Persistence of *E. coli* O157:H7 in urban recreational waters from Spring and Autumn: a comparison analysis

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Abstract
People might get infected by pathogens found in urban recreational waters during water-contact activities, such as swimming, boating, bathing, and yachting. However, the persistence of pathogenic bacteria in those waters was not well documented. In this study, persistence of *E. coli* O157:H7 (EcO157) in 48 water samples (24 Spring samples and 24 Autumn samples) from the 3 urban recreational waters was investigated. Multivariate statistical analysis was performed to correlate survival data with water physicochemical properties and bacterial communities. Our data showed that EcO157 survived longer in Spring samples than in Autumn samples regardless of the lakes. Results revealed that recreational water physicochemical properties and bacterial community in Spring samples were different from those in Autumn samples. Mantel and Partial Mantel tests, as well as co-occurrence network analysis illustrated that EC salinity, TOC, and bacterial community were correlated with survival time (*ttd*) (*p* < 0.05). Variation partition analysis (VPA) indicated that bacterial community, EC, TOC, and TN explained about 64.81% of overall *ttd* variation in Spring samples, and bacterial community, EC, pH, and TP accounted for about 56.59% of overall *ttd* variation in Autumn samples. Structural equation model (SEM) illustrated that EC indirectly positively affected *ttd* through bacterial community. The correlation between bacterial community and *ttd* was negative in Spring samples and positive in Autumn samples. TN appeared a direct positive effect on *ttd* in Spring samples. TP displayed a direct negative effect on *ttd* in Autumn samples. Our results concluded that there was seasonal variation in environmental factors that directly or indirectly affected the survival of EcO157 in urban recreational waters.

Keywords *E. coli* O157:H7 · Persistence · Bacterial community · Urban recreational water · Swimming · Seasonal variation

Introduction

*E. coli* O157:H7 (EcO157) is a pathogenic, rod-shaped, gram-negative, and Shiga toxins–producing bacterium belonging to phylum *Proteobacteria*. EcO157 was firstly identified during the investigation of a major gastrointestinal illness outbreak caused by undercooked hamburger patties in the USA (Riley et al. 1983). The infectious dose of EcO157 was very low, and as low as 10 cells were thought to be enough to cause infection in human beings (Bach et al. 2002). When get infected by this pathogen, people could display typical clinical symptoms including bloody diarrhea, vomiting, which could ultimately develop into hemolytic uremic syndrome (HUS) (Riley et al. 1983). It has been reported that in the USA alone, over 73,000 cases of human infection with EcO157 occur every year (Nataro and Kaper 1998). In 1999, an outbreak of EcO157 infection occurred in Jiangsu, China, over 20,000 people were infected, and among them 177 died (Cookson et al. 2006). EcO157 infections via waterborne transmission were also frequently documented, e.g. the beach bathing area near Rome in central Italy contaminated by a nearby sewage treatment plant posed a threat to the safety of tourists (O’Flaherty et al. 2019). It can be seen that the investigation of EcO157 infection is necessary to protect human health.
The major origin of EcO157 strains was cattle, and the count of this pathogen in the feces of super shedders could be as high as $10^6$ CFU (colony forming units)/g (Fukushima and Seki 2004). Other warm-blooded animals including pets, birds, sheep, as well as cold-blooded animals such as snakes, frogs, and fish (Ferens and Hovde 2011) were also potential EcO157 carriers. Animal manure containing EcO157, if improperly disposed or composted, could release this pathogen into other aqueous environment (e.g. reservoirs, lakes, and ponds) through wind and surface runoff (Halford et al. 2021), thereby causing EcO157 water contamination. Untreated sewage containing EcO157 from surrounding hospitals, farms, and residential areas, if directly discharged into water, would greatly increase the risk of human infection (Lopes et al. 2020). Plenty of evidence have indicated that EcO157 has a strong ability to survive in water. It has been demonstrated that EcO157 can survive for weeks and up to several months in water (Rice et al. 1992). Under strong rainstorm conditions, sediments could be resuspended and EcO157 concentrations might increase rapidly in overlying water (Stott et al. 2011; Chavez-Diaz et al. 2020). Obviously, waterborne transmission will increase the risk of human infection. Thus, it is of great significance to study the persistence of EcO157 in water environment to reduce the risk of human infection.

Urban recreational waters are used for leisure and entertainment and they are also the main places for citizens to take water activities. Recreational waters were considered to be prone to pathogen infections and outbreaks of EcO157 due to human recreational activities, such as swimming, fishing, boating, surfing, and water playgrounds (Swinscoe et al. 2018). Beaches and swimming areas overcrowded with people could cause large-scale infections (von Sperling and von Sperling 2013). The reports of recreational water outbreaks among pools and lakes have never been decreased (Hammer et al. 2019; Perebom et al. 2018). The sources of fecal pollution might be untreated sewage from upstream farms and industries, which polluted recreational waters through runoff (Hu et al. 2018). Sewage carrying EcO157 originated from camping, toilets and catering near recreational waters, if discharged without disinfection, might cause contamination (Zhao et al. 2014). Lake water agitated by runoff, rainfall, and rowing might aggravate the pollution diffusion. In addition, contaminated groundwater was also a potential pathway to contaminate urban recreational waters (Alm et al. 2003). Infection might be caused by swallowing water in contact recreation activities such as boating, and swimming (Dufour et al. 2017). As a potential biohazard, EcO157 could enter into the recreational waters in populous metropolitan area via different pathways as discussed above. Therefore, it is worthwhile to investigate its environmental behavior in the environment.

