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Population structure and attribution of human clinical *Campylobacter jejuni* isolates from central Europe to livestock and environmental sources

J. Kovac[1,2] | B. Stessl[3] | N. Čadež[1] | I. Gruntar[4] | M. Cimerman[5] | K. Stingl[6] | M. Lušicky[5] | M. Ocepek[4] | M. Wagner[3] | S. Smole Možina[1]

[1]Department of Food Science and Technology, Biotechnical Faculty, University of Ljubljana, Ljubljana, Slovenia

[2]Department of Food Science, The Pennsylvania State University, State College, PA, USA

[3]Institute of Milk Hygiene, Milk Technology and Food Science, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine, Vienna, Austria

[4]Institute of Microbiology and Parasitology, Veterinary Faculty, University of Ljubljana, Ljubljana, Slovenia

[5]National Laboratory of Health, Environment and Food, Department of Microbiological Analysis of Food, Water and Environmental Samples Maribor, Maribor, Slovenia

[6]Department of Biological Safety, National Reference Laboratory for Campylobacter, Federal Institute for Risk Assessment (BfR), Berlin, Germany

Correspondence
J. Kovac, Department of Food Science, The Pennsylvania State University, State College, PA, USA.
Email: jzk303@psu.edu

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Summary

Campylobacter jejuni is among the most prevalent causes of human bacterial gastroenteritis worldwide. Domesticated animals and, especially, chicken meat are considered to be the main sources of infections. However, the contribution of surface waters and wildlife in *C. jejuni* transmission to humans is not well understood. We have evaluated the source attribution potential of a six-gene multiplex PCR (mPCR) method coupled with STRUCTURE analysis on a set of 410 *C. jejuni* strains isolated from environment, livestock, food and humans in central Europe. Multiplex PCR fingerprints were analysed using Subclade prediction algorithm to classify them into six distinct mPCR clades. A subset of *C. jejuni* isolates (70%) was characterized by multilocus sequence typing (MLST) demonstrating 74% congruence between mPCR and MLST. The correspondence analysis of mPCR clades and sources of isolation indicated three distinct groups in the studied *C. jejuni* population—the first one associated with isolates from poultry, the second one with isolates from cattle, and the third one with isolates from the environment. The STRUCTURE analysis attributed 7.2% and 21.7% of human isolates to environmental sources based on MLST and mPCR fingerprints, respectively.

KEYWORDS
Campylobacter jejuni, fingerprinting, MLST, mPCR, population structure, source attribution

1 | INTRODUCTION

Campylobacter jejuni, transmitted via undercooked poultry meat, chicken liver paté and contaminated raw milk, is a major cause of foodborne diarrhoeal illness, which affects humans worldwide (Kaakoush, Castaño-Rodríguez, Mitchell, & Man, 2015; Lahti, Löfdahl, Ågren, Hansson, & Olsson Engvall, 2017). Despite domesticated animals, particularly poultry and cattle, being recognized as the main sources of infection (Levesque et al., 2013; Schallegger et al., 2016; Sheppard et al., 2009), the role of water and wildlife as sources and transmitting agents is not yet well understood (Cody et al., 2015; Hald et al., 2016; Skarp, Hänninen, & Rautelin, 2016). Environmental surface water is commonly contaminated with *Campylobacter* due to the sewage discharge, as well as domestic and wild animal faecal input (Marcheggiani et al., 2015; Rechenburg & Kistemann, 2009; Sales-Ortells, Agostini, & Medema, 2015a; Sales-Ortells & Medema, 2015b). It is therefore not surprising that the consumption of untreated water has been implicated in multiple outbreaks of campylobacteriosis over the past years (de Fraites et al., 2014; Gubbels et al., 2012; Kaakoush et al., 2015; Taylor et al., 2013). The gastrointestinal disease risk from oral intake of *C. jejuni*-contaminated surface water increases during the warmer season, after heavy rainfall and storms

in coastal areas, as the run-off water from large urban and agricultural areas raises the pathogen load (Rechenburg & Kistemann, 2009; Sales-Ortells & Medema, 2015b; Soneja et al., 2016). These events present an opportunity for transmission of environment-adapted genotypes to livestock and humans.

