinolone resistance in *Campylobacter* strains causing human disease is a matter of public health importance as has been noted previously in the Oxfordshire data set (5). The mean values for ciprofloxacin (30.1%) and erythromycin (1.7%) resistance phenotypes over the 6-year period were similar to those recently reported (5, 25), although even higher levels of fluoroquinolone resistance have been observed (17, 20, 30). Similarly, higher rates of ciprofloxacin-resistant isolates among patients reporting recent travel outside the United Kingdom confirm a previous study (37). The poultry production industry is often seen as the major source of antimicrobial resistance in *Campylobacter* (36).There was a statistically significant association between ciprofloxacin resistance and seven *C. jejuni* clonal complexes; ST-49, ST-206, ST-354, ST-446, ST-460, ST-464, and ST-607 (Table 3). Previous reports have identified an association be-

tween ST-21, ST-206, ST-353, and ST-354 complexes and ciprofloxacin resistance (15, 24, 25, 51), although each of these investigations included fewer than 200 geographically diverse isolates. Furthermore, variation occurred between studies in the methodology and breakpoints used to define ciprofloxacin-resistant strains. Eight of the clonal complexes associated with ciprofloxacin resistance have not been associated with a single reservoir of infection, two (ST-354 and ST-464) are chicken associated (41, 51, 53), and one (ST-206) is predominantly isolated from ruminants (41). Of nine clonal complexes associated with ciprofloxacin sensitivity, two (ST-257 and ST-283) are chicken associated (40) and one (ST-45) has been isolated from a variety of hosts, including poultry (41, 44).

These results suggest a complex acquisition pathway and raise the question whether the food industry, or one subset of it such as poultry production, is a dominating influence in the distribution of ciprofloxacin resistance among campylobacters. Levels of resistance to erythromycin remained static and low (<2%) throughout the study, and the only associations observed were between erythromycin resistance and *C. coli* isolates and between ST-257 complex and erythromycin sensitivity, the former in agreement with reports from others (51). However, unlike fluoroquinolones, the fitness cost to low-level erythromycin-resistant isolates results in a lack of stability in the absence of selective pressure (3, 23), which may in part explain the difference in levels of resistance to these antibiotics in clinical isolates.

The continuing high burden of human campylobacteriosis remains an unresolved public health problem of high importance. Genetic attribution studies have identified the principal source of infection as retail meat, especially chicken, in a number of countries, including the United Kingdom. Molecular data have led to interventions in the poultry industry in New Zealand which have proved to be effective in reducing human disease (40); however, New Zealand poultry production is limited to three major suppliers (33) and the extent to which this will be possible in countries with more complex industries remains unclear. In any case, ongoing surveillance of the genotypes causing human disease is essential if the impact of such interventions is to be measured and understood, particularly as the predominant genotypes in human disease show pronounced changes over time and new genotypes are frequently observed, reflecting the size and diversity of the *Campylobacter* populations that infect chickens. The present study demonstrates that such surveillance is feasible. In a country such as the United Kingdom where nationally distributed foods are the major source of infection, this can be achieved by intensive surveillance at relatively few sites.

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