Prevalence and persistence of *Escherichia coli* strains with uropathogenic virulence characteristics in sewage treatment plants

E. M Anastasi¹, B. Matthews², A. Gundogdu¹, T.L Vollmerhausen¹, N.L Ramos¹, H. Stratton², W. Ahmed³ and M. Katouli¹

¹Faculty of Science, Health and Education, University of the Sunshine Coast, Maroochydore DC 4558, ²School of Biomolecular and Physical Sciences, Griffith University, Nathan Campus, Queensland , Australia and ³Department of Environment and Resource Management, 80 Meiers Road, Indooroopilly, Brisbane, 4068, Australia

Running title: Uropathogenic *E. coli* in STPs

Key words: Sewage treatment plant, *E. coli*, Virulence factors, Biochemical phenotypes

Corresponding author: Dr Mohammad Katouli, Faculty of Science, Health and Education, University of the Sunshine Coast, Queensland, Maroochydore DC 4558, Australia.

Email: mkatouli@usc.edu.au

Phone: (+61) 7 5430 2845

Fax: (+61) 7 5430 2887
Abstract

We investigated the prevalence and persistence of *Escherichia coli* strains in four sewage treatment plants (STPs) in a sub-tropical region of Queensland, Australia. In all, 264 *E. coli* strains were typed using a high resolution biochemical fingerprinting method and grouped into either a single (S) or a common (C) biochemical phenotype (BPT). These strains were also tested for their phylogenetic groups and 12 virulence genes associated with intestinal and extraintestinal *E. coli* strains. Comparison of BPTs at various treatment stages indicated that certain BPTs were found in two or all treatment stages. These BPTs constituted the highest proportion of *E. coli* strains in each STP and mainly belonged to phylogenetic groups B2 and to a lesser extent group D. No virulence genes associated with intestinal *E. coli* were found among the strains, but 157 (59.5%) strains belonging to 14 C-BPTs carried one or more virulence genes associated with uropathogenic strains. Of these, 120 (76.4%) strains belonged to seven persistent C-BPTs and were found in all four STPs. Our results indicate that certain clonal groups of *E. coli* with virulence characteristics of uropathogenic strains can survive the treatment processes of STPs. These strains were common to all STPs and constituted the highest proportion of the strains in different treatment tanks of each STP.
Community sewage treatment plants (STPs) receive waste from diverse sources including residential, industrial and recreational facilities (31). Waste generated from these facilities contains the liquid and faecal discharges of humans and animals, household wastes, industry-specific materials and stormwater runoff (31). These materials are treated through primary, secondary and tertiary sedimentation processes (18). Following these processes, effluent is normally clear and thus often recycled for use in non-potable applications (20), with excess released into receiving waterways. However, due to possible malfunctions or poor management of wastewater systems (1), effluent often containing pathogenic bacteria can be discharged into receiving waterways (11, 34). It has been speculated that waters contaminated with faeces are regarded as a greater risk to human health as they are more likely to contain human-specific enteric pathogens, including Salmonella spp. (30), Shigella spp. (10), enteroviruses (12), hepatitis A (13) and pathogenic Escherichia coli (30).

E. coli, whilst widely used as an indicator bacterium (30, 35), can actually be pathogenic and be responsible for the cause of both intestinal and extraintestinal diseases (16). Intestinal pathogenic strains of E. coli are rarely encountered in the faecal flora of healthy hosts. Extraintestinal pathogenic E. coli (ExPEC) strains commonly cause infections of any organ or anatomical site (28). The ability of these pathogenic bacteria to cause disease is due to the acquisition of specialised virulence factors, in which commensal E. coli strains typically lack. These specialised virulence factors allow them to cause a broad spectrum of diseases (17, 28) such as gastroenteritis (34), diarrhoea (16), urinary tract infections and meningitis (29), soft-tissue infections and bacteremia (28). E. coli strains belong to four main phylogenetic groups (A, B1, B2 and D) (2) with pathogenic strains belonging mostly to phylogenetic...
groups B2 and to a lesser extent D. Another phylogenetic group (group E) has also been identified, however it is uncommon and is not widely used (5).