Some *C. jejuni* genotypes (e.g. MLST sequence type (ST) 45) seem to be better adapted to the environment, wild birds and to the water, presumably due to their better ability to survive outside the host (Cools et al., 2003; González & Hänninen, 2012; Sopwith et al., 2008), however their rapid host switching impedes the traceback of human infection to sources (Dearlove et al., 2016). To improve the source attribution signal, it is imperative to identify genomic markers that carry strong source signal. In a comparative phylogenomic study of the presence/absence of 1,574 predicted coding sequences, several genetic markers predictive of infection source have been identified (Champion et al., 2005). In a follow-up study, a six-gene multiplex PCR (mPCR) fingerprinting method indicative of environmental or domesticated livestock source has been developed based on genome-wide gene presence/absence data. Initial validation of mPCR demonstrated clustering of environmental isolates within mPCR clade C9ii, distinguishing them from isolates originating from livestock (Stabler et al., 2013). Methods such as this one have a potential to be used (i) for initial screening of large isolate sets to guide selection of isolates for further downstream sequence-based analyses and (ii) for genotyping and source attribution in lower-income countries where sequencing is not readily available. Here, we have used this high-throughput mPCR method to determine a population structure of 410 C. *jejuni* strains isolated from diverse sources in three Central European countries (Slovenia, Austria and Germany) and to estimate the proportion of human clinical isolates that may originate from environmental sources based on (i) their classification into environment-specific clade C9ii, and STRUCTURE source attribution using (ii) mPCR fingerprints and (iii) MLST allelic types.

Impacts

- mPCR clade data can be used to structure *Campylobacter jejuni* population in three groups associated with source of isolation;
- mPCR fingerprints were congruent with multilocus sequence types in 74% of the cases;
- 21.7% and 7.2% of human isolates were attributed to environmental sources with STRUCTURE based on mPCR fingerprints and MLST, respectively.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains

The 410 *Campylobacter jejuni* isolates originating from domesticated livestock sources (chicken, *n* = 210; turkey, *n* = 18; cattle, *n* = 44), environmental sources (surface waters, *n* = 12; wild birds, *n* = 15) and human clinical cases (*n* = 111) were included in this study. These isolates have been previously obtained from strain collections of the Biotechnical and Veterinary faculties at the University of Ljubljana, Slovenia, the National Institute of Public Health (NLZOH), Slovenia, the Austrian Agency for Health and Food Safety (AGES), the Institute of Milk Hygiene, Milk Technology and Food Science (Vetmeduni Vienna), Austria, and the Federal Institute for Risk Assessment (BfR), Germany, and were available for typing. Strains were isolated between 2002 and 2014, within the framework of national monitoring of retail meat and production animals, the monitoring of human clinical cases and other national and EU research projects. Data on epidemiological link between studied isolates were not available. The full list of these *C. jejuni* isolates is provided in the Supporting Information, along with the genotype and source information (Table S1).

2.2 | Bacterial growth conditions and DNA extraction

Frozen stocks (−80°C) of *C. jejuni* isolates were cultured on selective Karmali agar (Oxoid, Hampsire, UK) and incubated at 42°C for 24 h under microaerobic conditions (5% O_2, 10% CO_2 in N_2). Their DNA was extracted using PrepMan Ultra Sample Preparation Reagent (Applied Biosystems, Foster City, USA), according to manufacturer's protocol.

2.3 | Molecular species confirmation and multiplex PCR (mPCR) fingerprinting

C. jejuni species was confirmed by amplification of the 23S rRNA and *hipO* genes, according to Wang et al. (2002). PCR amplicons were visualized by gel electrophoresis on 1.5% agarose gels (Sigma, St. Louis, MO, USA).