The outbreak of EcO157 might have seasonal characteristics (Ailes et al. 2008). Previous studies reported the prevalence of this pathogen in countries with temperate climates in late Spring and early Fall (Hermos et al. 2011), suggesting seasonal variation in EcO157 infection. However, the seasonal variation of EcO157 in recreational water has been rarely studied. In northeast Changchun City, China, Spring and Autumn were the peak seasons for water recreational activities, including swimming, boating, yachting, and bathing. In winter, there are few visitors because the temperature is too low and lakes are frozen. Consequently, in order to protect public health, it is useful to investigate the survival profiles of EcO157 in different seasons in urban surface recreational waters.

In our study, 48 samples were collected from Nanhu Lake, Jingyue Lake, and Beihu Lake in Changchun City, respectively. The sampling time was October 2018 (early Autumn) and May 2019 (late Spring). These lakes are primary urban recreational waters for Changchuners. The vegetation of Nanhu Lake, Jingyue Lake, and Beihu Lake area is lawns and artificial forest. In addition, some restaurants and shopping areas have been built there to satisfy the need of the booming tourism (Cao et al. 2021). Nanhu Lake, Jingyue Lake, and Beihu Lake serve a population of 7,580,000, 764,000, and 663,000, respectively (Zhao and Qin 2017). The recreational waters are managed in accordance with Environmental Quality Standards for Surface Water Class III in China (GB 3838–2002), which is compatible for recreational purpose. The source of pollution might be sewage from surrounding restaurants and public toilets. Other pollution sources might also include nearby surface runoff, as well as sewage from upstream residential areas (Liang 2013). Pollutants might easily accumulate in these recreational waters due to poor mobility and self-purification of the lakes (Liu 2005). Unfortunately, there were no health instructions and specific advisories for water-contact activities (Cao et al. 2021) due to lack of adequate background information, e.g. persistence data of pathogens in those waters. Therefore, the purposes of this study were to, (1) compare physicochemical properties, bacterial community and EcO157 persistence in urban recreational waters between Spring and Autumn, and (2) reveal the possible mechanisms causing the seasonal variation in EcO157 persistence in urban recreational waters in NE China.

Materials and methods

Water sampling and characterization

According to China Meteorological Network of National Bureau of China (http://www.cma.gov.cn), the mean temperature, precipitation, and sunshine duration of Spring in
Changchun City were 9.6 °C, 32.8 mm, and 7.9 h, while the values for Autumn were 7.7 °C, 43.4 mm, and 6.4 h. A total of 48 water samples were collected in Spring and Autumn from three major urban recreational waters in Changchun City, including Nanhu Lake (NH), Jingyue Lake (JY), and Beihu Lake (BH). Eight samples were respectively obtained in Autumn 2018 and Spring 2019 from each of the lakes. The sampling sites in Spring and Autumn were the same. The specific longitude and latitude coordinates were shown in Table S1. Distribution of those lakes and their sampling stations was shown in Fig. S1. Water samples were collected at 0.2 m below the surface of the lakeshore with a fixed-depth water sampler. A total of 5-L water sample was collected from each site. The samples were transported to the laboratory on ice within 8 h. 1-L sample was saved under 4 °C temporarily for survival study. 2-L water sample was filtered through 0.22-μm membrane, and the samples were saved in −80 °C fridge for community DNA extraction. Water physicochemical properties were determined with well-developed methods (see below). TN (total soluble nitrogen) was qualified by potassium persulfate oxidation-double wavelength spectrophotometry method. NH4+–N (ammonia nitrogen) was qualified by Nessler reagent spectrophotometry. TP (total phosphorus) was measured by molybdenum-antimony-ascorbic acid spectrophotometric. TOC (total organic carbon) was measured by a total organic carbon analyzer (TOC-VCPH, Shimazu, Japan). The EC (electrical conductivity) was determined by s conductivity meter (DDS-11A, Rex, China). The pH was determined by a pH meter (FE20, Mettler, China).

Bacterial strains

The strain used in this experiment was E. coli O157:H7 EDL931 (ATCC 35150), conferring toxic genes including stx1, stx2, and eae. The EDL931 was originally obtained from human feces (Beery et al. 1984). The EDL931 wild type was tagged with rifampicin resistance for ease of counting (Ma et al. 2011).