Presently, chlorination is an extremely widespread practice aimed at reducing the pathogen load in the final effluent to levels low enough to ensure that they will not be present in sufficient quantities to cause disease when discharged (31). Despite this, some pathogenic strains of *E. coli* may survive to become a significant public health risk (14, 35). The aim of this study was to investigate the presence and survival of these pathogenic *E. coli* strains during the treatment processes of four community STPs with different capacities in South East Queensland, Australia.

**Materials and Methods**

**Sewage treatment plants and sample collection**

Four STPs that employ the activated sludge process were sampled between May 2006 and October 2007. Samples were collected in 50 ml centrifuge tubes from secondary sedimentation effluent, plant effluent and final lagoon effluent from STPs 1-3 and from secondary sedimentation effluent (composite stage 1-5), two plant effluent stages (stages 1 to 3 and 5) and final lagoon effluent from STP 4. These STPs service an equivalent population of 60,000 (STP 1), 300,000 (STP 2), 100,000 (STP 3) and 170,000 (STP 4). The average time for water to pass through the treatment process i.e. hydraulic residence time, varied among the STPs and ranged between 20 to 30h.

Samples were transferred to the laboratory on ice where they were processed within 24h of collection by diluting (if necessary) and filtering through 0.45μm membrane filters (Millipore, Bedford, Massachusetts, USA) and placed on m-FC (Oxoid, Basingstoke, UK) agar plates. The plates were then incubated at 44.5°C for 24h and from each sample up to 28 colonies (where possible) suspected as *E. coli* were saved
in nutrient broth (Oxoid) containing 20 % v/v glycerol (Pronalys) and stored at -80°C for
further analysis. In all 367 isolates were saved.

**DNA Extraction and *E. coli* confirmation**

Chromosomal DNA was extracted using a Genomic DNA Extraction Kit (Blood/Bacterial/Cultured Cells) (Real Biotech Corporation, Taiwan), 100 mini preps kit, with minor adaptations. Briefly, cells were harvested from 3h grown nutrient broth (Oxoid) cultures, centrifuged for 1 min at 14 000 rpm, resuspended in 200µL of GT buffer and lysed with 200µL of GB buffer for 10 min. Cells were treated with 200µL of absolute ethanol (96-100%), washed in a Wash Buffer twice and centrifuged at 8000 rpm for 2 min. The GD column was dried using a 14000 rpm centrifugation step for 3 min. DNA was eluted using 100µL of Elution Buffer and centrifuged at 14000 rpm for 60s to elute the purified DNA. The purified DNA was stored at -20 °C.

Strains were confirmed as *E. coli* by PCR amplification of the universal stress protein (*uspA*) gene according to Chen and Griffiths (1998) (4). The reaction included a mastermix of 1730µL filtered sterilised, autoclaved Milli Q water, 250µL 10 x PCR Buffer (Bioline), 100µL dNTP (10mM) (Fisher Biotech), 150µL MgCl₂ (50 mM) (Bioline), 25µL of forward and reverse *uspA* primers (50µM) (Invitrogen), 20µL Taq polymerase (Bioline), and 2.0µL of purified bacterial DNA. The PCR was performed under the following conditions: denaturation for 5 min at 95 °C; 30 cycles of 30s at 94°C, 30s at 56°C and 30s at 72°C; and a final extension step of 5 min at 72°C.

The primer sequences used were (F 5’-CCGATACGCTGCAATCAGT-3’) and (R 5’-ACGCAGACCGTAGGCCCAGAT-3’) which are specific to the *uspA* gene expressed in *E. coli* K-12 strain W3110 (24) generating a 884-bp fragment. Amplified PCR products were electrophoresed on 2% agarose (AMRESCO, Astral Scientific) gels in 0.6 x TrisBase EDTA (TBE) buffer and subsequently stained with ethidium bromide. In all,
265 strains were confirmed as *E. coli* using this method. These strains were then typed using a biochemical fingerprinting method and tested for their phylogenetic groups.

### Biochemical fingerprinting

A biochemical fingerprinting method (PhPlate system, PhPlate AB, Stockholm, Sweden) was used to type *E. coli* strains. In this study we used PhP-RE plates, specifically designed to type *E. coli* strains. Briefly, *E. coli* colonies were suspended in the first well of each row containing 325µL of growth medium, comprised of 0.011% w/v bromothymol blue and 1% w/v proteose peptone (Bactus AB, Blackaby Diagnostics).