The *C. jejuni* subtyping was performed by mPCR according to Stabler et al. (2013). In detail, six source-discriminatory genes were amplified in two 20-μL mPCR reactions (M1, M2), using 1 μL DNA template and QIAGEN multiplex PCR kits with Q-solutions (Qiagen, Venlo, Netherlands). Reaction M1 amplified genes *Cj0056c* (hypothetical protein), *Cj1139c* (beta-1,3 galactosyltransferase) and *Cj1422c* (putative sugar transferase), and reaction M2, genes *Cj0485* (putative oxidoreductase), *Cj1324* (hypothetical protein) and *Cj1720* (hypothetical protein). The amplicons were visualized by gel electrophoresis on 2% agarose gels (Sigma). The reference strain NCTC 11168 served as a positive control with all target genes present, and PCR water with no DNA template was used as a negative control. The obtained mPCR fingerprints represented a combination of positive (1) and/or negative (0) mPCR reactions.

2.4 | Multilocus sequence typing (MLST)

The multilocus sequence types (ST) were determined for a subset of isolates (*n* = 287; 70%) following the previously described MLST protocol (Dingle et al., 2001). Briefly, seven housekeeping genes (*aspA*, *glnA*,

gltA, *glyA*, *pgm*, *tkt* and *uncA*) were amplified using GoTaq polymerase (Promega). The purified PCR products were sent for bidirectional Sanger sequencing (Macrogen, South Korea), and the Bionumerics version 7.1 MLST plugin (Applied Maths NV, Sint-Martens-Latem, Belgium) was used for sequence editing, MLST profile identification and minimum spanning tree construction. The MLST profiles identified in this study were deposited at the PubMLST database at http://pubmlst.org/campylobacter/.

2.5 | mPCR Subclade prediction

The Subclade prediction algorithm was applied to classify *C. jejuni* isolates into mPCR clades based on the mPCR fingerprints (http://onlinelibrary.wiley.com/doi/10.1111/1462-2920.12111/suppinfo; Stabler et al., 2013). In some cases, the algorithm was unable to cluster the fingerprint input unequivocally; therefore, the mPCR clades are presented as combined, such as C1/C2/C3, C4/C6 and C7/C8.

2.6 | Source attribution

Source attribution of 69 human *C. jejuni* isolates of 287 isolates with determined mPCR fingerprints and MLST sequence types was performed with STRUCTURE 2.3.4 (Pritchard, Stephens, & Donnelly, 2000) based on (i) mPCR fingerprints and (ii) MLST allelic types. Assignment of the individuals to one of the three pre-defined populations (i.e. environment, poultry, cattle) was carried out using the USEPOPINFO option, where the individuals with known sources (218 of 287) were used as a training set, and the source of the individuals from human clinical cases was assumed to be unknown. Simulations were performed with 100,000 Markov Chain Monte Carlo (MCMC) iterations and 1,000 burn-in periods. The no-admixture model (GENSBACK = 2, MIGPRIOR = 0) was applied to test for migrants, and the allele frequencies were assumed to be independent ($\lambda = 1$) (McCarthey et al., 2007).

2.7 | Statistical analysis, discriminatory power and congruence between mPCR and MLST

The Simpson's index of diversity was calculated to determine the discriminatory power (Hunter & Gaston, 1988), and Adjusted Wallace coefficient (Severiano, Pinto, Ramirez, & Carriço, 2011) for assessing the level of congruence between mPCR and MLST based on mPCR fingerprints and MLST sequence types. Null hypothesis of overall even distribution of MLST clonal complexes among mPCR clades, and isolate sources among mPCR clades were tested using chi-square or Fisher's exact test, when appropriate, using R version 3.1.2 (R Core Team, 2015). Statistical relevance was set at $p < 0.05$. If null hypothesis was rejected, further individual chi-square or Fisher's exact tests were performed testing for significance of specific categorical associations for categories with > 5 representatives. The p values of these further tests were corrected for multiple comparisons using Bonferroni correction. The association of specific isolate sources with mPCR clades was evaluated and visualized based on correspondence analysis in Orange data mining tool (Demšar et al., 2013).