Survival experiment of E. coli O157:H7

The EcO157 cells were initially inoculated in LB liquid medium and incubated at 37 °C with shaking (220 r·min−1) for 15 h. The cells were harvested by centrifugation at 4 °C, 18,000 g for 10 min. Then, the cell pellet was washed three times by 0.9% sodium chloride buffer, and finally resuspended in sterile deionized water. The cells were then starved for 2 h in dark under 4 °C. The starved cells were then inoculated into lake water samples, and the concentration of EcO157 solution was about 1.0×108 CFU/mL. The experiments were conducted at room temperature of 20 ± 1 °C. The water samples were exposed to household illumination fluorescent lamp (TL8/36 W, Philips, China) on room ceiling with light intensity being about 100 Lux at bench top height (10 h light alternated with 14 h dark). The inoculated water samples were sampled periodically, and subjected to tenfold serial dilution. Fifty microliters of the two highest dilutions were plated in duplicate onto the SMAC (sorbitol MacConkey) agar (Lab M, Lancashire, UK) containing 100 mg/L of rifampicin (Ma et al. 2011). The plates were incubated at 37 °C for EcO157 counting. The detailed process of counting could be found in our previous publications (Han et al. 2021).

Survival data modeling

Modeling EcO157 survival was achieved by fitting the experimental data to the Weibull model using GInaFiT Excel add-in (Geeraerd et al. 2005). The model was established based on the assumption that the survival of EcO157 follows the Weibull distribution. The number of the survivors can be quantified by using the following equation:

\[ \log(N_t) = \log(N_0) - (t/\delta)^p \]

where \( N_0 \), \( N_t \), and \( t \) represent survivor counts, inoculum size, and inoculation time, respectively. The \( \delta \) is scale parameter representing the time needed for the first decimal reduction, \( p \) is the shape parameter. Survival curves display a convex, concave, and linear shape when \( p > 1 \), \( <1 \), and \( =1 \), respectively. The survival time (\( t_{50} \)) represents time (days) needed to reach the detection limit could also be obtained by fitting the Weibull model.

Water DNA extraction, sequencing, and sequencing data processing

Water community DNA was extracted from prepared fiber membranes from each of the 48 water samples. V3 and V4 regions of 16S rDNA were amplified using forward primers containing the sequence “5’-CCTACGGGGRBGCASCAG-3’” and reverse primers containing the sequence “5’-GGACTACNVGGGTWTCTAATCC-3’”. PCR products were detected by 1.5% agarose gel electrophoresis. DNA libraries concentration was validated by Qubit 3.0 fluorometer. DNA libraries were multiplexed and loaded on an Illumina MiSeq instrument according to the instructions (Illumina, San Diego, CA, USA) (Caporaso et al. 2012). PE250/300 paired-end was used to sequence. Image analysis and base calling were conducted by the MiSeq Control Software (MCS) embedded in the MiSeq instrument. The purified chimeric sequences were used for OTU clustering by VSEARCH clustering (1.9.6) (sequence similarity is set to 97%). The used 16S rRNA reference database was Silva, 132. Bayesian algorithm was used to analyze representative
sequences and classify community composition under different species classification level. The sequencing data have been deposited with links to NCBI under accession number PRJNA725369.

**Statistical analysis**

Bar charts of survival parameters, physicochemical properties, and relative abundances of major bacterial phyla were plotted by OriginPro 9.1 (OriginLab, USA). Principal component analysis (PCA) was constructed to visualize the differences of physicochemical properties in different samples with the vegan package of R 4.0.4. Principal coordinate analysis (PCoA) was performed to appear the differences of bacterial community in Bray–Curtis conversion by vegan package. Multivariate analysis of variance (MANOVA) was conducted with SPSS 26 (IBM, USA) in order to test the effects of lake, season and interaction (Lake × Season) on survival parameters of EcO157 and water physicochemical properties. Dissimilarity analysis was applied to indigenous bacterial community by Multi-Response Permutation Procedure (mrpp), Permutational MANOVA (adonis) and Analysis of Similarities (anosim) based on four distance conversion measures of Bray–Curtis, Horn, Gower and Jaccard (vegan package of R 4.0.4).

Mantel and partial Mantel tests (non-parametric statistical methods) were calculated with the vegan package of R 4.0.4. The Mantel test is a correlation test between two matrices including measured variables (Guillot and Rousset, 2013). Partial mantel test also measures correlation, but controls for the effect of a third matrix including other variables (Guillot and Rousset, 2013). The influence of bacterial community and physicochemical properties on the survival of EcO157 was tested by Mantel and partial Mantel. In addition, Pearson correlation between ttd and relative abundances of phylum bacterial taxa in Spring and Autumn samples was calculated respectively.

To clearly display the correlations between survival parameters, physicochemical properties and bacterial community, co-occurrence network analysis based on Pearson correlation (p < 0.05) was conducted. In order to simplify the network, the correlations between bacteria were deliberately removed. Co-occurrence network analysis and plotting were jointly completed through the Hmisc package of R 4.0.4 and Gephi 0.9.2.