Aliquots of 25 µL of bacterial suspensions were transferred into each of the other 11 wells containing 150 µL of growth medium. Plates were then incubated at 37 °C and read at intervals of 7, 24 and 48 h. Images of plates at corresponding times were scanned using a HP Scanjet 4890 scanner. After the final reading of plate images, the mean of the absorbance values from all individual readings was calculated for each reagent, creating the biochemical fingerprint for each isolate (19). Similarity among the isolates was calculated as a correlation coefficient and strains showing an identity (ID) level of >0.965 were regarded as identical and assigned to the same biochemical phenotype (BPT). The ID level of the system was established based on the reproducibility after testing 60 isolates in duplicate. Isolates showing similarity to each other above the ID level were regarded as identical and assigned to the same BPT. BPTs with more than one isolate were termed common (C) and those with a single isolate were termed single (S) BPTs. Diversity among the isolates was calculated using Simpson's index of diversity.

All data handling, including calculations of correlations and coefficients, as well as diversity indices were performed using the PhPlate software version 4002 (PhPlate AB, Stockholm, Sweden).
Phylogenetic grouping and testing for virulence genes

All confirmed *E. coli* strains were tested for phylogenetic groups using multiplex PCR with the *chuA* and *yjaA* genes and the DNA fragment TSPE4.C2 according to Clermont et al (2000) (5).

Representative C-BPTs (n=25) from all 4 STPs were tested for the presence of 12 virulence genes associated with intestinal and extraintestinal *E. coli* strains using a series of three multiplex and five uniplex PCR sets as previously described (3). The virulence genes included *papC*, *papAH*, *papEF*, *iroN*<sub>E.coli</sub>, *cnf1*, *hlyA*, *eltA*, *estII*, *ipaH*, *eaeA*, *stx1* and *stx2*. The PCR protocol for the genes, *papC*, *iroN*<sub>E.coli</sub>, *cnf1*, *papAH*, *papEF* and *hlyA* were modified to the following conditions: denaturation for 30s at 94°C for 25 cycles; 25 cycles of 30s at 63°C; 25 cycles of 3 min at 68°C (1min for *iroN*<sub>E.coli</sub> and *cnf1* primers), and a final extension step of 10 min at 72°C. The uniplex PCR reaction volume for *papC*, *hlyA*, *iroN*<sub>E.coli</sub> and *cnf1* virulence genes consisted of 2.5µl 10 x reaction buffer (Bioline) (3µl for *iroN*<sub>E.coli</sub>), 1.25µl 50mM MgCl<sub>2</sub> (Bioline) (1.0µl for *papC* and *iroN*<sub>E.coli</sub>), 5.0µl 2mM dNTPs (Fisher Biotech) (2.5µl for *papC*), 0.3µl of each primer (Invitrogen) from 50pM/µl stock solution, 0.15µl *Taq* polymerase (Bioline), 2.0µl DNA and sterile MilliQ to make the final volume to 30µl for *cnf1*, *iroN*<sub>E.coli</sub> and 25µl for *hlyA* and *papC*. The reaction volume for the multiplex procedure for *papAH* and, *papEF* genes had a final volume of 30µl and consisted of 15.35µl sterile MilliQ, 5.0µl 10 x reaction buffer, 1.5µl 50mM MgCl<sub>2</sub>, 5.0µl 2mM dNTPs, 0.3µl of primers from 50pM/µl stock solution. All PCR products were separated electrophoretically as described above.

Statistical analysis

The Chi square test ($X^2$) was used to compare the significance of difference between the mean number of BPTs and diversity found in different tanks and STPs.
Results

Of the 367 *E. coli*-like colonies tested, 264 were confirmed as *E. coli* using the uspA gene. The range of confirmed *E. coli* strains in all STPs varied from 72% to 83%.

Biochemical fingerprinting of these isolates showed the presence of both common (C) and single (S) BPTs in each STP and also within different treatment tanks of each plant. Within each STP, *E. coli* strains from the secondary sedimentation tanks, plant effluent tanks and final lagoon effluent were compared to each other. It was found that some strains, originally found in the secondary sedimentation tank were also present in the plant effluent and final lagoon effluent, therefore these strains were regarded as persistent strains. Of the 162 strains tested in STP 1, 49 (30.2%) belonged to two C-BPTs (i.e. C4 and C5) and were found in all three treatment tanks (Table 1). Some strains were also found in two treatment tanks and although their numbers were small, they were considered as less persistent mainly due to the fact that they were inconsistently found in two consecutive tanks (Table 1). Phylogenetic grouping of all C-BPTs found in STP 1 (104 isolates) showed that they belonged to either group B2 (n=56) or D (n=48) (Table 1). Similar results were found in other STPs, which contained 7 (STP 2), 2 (STP 3), and 3 (STP 4) C-BPTs. The most common C-BPTs in these STPs consisted of 20 (19%), 10 (29%) and 21 isolates (32%) respectively.