3 | RESULTS

In the present set of 410 *C. jejuni* isolates, we identified 39 different mPCR fingerprints that were assigned to clades C1 to C9ii using Subclade prediction algorithm (Stabler et al., 2013). The clades C1/C2/C3, C4/C6 and C7/8 were predicted as combined clades (Table 1), as the algorithm did not always distinguish among them based on the fingerprint input. Clade C9i was by far the most prevalent (*n* = 122; 30%), followed by C1/C2/C3 (*n* = 74; 18%), C5 (*n* = 73; 18%), C4/C6 (*n* = 48; 12%), C7/C8 (*n* = 64; 16%) and C9ii (*n* = 29; 7%).

MLST genotyping of a subset of 287 *C. jejuni* resulted in identification of 88 different STs that were assigned to 22 clonal complexes. A total of 36 *C. jejuni* isolates with 13 different STs could not be assigned to any existing clonal complexes. The most frequent sequence type was ST 5205 (*n* = 34; clonal complex ST-353), which has been identified for the first time in our past study (Kovač et al., 2014) and is so far unique to Slovenia (Kovač et al., 2015). The second most prevalent sequence type was ST 50 (*n* = 33), which belongs to the widespread clonal complex ST-21. Isolates from this study were most frequently assigned to clonal complexes ST-21 (*n* = 85) and ST-353 (*n* = 47).

3.1 | Congruence and discriminatory power of mPCR fingerprints and MLST sequence types

The discriminatory power of mPCR fingerprints was somewhat lower (0.927) than that of MLST sequence types (0.958) according to the

TABLE 1 Distribution of *Campylobacter jejuni* isolates (*n* = 410) from different sources among mPCR clades

mPCR clade	Human *n* (%)	Environment *n* (%)	Livestock *n* (%)	Total *n* (%)
C1/2/3	17 (15.3)	1 (3.7)	56 (20.6)	74 (18.0)
C4/6	22 (19.8)	1 (3.7)	25 (9.2)	48 (11.7)
C5	19 (17.1)	1 (3.7)	53 (19.5)	73 (17.8)
C7/8	26 (23.4)	1 (3.7)	37 (13.6)	64 (15.6)
C9i	26 (23.4)	10 (37.0)	86 (31.6)	122 (29.8)
C9ii	1 (0.9)	13 (48.1)	15 (5.5)	29 (7.1)
Total	111 (27.1)	27 (6.6)	272 (27.1)	410 (27.1)

Simpson's index of diversity. Similarly, lower discriminatory power was achieved using mPCR clades (0.807) than with MLST clonal complexes (0.824). The two methods reached relatively high congruence level according to the Adjusted Wallace coefficient (0.738; Fig. 1). Independence of the mPCR clades and MLST clonal complexes was rejected by the chi-square test (p-value < 0.001). Significant associations ($p < 0.05$ after Bonferroni correction for multiple comparisons) were confirmed between seven clonal complexes and mPCR clades (e.g. ST-206 and C4/6; ST-21, C1/2/3 and C5; ST-353 and C9i, ST-354 and C1/2/3, C7/8; ST-45 and C9ii).