In order to further reveal the influence of environmental factors, variation partition analysis (VPA) was used to quantify the explanatory contribution of partitioning factor matrices to the overall ttd variation of EcO157 (Borcard et al. 1992). VPA was performed after obtaining the corrected R² (total explanatory proportion) of Redundancy analysis (RDA) with R 4.0.4 (Peres-Neto et al. 2006) using vegan package. VPA can be output in the style of Venn diagram.

The overlap in the Venn diagram indicated the combined contributions of multiple matrices (Peres-Neto et al. 2006).

Compared to linear regression analysis, the structural equation model (SEM) can more accurately identify the direct and indirect effects of single environmental factors on the persistence of EcO157 (Alexandrowicz et al. 2016; Shih and Tu 2019). SEM was performed by AMOS 22.0 (Amos Development, Spring House, PA, USA). Direct effect, indirect effect and total effect were calculated based on standard path coefficients (λ) which reflect the strength of the relationship between variables (Igolkina and Samsonova 2018). Model fitness is the degree of consistency between the hypothetical theoretical model and the actual data. The model fitness parameters and criteria are as follows: χ^2 > 0.05, chi-square; p > 0.05, p value; CMIN/DF < 3, ratio of χ^2 and degrees of freedom; GFI > 0.9, goodness of fit index; RMSEA < 0.08, root mean square error of approximation.

**Results**

**Water properties, dominant bacterial phyla and E. coli O157:H7 survival parameters**

The water physicochemical properties were listed in Table S2. Figure 1 displayed the variation of physicochemical properties. The result showed that EC levels were higher in Spring samples than in Autumn samples (p < 0.05) (Fig. 1b). Regardless of seasons, EC levels were the highest in BH (Fig. 1b). TN levels were higher in Autumn samples than in Spring samples (p < 0.05) (Fig. 1c). TOC was higher in Spring samples than in Autumn samples (p < 0.05). In Spring samples, TOC was the highest in BH (p < 0.05). However, TOC were not significantly different in Autumn samples (Fig. 1d). For TP and NH₄⁺-N, the highest concentrations were showed in NH (p < 0.05) (Fig. 1e and f).

Figure 2 showed the variation of relative abundances of major bacterial phyla. Relative abundance of Proteobacteria was the highest in BH for Spring samples (p < 0.05) and NH for Autumn samples (p < 0.05) (Fig. 2a). For Spring samples, BH had the lowest relative abundance of Bacteroidetes (p < 0.05), while in Autumn samples JY had the lowest one (p < 0.05) (Fig. 2b). Relative abundance of Actinobacteria was highest in BH in Autumn samples (p < 0.05) (Fig. 2c).

Survival parameters (ttd, δ, p) were calculated according to survival profiles of EcO157 (Fig. S2). Variation of EcO157 survival parameters was shown in Fig. 3. On average, the survival time (ttd) in urban recreational waters was 64.6 days when both seasonal samples were taken into account. For Spring samples, ttds in NH, JY, and BH were 70 days, 58.9 days, and 80 days, respectively, with average being 69.7 days. For Autumn samples, the ttds in NH, JY, and BH were 52.8 days, 51.6 days, and 75 days, respectively.
Fig. 1 The variation of physicochemical properties in Spring and Autumn among different lakes. Legend s and a represented Spring and Autumn, respectively. NH, JY, and BH represented Nanhu Lake, Jingyue Lake, and Beihu Lake, respectively. NH$_4$$^+$-N, ammonium nitrogen; EC, electrical conductivity; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus. Samples with different capital letters indicated significant difference at 0.05 level.

Fig. 2 The variation of relative abundances of major bacteria at phylum level in Spring and Autumn among different lakes. Legend s and a represented Spring and Autumn, respectively. NH, JY, and BH represented Nanhu Lake, Jingyue Lake, and Beihu Lake, respectively. Samples with different capital letters indicated significant difference at 0.05 level.

Fig. 3 The variation of survival parameters ($t_{td}$, $\delta$, and $p$) of E. coli O157:H7 in Spring and Autumn among different lakes. Legend s and a represented Spring and Autumn, respectively. NH, JY, and BH represented Nanhu Lake, Jingyue Lake, and Beihu Lake, respectively. $t_{td}$, time needed to detection limit; $\delta$, scale parameter; $p$, shape parameter. Samples with different capital letters indicated significant difference at 0.05 level.
and the average was 59.8 days. Overall, the survival time was longer in Spring samples compared with Autumn ones (Fig. 3a). In individual recreational water, EcO157 survived longest in BH, regardless of seasons (Fig. 3a). The $\delta$ of EcO157 was longer in Autumn samples than in Spring samples (Fig. 3b). Survival profiles of EcO157 mostly displayed a concave curve, and $p$ was less than 1 in most cases (Fig. 3c).

Principal component analysis visualized the variation in physicochemical properties of different samples. The closer the distance between sample points, the more similarity shared by those samples, and vice versa. PCA showed that the most Spring samples could be distinguished from the Autumn samples (Fig. 4a). However, the variation between lakes was not observed since the sampling points were not well separated from each other. Similarly, seasonal variation was observed among bacterial communities in all samples (Fig. 4b).