In all, 42 C-BPTs representing 214 isolates and 50 S-BPTs were identified among the four STPs. Of these, 212 (80%) belonged to phylogenetic groups B2 and D (Table 2). The remaining 20% of isolates that belonged to other phylogenetic groups were spread among 18 BPTs (Table 2). Comparison of these BPTs showed that 23 C-BPTs were found in more than one STP with 62.4% belonging to phylogenetic group B2 (data not shown).

Representative strains belonging to C-BPTs in all four STPs (n=25) were tested for the presence of 12 virulence genes found in *E. coli* strains causing intestinal (i.e. eltA,
estII, ipaH, eaeA, stx1 and stx2) or extraintestinal infections (i.e. papC, papAH, papEF, iroN, cnf1, hlyA). None of the strains carried virulence genes associated with intestinal infections. However, 14 C- BPTs comprising 157 (59.5%) isolates carried one or more of the virulence genes associated with uropathogenic E. coli strains (Table 3). Of these, 120 (76.4%) strains belonged to seven persistent C-BPTs and were found in all four STPs (data not shown).

Discussion

To our knowledge this is the first study that investigates the occurrence and persistence of pathogenic E. coli strains in different treatment tanks of STPs. Of the 367 E. coli-like colonies tested in four STPs, only 264 were confirmed as E. coli using the uspA gene. Identification of E. coli hardly causes any problems in microbiological laboratories. An additional criteria used for identification of E. coli was the test cellobiose, which is a reagent of the 11 tests in the PhP-RE plates used in this study. Strains that were negative for this test during the biochemical fingerprinting of the isolates were disregarded as E. coli. In our study, three isolates that were positive for E. coli in the cellobiose test were negative for the uspA gene, but positive for phylogenetic grouping. To be consistent with identification criteria for E. coli these strains were not included in the study. This however, may indicate that the sequences of primers used to detect the uspA gene may not efficiently represent all groups of E. coli strains and thus, a possibility that we have underestimated the number of E. coli strains in our samples.

The prevalence of E. coli in receiving waterways can be due to an array of sources including domestic and/or wild animals, malfunctioning septic systems, industrial outlets, combined sewer overflows and wastewater effluents, as well as ineffectively controlled treatment stages and to an extent, persistence of bacteria after disinfection (1). The net outcome of the STP treatment process is recycled water with a reduced
number of bacteria and ideally, free of pathogens (15). In a typical STP, the bacterial count is normally reduced by 90% (27, 35); however, studies by Harwood et al (2005) and Kay et al (2008) have shown that approximately 67% of the initial number of total coliforms present in the primary and/or secondary sedimentation effluent can still be detected in disinfected tertiary effluent samples (15, 18). Moreover, organisms with initial high concentrations (in influent) can still be retained at detectable levels in disinfected effluents.

In our study the number of *E. coli* strains sampled from each treatment tank varied among the STPs, and despite the fact that some STPs (e.g. STP 1) were sampled more extensively than others, only those *E. coli* strains that were more prevalent in the secondary sedimentation tanks were detected in the final lagoon effluent of each STP. Interestingly, most C-BPTs found in a final lagoon effluent were also found in other STPs, indicating that either strains belonging to these BPTs persist in STPs due to their higher numbers or have a better ability to survive the treatment processes, including disinfection, or a combination of both. An alternative explanation is that some BPT patterns may be common to several *E. coli* strains. It has been shown that whilst there is a dramatic reduction in the number of *E. coli* during the treatment processes of STPs, up to $10^2$ colony forming units (CFU)/mL may enter receiving waterways after the disinfection process (26). Other studies also indicate that the sewage treatment process reduces the number of pathogens insufficiently (32) with further treatment and disinfection required to render the water safe for release. In the present study we found that the majority of the surviving *E. coli* strains belonged to phylogenetic groups B2 and D. The association of the phylogenetic groups for *E. coli* with diseases at specific sites of the human body has been widely reported and allows for separation of commensal (A and B1) and pathogenic (B2 and D) strains (2, 6). In our study, the efficacy of water treatment to remove the bacterial pathogens responsible for a range of waterborne diseases was indexed by the presence or absence of these *E. coli* strains (7). It has to
be noted that the prevalence of pathogens in different phylogenetic groups may vary in
different geographical regions, and could possibly alter over time (9). Regardless,
phylogenetic grouping of *E. coli* can be used as a simple tool to identify whether the
strains isolated from surface waters are pathogenic or not.