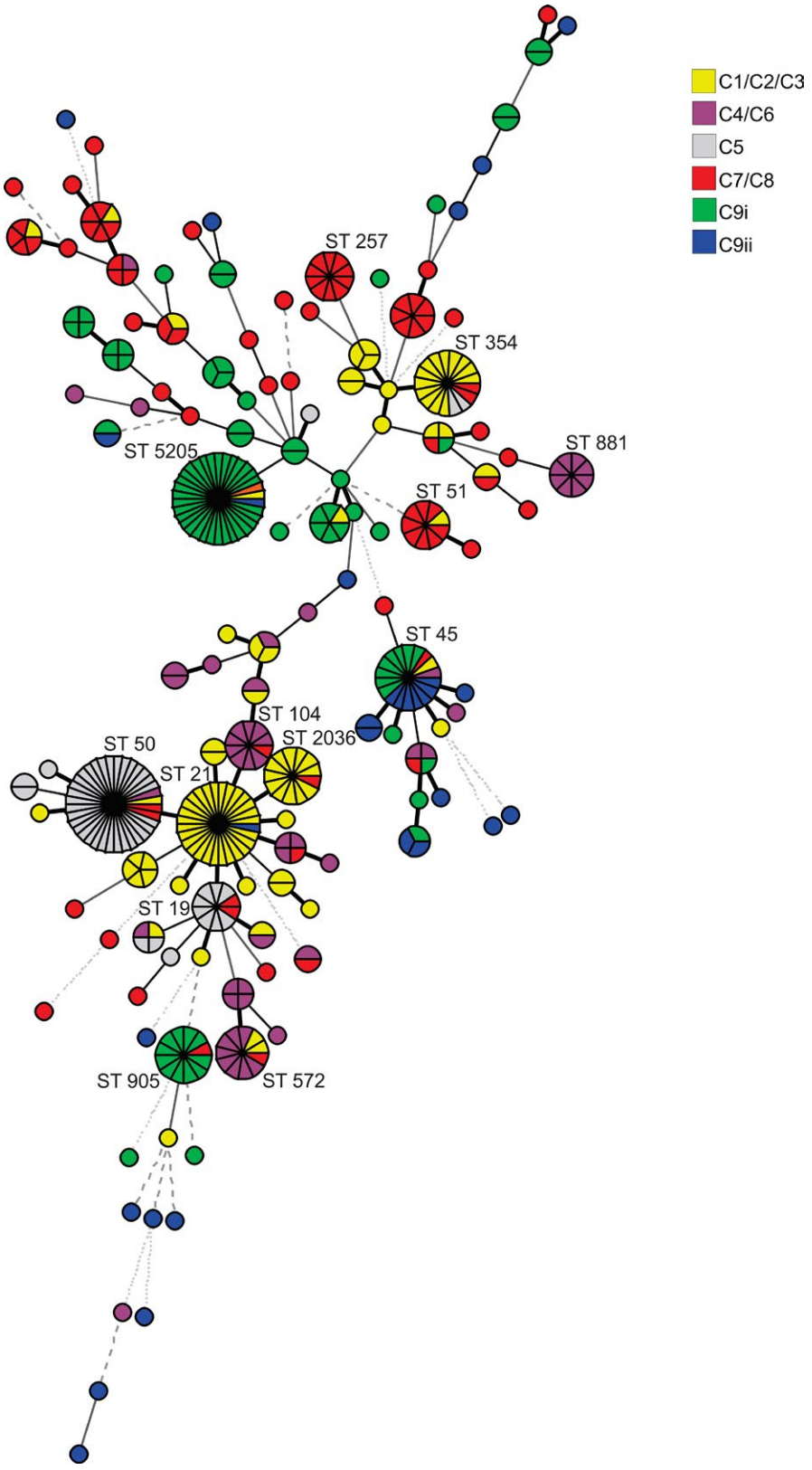

FIGURE 1 MLST minimum spanning tree demonstrating the congruence between MLST sequence types and mPCR clades. The minimum spanning tree was built based on the allele types of the seven MLST housekeeping genes, for 287 *C. jejuni* isolates. Circles represent isolates of the same sequence type (ST), and each isolate is colour-coded according to the mPCR clade. [Colour figure can be viewed at wileyonlinelibrary.com]

3.2 | Population structure

Isolate sources were not equally distributed among mPCR clades ($p < 0.001$). The majority of the environmental *C. jejuni* isolates in this study ($n = 13/27$; 48%) was assigned to clade C9ii ($p < 0.001$), suggesting possible adaptation of this genotype to the water environment and wildlife. However, the cause of these associations is not yet known. The second most common mPCR clade to which environmental isolates were assigned was C9i ($n = 10/27$; 37%). Fingerprints from clade C9ii were rarely observed among livestock (5.5%) and human isolates (0.9%), while fingerprints from C9i were commonly found in both livestock (31.6%) and human (23.4%) isolates. This may be due to an impaired ability of genotype C9ii to colonize a gut; however, this hypothesis needs to be further investigated (Table 1). The common fingerprint of the clade C9ii [000001] was not identified among human clinical isolates (Table S1).