MANOVA was performed on survival parameters and physicochemical properties of all 48 samples. MANOVA results revealed that NH$_4^+$-N, pH, EC, TOC, TN, $ttd$ and $\delta$ were largely affected by seasons ($p < 0.05$) and the interaction (Lake $\times$ Season) could be observed for NH$_4^+$-N and pH (Table 1). The $p$ was neither affected by lakes nor seasons. Dissimilarity test revealed that bacterial community was significantly different ($p < 0.05$) between Spring and Autumn samples (Table 2). Overall, seasonal variation was noticed in recreational water physicochemical properties, bacterial community, and EcO157 survival time.

| Variables | Lake $\times$ Season |
|-----------|----------------------|
| NH$_4^+$-N | 0.001 0.001 0.025 |
| pH        | 0.464 0.001 0.001 |
| EC        | 0.001 0.001 0.222 |
| TOC       | 0.006 0.001 0.076 |
| TN        | 0.050 0.001 0.809 |
| TP        | 0.001 0.948 0.380 |
| $ttd$     | 0.001 0.019 0.416 |
| $\delta$  | 0.362 0.005 0.864 |
| $p$       | 0.465 0.506 0.309 |

Table 1 MANOVA of water properties and survival parameters with different lakes and seasons

$NH_4^+\text{-N}$, ammonium nitrogen; EC, electrical conductivity; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; $ttd$, time needed to reach the detection limit; $\delta$, scale parameter; $p$, shape parameter. $p$ with value less than 0.05 was bolded

Multivariate statistics on environmental factors and E. coli O157:H7 survival parameters

The result of Mantel and partial Mantel tests for all 48 samples revealed that $ttd$ was correlated with the indigenous bacterial community, EC, and TOC ($p < 0.05$) (Table 3). When the effects of other factors were partialled out, coefficients between $ttd$ and bacterial community, EC, and TOC became smaller, but remained statistically significant. Bacterial community and physicochemical properties showed no significant effect on $p$ and $\delta$ (data not shown).

Pearson correlation showed that the bacterial phyla correlated with $ttd$ varied greatly in Spring and Autumn samples (Table 4). In Spring samples, relative abundances of Proteobacteria and Bacteroidetes were positively and negatively correlated with $ttd$, respectively ($p < 0.05$) (Table 4).

![Fig. 4](image_url)

Fig. 4 Principal component analysis (PCA) of physicochemical properties (a). The contributions of PC1 and PC2 were 39.47% and 23.69% (a). Principal coordinate analysis (PCoA) of bacterial community (b). The contributions of PC1 and PC2 were 23.20% and 19.58% (b). sNH, sJY, and sBH represented Spring water samples from Nanhu Lake (NH), Jingyue Lake (JY), Beihu Lake, respectively. aNH, aJY, and aBH represented Autumn water samples from Nanhu Lake (NH), Jingyue Lake (JY), and Beihu Lake (BH), respectively.
Tables:

**Table 2** Dissimilarity analysis of indigenous bacterial community between Spring and Autumn samples

|         | mrpp  | adonis | anosim |
|---------|-------|--------|--------|
|         | δ     | p      | F      | p      | R      | p      |
| Spring and Autumn samples | Bray–Curtis | 0.5239 | **0.001** | 10.55 | **0.001** | 0.4651 | **0.001** |
|        | Horn  | 0.4115 | **0.001** | 9.4247 | **0.001** | 0.2739 | **0.001** |
|        | Gower | 0.1830 | **0.001** | 8.8664 | **0.001** | 0.3858 | **0.001** |
|        | Jaccard | 0.6759 | **0.001** | 8.2248 | **0.001** | 0.4651 | **0.001** |

Four distance measures of Bray–Curtis, Horn, Gower and Jaccard were used. Three distance tests of mrpp, adonis and anosim were calculated. p with value less than 0.05 was bolded.

**Table 3** Mantel and partial Mantel tests between ttd and environment factors for total 48 samples

| Bacterial community | NH₄⁺-N (mg/L) | pH | EC (mS/cm) | TOC (mg/L) | TN (mg/L) | TP (mg/L) |
|---------------------|---------------|----|------------|-------------|-----------|-----------|
| ttd Mantel          | 0.1265*       | −0.0470 | −0.0419 | 0.1486** | 0.0890* | −0.0513 | −0.0518 |
| Partial Mantel      | 0.1046*       | −0.0724 | −0.0567 | 0.1230* | 0.0833* | −0.0785 | −0.0482 |

The first and second coordinates of principal coordinate analysis (PCoA) were used to represent bacterial community. NH₄⁺-N, ammonium nitrogen; EC, electrical conductivity; TOC, total organic carbon; TN, total nitrogen; TP, total phosphorus; ttd, time needed to reach the detection limit. Asterisk meant the following: *p < 0.05, **p < 0.01.