A comparison of the C-BPTs found in all four STPs indicated that 37.4% of the strains
were also present in other STPs and at higher proportion than others. Interestingly, all
(except one) C-BPTs contained strains belonging to phylogenetic group B2.

Our search for the presence of virulence genes associated with intestinal *E. coli* proved
negative but 14 C-BPTs harboured one or more virulence genes associated with
extraintestinal *E. coli*. The high prevalence of strains with uropathogenic virulent genes
and belonging to the same C-BPTs in all STPs indicates that these strains may have a
better ability to survive the treatment process. Some of the strains belonging to
phylogenetic groups A and B1 also showed identical BPTs to strains belonging to B2
and D groups. These strains however, had generally a lower prevalence than strains
belonging to B2 and D groups in the same BPTs.

Community STPs normally serve somewhere between hundreds of thousands to
millions of residents (25). In these STPs, the population of *E. coli* strains can be quite
diverse, depending on a variety of factors including health or hygiene status (8),
geographical region/environment (6, 9) and the host diet (8). Additionally, the niche of
the sewage and sludge in these STPs provides an excellent environment for the growth
of *E. coli* and other bacteria due to the presence of variable mixtures of bacteria and
nutrients from organic matter (21). These environments may therefore become a ‘hot
spot’ (23) for the genetic exchange between certain bacterial species. An example of
such is the transfer of the *chuA* gene, used for phylogenetic grouping, between *E. coli*
O157:H7 and *S. dysenteriae* strains (33). The fact that some *E. coli* strains belonging
to the same BPTs demonstrated different phylogenetic groups may be due to the loss
or horizontal transfer of genes used for classification of *E. coli* into different phylogenetic groups (22).

In conclusion, our findings indicate that *E. coli* strains carrying uropathogenic virulent genes belonging to phylogenetic groups B2 and D, can survive all treatment processes of STPs, with these particular strains establishing dominance in other STPs. This suggests an enhanced ability for these strains to persist the treatment processes of STPs. From a public health point of view, the presence of these strains in the final lagoon effluent of STPs increases the likelihood of their release in surface waters, presenting a significant risk and should therefore be considered in risk management regimes.

### Acknowledgements

We thank Mr. Daniel Powell and Mr. Dan Shelley for their technical assistance.
References


8. Escobar-Páramo, P., A. Le Menac’h, T. Le Gall, C. Amorin, S. Gouriou, B.
   Picard, D. Skurnik and E. Denamur. 2006. Identification of forces shaping the
   commensal Escherichia coli genetic structure by comparing animal and human

   Amorin, S. Gouriou, B. Picard, M. Chérif Rahimy, A. Andremont, E.
   Denamur and R. Ruimy. 2004. Large-scale population structure of human

10. Faruque, S.M., R. Khan, M. Kamruzzaman, S. Yamasaki, Q. Shafi Ahmad,
    T. Azim, G. Balakrish Nair, Y. Takeda, and D.A. Sack. 2002. Isolation of
    Shigella dysenteriae type 1 and S. Flexneri strains from surface waters in
    Bangladesh: comparative molecular analysis of environmental Shigella isolates

    individual and small community sewage systems, St Joseph, Michigan:
    American Society of Agricultural Engineers.

    E&FN Spon, London.

    1999. Detection of viral pathogens by reverse transcriptase PCR and of
    microbial indicators by standard methods in the canal of the Florida keys. Appl.
    Environ. Microbiol. 65(9):4118-4125.

    watershed with antibiotic resistance patterns in fecal streptococci. Appl.