Majority of livestock isolates ($n = 86$; 31.6%) were assigned to clade C9i, followed by C1/2/3, C5, C7/8, C4/6 and C9ii (Table 1), while human isolates were more evenly distributed among these clades, with exception of clade C9ii to which only one human isolate was assigned. Livestock and human isolate groups were not significantly associated with any of the mPCR clades, although clades C1 to C6 have been previously described as livestock-associated clades (Champion et al., 2005; Stabler et al., 2013). Nevertheless, within the livestock group, isolates from cattle were associated with clades C1/2/3 ($p = 0.002$) and isolates from chicken with clade C9i ($p = 0.01$). Two fingerprints from clade C9i, 000100 ($n = 35$) and 000101 ($n = 17$) were found in human, environmental and livestock isolates. Ten of 12 (83%) isolates with the fingerprint 000100 that was MLST typed were classified into MLST clonal complex ST-45, which has a broad host range that may obstruct the source attribution (e.g. Dearlove et al., 2016; Sheppard et al., 2014). Most environmental isolates assigned to clade C9i carried this fingerprint, which may have influenced different source attribution using mPCR fingerprints and MLST allelic types described in the following paragraph. Seven of 12 (58%) isolates with the fingerprint 000101 that were MLST typed belonged to clonal complex ST-353, which seems to be adapted to chicken host (e.g. Sheppard et al., 2014).

Correspondence analysis (CA) was carried out based on source and mPCR clade data in Orange. The environmental water isolates were clearly separated from chicken and cattle isolates based on the CA. The analysis also indicated the association of environmental water and wild bird isolates with clade C9ii, and the remaining nine clades with domesticated livestock sources, including chicken and cattle (Fig. 2). Based on CA, the human isolates were plotted in the close proximity of chicken source of isolation, indicating the association between them (Fig. 2).

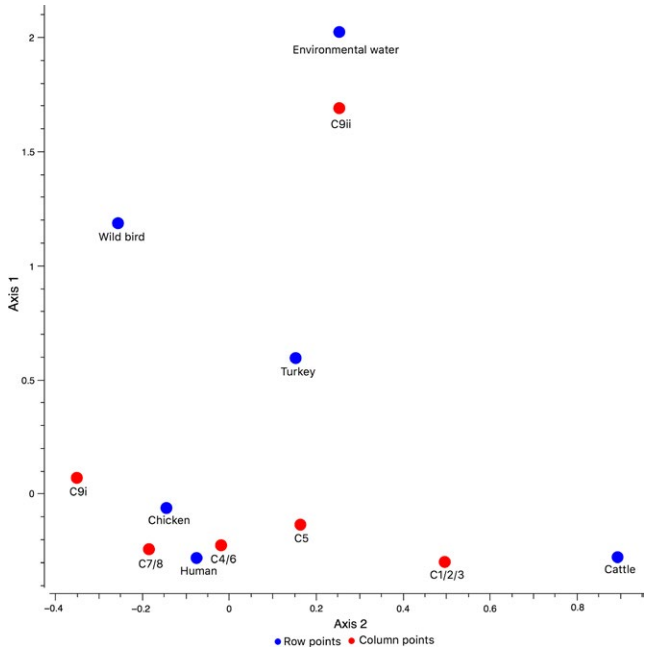

FIGURE 2 Population structure of *C. jejuni* isolates from different sources based on the mPCR clades. The figure demonstrates the structure of *C. jejuni* population ($n = 410$). Associations between isolates from different sources and mPCR clades were visualized based on correspondence analysis in a plot that shows a global similarity pattern in the data. The dimension 1 (Axis 1) contributes 24.5% and dimension 2 (Axis 2) 60.8% to the total variance. The points that are closer to each other are similar in terms of relative frequencies across the columns or rows of a source-genotype contingency table; the distance between any isolate source (marked in blue) and mPCR clade (marked in red) points therefore denotes a measure of their association. [Colour figure can be viewed at wileyonlinelibrary.com]