**Table 4** Pearson correlation between ttd and relative abundances of bacterial taxa (Pearson’s p < 0.05)

| Taxa at phylum level | Relative abundance (%) | ttd | r    | p    |
|----------------------|------------------------|-----|------|------|
| Spring season        |                        |     |      |      |
| Proteobacteria       | 33.357                 | 0.439 | 0.032 |
| GammaProteobacteria  | 26.400                 | 0.486 | 0.016 |
| Bacteroidetes        | 26.242                 | −0.413 | 0.045 |
| Chloroflexi          | 0.393                  | −0.414 | 0.047 |
| Planctomycetes       | 0.018                  | −0.500 | 0.013 |
| Latescibacteria      | 0.002                  | −0.497 | 0.047 |
| Autumn season        |                        |     |      |      |
| Actinobacteria       | 30.048                 | 0.454 | 0.026 |
| Gemmatimonadetes     | 0.289                  | 0.519 | 0.011 |
| Spirochaetes         | 0.021                  | 0.529 | 0.008 |

For Spring samples, most bacterial taxa excluding Proteobacteria were negatively correlated with ttd (p < 0.05). In Autumn samples, Actinobacteria was positively correlated with ttd (p < 0.05) (Table 4). Almost all major bacterial taxa in Autumn samples were positively correlated with ttd (p < 0.05).

Co-occurrence network analysis displayed the interactions among EcO157 survival parameters, water physicochemical properties and indigenous bacterial community. When all 48 samples were considered, ttd was positively linked to TOC and EC (Fig. 5a). Networks of Spring (24 samples) and Autumn (24 samples) were less complex with fewer links, lower average degree, and lower density (Fig. 5d). Connections profiles between ttd and other nodes representing water properties and bacterial phyla were seasonally differentiated between Spring and Autumn samples (Fig. 5b and c). On the other hand, δ and p had fewer links in the networks (Fig. 5b and c). All Pearson correlations (p < 0.05) can be seen in Table S3.

Variation partition analysis (VPA) was used to quantify the contribution of environmental factors to the ttd variation of EcO157. When all 48 samples were considered, VPA showed that the measured environmental factors explained approximately 47.49% of the overall ttd variation, leaving 52% unexplained. The combined contribution by bacterial community and water properties explained about 23.74% of ttd variation (Fig. 6a). Figure 6b and 6c highlighted the seasonal differences in VPA between Spring and Autumn samples. In Spring samples, TOC, and TN accounted for 36% of overall ttd variation. The individual contribution of bacterial community was only 3.88%, but the combined contribution reached 22.09%. About 64.81% of overall ttd variation was explained by measured environmental factors (Fig. 6b). In Autumn samples, pH and TP explained 10.42% of overall ttd variation (Fig. 6c). The bacterial community alone explained 24.11%. About 56.59% of overall ttd variation was explained by measured environmental factors (Fig. 6c).

The effects of bacterial community and water properties on the ttd of EcO157 could be either direct or indirect effects through SEM (Fig. 7). TOC had a direct positive effect on ttd (p < 0.05) and EC had an indirect effect on ttd through bacterial community (Fig. 7a). Figure 7b and c emphasized the seasonal differences in SEM between Spring and Autumn samples. It was showed that TN had a positive effect on ttd (p < 0.05) in Spring samples, while TP had a negative effect on ttd (p < 0.05) in Autumn samples (Fig. 7b and c).
The effect of EC on bacterial community was negative in Spring samples and positive in Autumn samples, respectively. Similarly, EC had an indirect positive effect on ttd through the co-occurrence network based on Pearson correlation (p < 0.05) was constructed. The network depicted the interactions of bacterial community (at phylum level), survival parameters, and water physicochemical properties. Correlations between bacteria were not considered. Bacteria phyla (relative abundance > 0.1%), physicochemical properties and survival parameters were labeled. The nodes of bacteria, physicochemical properties, and survival parameters were filled with purple, orange, and green, respectively. The size of the node was proportional to the degree of the node (sum of the weights of correlated links). The links were undirected and weighted. The thickness of link represented the absolute value of Pearson’s correlation coefficient. Red link represented a positive correlation. Green link represented a negative correlation. ttd, time needed to reach detection limit; δ, time needed for first decimal reduction; p, shape parameter; EC, electrical conductivity; TN, total soluble nitrogen; TP, total phosphorus; NH₄⁺-N, ammonium nitrogen; TOC, total organic carbon.

**Fig. 5** Co-occurrence network based on Pearson correlation (p < 0.05) was constructed. The network depicted the interactions of bacterial community (at phylum level), survival parameters, and water physicochemical properties. Correlations between bacteria were not considered. Bacteria phyla (relative abundance > 0.1%), physicochemical properties and survival parameters were labeled. The nodes of bacteria, physicochemical properties, and survival parameters were filled with purple, orange, and green, respectively. The size of the node was proportional to the degree of the node (sum of the weights of correlated links). The links were undirected and weighted. The thickness of link represented the absolute value of Pearson’s correlation coefficient. Red link represented a positive correlation. Green link represented a negative correlation. ttd, time needed to reach detection limit; δ, time needed for first decimal reduction; p, shape parameter; EC, electrical conductivity; TN, total soluble nitrogen; TP, total phosphorus; NH₄⁺-N, ammonium nitrogen; TOC, total organic carbon.