3.3 | Source attribution

We used STRUCTURE for source attribution using a subset of 287 (218 with known source and 68 with unknown [human] source) isolates that had both mPCR fingerprints and MLST determined. This allowed for comparison of results obtained using these two typing methods. Based on CA results, we have grouped 218 environmental and livestock isolates into i) poultry (chicken and turkey), ii) cattle and iii) environmental (water and wild bird) population classes and used them as a training set for source attribution of 69 human clinical isolates with STRUCTURE based on (i) mPCR fingerprints or (ii) MLST allelic types. We attributed 37.7, 40.6 and 21.7% of human isolates to poultry, cattle and environmental sources based on mPCR fingerprints, respectively (Table S2). In an independent analysis based on MLST allelic types, we have attributed 58.0, 34.8 and 7.2% of isolates to poultry, cattle and environmental sources, respectively. Attribution of four out of 15 isolates to environmental source was consistent using mPCR fingerprints and MLST allelic types. These isolates belonged to clonal complexes ST-45, ST-22 and ST-362, while isolates attributed to environmental sources just based on mPCR belonged to clonal complexes ST-446, ST-257, ST-49, ST-354, ST-353 and ST-206.

4 | DISCUSSION

Human *Campylobacter* infections are causing extensive economic and public health burden (Kaakoush et al., 2015). The understanding of

the infection source of this pathogen with a sporadic nature is essential for implementation of effective measures to decrease the disease exposure. Although poultry is currently recognized as the main source of campylobacteriosis, isolates from up to 10% of disease cases have been estimated to originate from environmental sources and wildlife (*e.g.* Gras et al., 2012; Mughini-Gras et al., 2016). Evaluating the relative contribution of different sources to the overall campylobacteriosis burden is therefore important to form effective control strategies. Here, we have used a six-gene multiplex PCR (mPCR) method, which produces fingerprints indicative of *C. jejuni* isolates source, to estimate the proportion of human clinical isolates associated with environmental sources such as surface waters and wild birds.

The discriminatory power of mPCR was, as expected, lower compared to the MLST, yet sufficient enough to structure the *C. jejuni* population in three distinct groups as demonstrated by the correspondence analysis. The two methods were also found to be moderately congruent. As expected, the human isolates were most closely associated with chicken source according to correspondence analysis based on mPCR clades.

Approximately half of the isolates from environmental sources included in this study were assigned to mPCR clade C9ii (nine from water and four from wild birds), approximately one-third to C9i (one from water and nine from wild birds) and one isolate to each, C1/2/3, C4/6, C5 and C7/8. Overall, higher proportion of wild bird isolates were assigned to C9i, compared to C9ii. Clade C9i was found to be associated with chicken source, suggesting that wild birds may be important factor in transmission of *C. jejuni* isolates from poultry farms to environmental waters or *vice versa*. This has a potential to complicate isolate source attribution, as poultry prone to *Campylobacter* infections can serve as a vehicle for dissemination of environmental genotypes. A recent study investigating the source of *Campylobacter* contaminating surface waters based on MLST and asymmetric island model found that majority (61.0%) of *Campylobacter* surface water isolates in Luxembourg originate from wild birds, whereas in Dutch waters from poultry (51.7%), which may reflect the magnitude of local poultry production (Mughini-Gras et al., 2016). Another Danish study supported this hypothesis by finding associations between prevalence of *C. jejuni* in wild birds and in the manure on poultry and cattle farms (Hald et al., 2016). The risk posed by wild birds may be greater in warmer months, as these months were significantly associated with higher prevalence of *C. jejuni* in United Kingdom (Cody et al., 2015).

Based on the number of human clinical isolates carrying environmental clade C9ii-specific fingerprint, only 0.9% of human isolates from our study were attributed to environmental waters and wildlife. Low attribution of human isolates to the environmental source using mPCR clades likely arises from the fact that approximately half of the environmental isolates from our data set belong to mPCR clades other than C9ii (especially C9i), which may have lead to underestimation of association between human and environmental genotypes using this approach.

The second, STRUCTURE-based source attribution approach we took in the present study resulted in different proportions of isolates attributed to environmental sources using mPCR fingerprints (21.7%) compared to MLST allelic types (7.2%). Comparing mPCR- and MLST-based source attribution with STRUCTURE showed consistent source attribution to environmental source in just four of 15 cases. These four isolates belonged to clonal complexes ST-45, ST-22 and ST-362, while isolates attributed to environmental sources only based on mPCR fingerprint belonged to clonal complexes ST-446, ST-257, ST-49, ST-354, ST-353 and ST-206. Clonal complex ST-45 is recognized as a generalist that is not specialized for any specific source, and isolates of this genotype can confound the source attribution (e.g. Dearlove et al., 2016; Sheppard et al., 2014). Similarly, isolates from clonal complex ST-22 reported in PubMLST database (n = 501; as of 30 October 2016) originated mostly from human, chicken, cattle, dog and environment. Isolates from clonal complex ST-362 were strongly underrepresented in the PubMLST database (n = 58; as of 30 October 2016); the vast majority of them were isolates from humans, and chicken was reported as the only non-human source of isolation. It is important to note, though, that the isolate population in the database does not reflect the true prevalence and source distribution in the environment due to the sampling and reporting biases. The differences between source attribution based on mPCR and MLST may also arise from the small proportion of environmental isolates compared to livestock isolates in a training set that we used, which makes source attribution less robust.

Other studies have attributed 4, 7.4, 10.1 and 12% of human isolates to environmental sources in United Kingdom, Netherlands, Canada and New Zealand, respectively, based on the MLST typing and using the asymmetric island model or STRUCTURE (Gras et al., 2012; Levesque et al., 2013; Mullner et al., 2009; Sheppard et al., 2009). Italian study attributed 69.75% of 56 *C. jejuni* isolates to chicken source, 8.25% of isolates to cattle and 6.28% of isolates to environmental sources using asymmetric island model (Di Giannatale et al., 2016). The same model was used also in a recent study from New Zealand, in which 55, 38 and 7% of human clinical isolates were attributed to ruminants, poultry and environmental water, respectively (Nohra et al., 2016).

All isolates attributed to environmental source in our study had variations of mPCR profile with missing *cj1422c*, which is also characteristic of clade C9ii. Besides *cj1422c* (putative sugar transferase), analysed isolates with determined C9ii genotype did not carry genes *cj0485* (putative oxidoreductase), *cj1139c* (beta-1,3 galactosyltransferase) and *cj1324* (hypothetical protein). The role of these genes in the survival in different environments (e.g. inside or outside of the host) is not yet well understood, however they do seem to be more prevalent in isolates that have been isolated from different animal or human hosts. One of these genes, *cj1139c* seems to be subjected to phase variation during the chicken colonization and has been previously demonstrated to impact the colonization of mice, which has been used as a model for human infection (Wilson et al., 2010). The hypothetical protein encoded by *cj1324* has been found in O-linked glycosylation locus *cj1239-cj1342* and is required for legionaminic acid modification of flagellin. Mutants defected in this gene have been demonstrated to be less capable of chicken gut colonization and biofilm formation; however, they were not attenuated in *Galleria mellonella* larvae model

(Champion et al., 2010; Howard et al., 2009). Absence of these genes in C9ii isolates may be associated with impaired ability for host colonization and possibly decreased virulence, however not enough experimental evidence exist to support this hypothesis.

In conclusion, mPCR fingerprints can structure the *C. jejuni* population in the environmental and domesticated poultry and cattle livestock clusters and can be used to infer the source of human isolates. We attributed 0.9, 21.7 and 7.2% of human clinical *C. jejuni* isolates to environmental source using mPCR clade classification, and mPCR fingerprint and MLST allelic type-based STRUCTURE source attribution, respectively. As source attribution is sensitive to the data set biases, further validation of mPCR-based source attribution is needed on more source-balanced data sets.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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