**Fig. 6** Variation partition analysis (VPA) was used to quantify the contribution of environmental factors to ttd variation of E. coli O157:H7. The first and second coordinates of principal coordinate analysis (PCoA) were used to represent bacterial community. The size of each area had no correlation with the contribution proportion. EC, electrical conductivity; TN, total soluble nitrogen; TP, total phosphorus; TOC, total organic carbon. (a) Total 48 samples, (b) Spring 24 samples, and (c) Autumn 24 samples.
bacterial community, regardless of seasons (Fig. 7e and f). Interestingly, although bacterial community had effects on ttd, the effect was negative in Spring samples and positive in Autumn samples, respectively (Fig. 7b and c).

Discussion

Seasonal variation in water properties and bacterial community

Our data revealed that EC and TOC levels were higher in Spring samples and TN levels were higher in Autumn samples. The possible reasons might lie in the changes of climate conditions associated with different seasons. As a rainy season of Changchun City, Autumn had higher precipitation and lake water volume, which might directly change some water physicochemical properties (e.g. pH, EC) (Stocker et al. 2019). Chemical components that reflected lake water EC salinity may be diluted due to increased water volume. The nutrients (e.g. nitrogen, phosphorus) from lake sediment and surrounding environment might be released into the lake with direct rainfall and surface runoff (Shen et al. 2021). Low temperature in Autumn was considered to be beneficial to maintain TN concentration (Mu et al. 2021). In Spring, there was plenty of sunlight and the temperature is higher. Although temperature had little direct effect on water total organic carbon, a recent study showed that the temperature was related to carbon efficiency of bacteria (Smith et al. 2021). Therefore, high temperature in Spring might indirectly affect the concentration and composition of total organic carbon in the water.

We also found that different bacterial phyla varied seasonally, e.g. Bacteroidetes. As the dominant bacterial phylum, the relative abundance of Bacteroides was higher in Spring samples than in Autumn samples. Previous research showed that Bacteroidetes might be more suitable for growth at the intermediate temperature (Wang et al. 2021a) in Spring rather than the low temperature in Autumn. Temperature variation would cause bacteria to adjust the transport and metabolism of amino acid and carbohydrate, which ultimately led to changes in bacterial community structure and behavior (Wang et al. 2021c). Available nutrients variation would significantly affect bacterial diversity and composition, especially on the dominant bacterial groups (e.g. Proteobacteria, Actinobacteria) (Wang et al. 2021b). In addition, seasonal variation in industrial wastewater and domestic sewage generated by human activities might also have unpredictable influence on urban recreational waters (Filgueiras Ferreira et al. 2021). A longer rainy season might lead to fewer tourists and therefore less sewage generated (de Melo Nobre et al. 2021).

Seasonal differences in environmental factors correlated with E. coli O157:H7 survival

Our data showed that EcO157 survival time (ttd) was longer in Spring samples (69.7 days) than in Autumn samples (59.8 days), both of which were longer than 30.1 days in well water from another research (Ding et al. 2018). Therefore, the risk of EcO157 transmission through recreational water was worth noting. Many studies have suggested that seasonal variation was related to the infection of E. coli (Sarr et al. 2020). Research based on epidemiology revealed that E. coli outbreaks linked to green leafy vegetables had seasonal characteristics, with more cases in Spring and Autumn than other seasons (Marshall et al. 2020). Compared with dry season, the prevalence of E. coli was higher in the rainy season, which might be due to climate conditions such as humidity and temperature (Desiree et al. 2021). Our results demonstrated that the reasons might be that different seasons had the differences in multiple environmental factors that affected the survival rate of EcO157 as discussed below.

Our results showed that most bacterial phyla (e.g. Bacteroidetes) were negatively correlated with ttd in Spring samples (p < 0.05). In spring samples, bacteria, especially dominant bacteria grew vigorously and demanded more resources. As an invading pathogen, EcO157 might have antagonism, predation and resource competition relationships with parts of dominant bacterial phyla (e.g. Bacteroidetes) (Ishii et al. 2021). These relationships weakened the ability of EcO157 in nutrients competition (Williams et al. 2012), leaving fewer niches for EcO157 to occupy. Thus, greatly inhibited its survival (Xing et al. 2019). Previous research found that EcO157 had many similarities with GammaProteobacteria in physiology and ecology and inhibited the persistence of EcO157 (Ma et al. 2013). It was reported that GammaProteobacteria showed negatively correlated with ttd (Han et al. 2021). However, in our study, GammaProteobacteria exhibited a positive correlation with ttd in Spring samples, which was contrary to previous studies. The reason might be that the combination of environmental factors including the relative abundance of GammaProteobacteria might vary simultaneously, and such variation would have opposite effects on the EcO157 persistence (Stocker et al. 2019).

In Autumn samples, most bacterial phyla (e.g. Actinobacteria) were positively correlated with ttd (p < 0.05). Compared with Spring samples, the activity of bacterial communities in Autumn samples dropped and the nutrient requirement decreased. EcO157 might benefit from nutrients released by certain bacteria during cell lysis and death (Anderson et al. 2021). In addition, research showed that Actinobacteria had the ability to decompose high-molecular organic matter, which could provide the required low-molecular nutrients for EcO157 persistence (Ma et al. 2013).
Therefore, the persistence of EcO157 was enhanced due to increased resource availability and competition release (Xing et al. 2019), which explained why ttd was positively correlated with bacterial community in Autumn samples.

We found that the similarity between the Spring and Autumn samples was that EC exhibited an indirect positive effect on ttd through bacterial community. EC salinity was considered as a powerful factor affecting bacterial composition, diversity, richness and function (Rath and Rousk 2015). Previous studies showed that higher EC level directly inhibited the ttd of EcO157 (Ma et al. 2012). Because osmotic effect or certain ion toxicity of EC would interfere with EcO157 cell metabolism by suppressing necessary enzymes activity (Khezri et al. 2020), but EC level in water was much lower than that in soil, it had little direct inhibition on the EcO157 persistence. Dissimilarly, EC had a negative effect on the indigenous bacterial community in Spring samples, but a positive effect in Autumn samples. It was possible that not only the EC levels but also the chemical composition that made up EC had changed greatly between Spring and Autumn samples. In different habitats, the correlation between bacterial community and EC might show an obvious opposite trend due to the difference in the composition of EC (Stocker et al. 2019).

In Spring samples, TN displayed a significant positive effect on the persistence of EcO157, which was consistent with previous studies (Ma et al. 2012). A recent study demonstrated that NH$_4^+$-N had the ability to influence the persistence of pathogens in well water (Li et al. 2018). But in our study, the correlation between NH$_4^+$-N and ttd was not observed in urban recreational waters. Previous studies have pointed out that dissolved organic nitrogen (DON) and NO$_3^-$-N could contribute to EcO157 persistence (Franz et al. 2008). The composition of TN must be further investigated to ascertain which forms of nitrogen affect ttd of EcO157 in recreational waters.

In Autumn samples, TP exhibited an adverse effect on ttd. Surprisingly, TN showed no effect on ttd, although TN concentration was higher in Autumn samples than in Spring samples. Due to high TN concentration, TP might act as a limiting factor for microbial growth in recreational waters in Autumn samples (Brailsford et al. 2019). In addition, TP was negatively correlated with Actinobacteria. The partial negative impact of TN on ttd might be manifested in the inhibition of certain beneficial bacterial phyla that had positive effects on ttd.

When all 48 samples were considered, TOC was positively correlated with ttd. TOC was identified as an important factor in determining microbial growth, including the assimilable organic carbon (AOC) (Li et al. 2020) and dissolved organic carbon (DOC) (Vila-Costa et al. 2020), which are easy to be absorbed and utilized by microorganisms. It was established that AOC and DOC were both positively correlated with the persistence of EcO157 in sterile freshwater (Vital et al. 2008). It has been reported that higher levels of available carbon sources may relax biological competition for energy, thereby promoting the persistence of EcO157 (Franz et al. 2008). As one of the most important nodes of co-occurrence network, TOC was also crucial to indigenous bacterial community. When the available carbon sources are not enough for both EcO157 and indigenous bacterial community, the impact of TOC on ttd may be weakened due to competition.

It should be noted that some abiotic and biotic factors were not characterized, and their contribution to the overall seasonal variation on persistence of EcO157 could not be ignored. The biotic factors include, but not limited to fungal, algal, viral, protist communities, and even zooplankton, could exert significant influence on the persistence of EcO157 in urban recreational waters. The effect of the fungal community on the persistence of EcO157 has also been confirmed (Huang et al. 2020). The effect of algae on EcO157 persistence is also worth studying (Lin and Ju 2017). For abiotic factors, e.g. dissolved oxygen, should be considered when conducting EcO157 survival studies.

**Conclusions**

Our study showed that EcO157 had a high survival potential in three recreational waters in Spring and Autumn, which could cause serious public health problems once these waters are contaminated. On average, EcO157 survived longer in Spring samples than Autumn samples, and the greatest ttd was also observed in Spring samples, implying that this pathogen might pose more health risks to visitors in Spring season. EC was the shared water parameter influencing the EcO157 survival in both Spring and Autumn samples. In addition to EC, TN, Proteobacteria and Bacteroidetes in
Spring samples and pH, TP and Actinobacteria in Autumn samples were found to be major environmental factors in determination of EcO157 survival in three lakes.

Our results might provide useful information for policy makers to reduce the health risks caused by EcO157. The possible solutions might include the following. Identification of potential EcO157 sources, and block the pathways of this pathogen to enter into the recreational waters by better planning sewage treatment facilities, controlling water parameters (e.g. lowering the N and P load), and monitoring the levels of pathogens (e.g. EcO157). In addition, design of guidance or advisories to visitors regarding potential health risks associated with water-contact activities, would also be helpful to limit the occurrence of EcO157 infection among visitors.

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Declarations

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