15. Harwood, V.J., A.D. Levine, T.M. Scott, V. Chivukula, J. Lukasik, S.R.
    Farrah and J.B. Rose. 2005. Validity of the indicator organism paradigm for


**TABLE 1.** Prevalence of common (C1-C20) and single (S1-S58) biochemical phenotypes (BPTs) and their phylogenetic groups among 162 *Escherichia coli* isolates obtained from three different treatment tanks in sewage treatment plant (STP). 1. n: number of isolates belonging to each C-BPT and the number of isolates found in each treatment tank is also given. All C- and S-BPTs were numbered consecutively. NT: not tested. Di: Diversity Index.

<table>
<thead>
<tr>
<th>BPTs</th>
<th>Secondary Sedimentation Tank (n=77)</th>
<th>Plant Effluent Tank (n=31)</th>
<th>Final Lagoon Effluent (n=54)</th>
<th>Number (%) of each BPT in the STP population</th>
<th>Phylgenetic group (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C1</td>
<td>5</td>
<td>-</td>
<td>1</td>
<td>6 (3.7)</td>
<td>B2 (6)</td>
</tr>
<tr>
<td>C2</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2 (1.2)</td>
<td>B2 (2)</td>
</tr>
<tr>
<td>C3</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>3 (1.8)</td>
<td>B2 (3)</td>
</tr>
<tr>
<td>C4</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>15 (9.2)</td>
<td>D (15)</td>
</tr>
<tr>
<td>C5</td>
<td>19</td>
<td>3</td>
<td>12</td>
<td>34 (21.0)</td>
<td>B2 (34)</td>
</tr>
<tr>
<td>C6</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td>3 (1.8)</td>
<td>D (3)</td>
</tr>
<tr>
<td>C7</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2 (1.2)</td>
<td>B2 (2)</td>
</tr>
<tr>
<td>C8</td>
<td>6</td>
<td>-</td>
<td>1</td>
<td>7 (4.3)</td>
<td>D (7)</td>
</tr>
<tr>
<td>C9</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2 (1.2)</td>
<td>D (2)</td>
</tr>
<tr>
<td>C10</td>
<td>2</td>
<td>-</td>
<td>-</td>
<td>2 (1.2)</td>
<td>D (2)</td>
</tr>
<tr>
<td>C11</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2 (1.2)</td>
<td>B2 (2)</td>
</tr>
<tr>
<td>C12</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2 (1.2)</td>
<td>D (2)</td>
</tr>
<tr>
<td>C13</td>
<td>-</td>
<td>2</td>
<td>-</td>
<td>2 (1.2)</td>
<td>B2 (2)</td>
</tr>
<tr>
<td>C14</td>
<td>1</td>
<td>3</td>
<td>-</td>
<td>4 (2.5)</td>
<td>D (4)</td>
</tr>
<tr>
<td>C15</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>4 (2.5)</td>
<td>B2 (4)</td>
</tr>
<tr>
<td>C16</td>
<td>-</td>
<td>-</td>
<td>4</td>
<td>4 (2.5)</td>
<td>B2 (4)</td>
</tr>
<tr>
<td>C17</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>4 (2.5)</td>
<td>B2 (3)</td>
</tr>
<tr>
<td>C18</td>
<td>-</td>
<td>-</td>
<td>2</td>
<td>2 (1.2)</td>
<td>D (2)</td>
</tr>
<tr>
<td>C19</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>2 (1.2)</td>
<td>D (2)</td>
</tr>
<tr>
<td>C20</td>
<td>-</td>
<td>1</td>
<td>1</td>
<td>2 (1.2)</td>
<td>D (2)</td>
</tr>
<tr>
<td>S1-S7</td>
<td>7</td>
<td>-</td>
<td>-</td>
<td>7 (4.3)</td>
<td>A (7)</td>
</tr>
<tr>
<td>S8-S11</td>
<td>4</td>
<td>-</td>
<td>-</td>
<td>4 (2.5)</td>
<td>B2 (4)</td>
</tr>
<tr>
<td>S12-S27</td>
<td>16</td>
<td>-</td>
<td>-</td>
<td>16 (9.9)</td>
<td>D (16)</td>
</tr>
<tr>
<td>S27-S38</td>
<td>-</td>
<td>12</td>
<td>-</td>
<td>12 (7.4)</td>
<td>NT</td>
</tr>
<tr>
<td>S39-S58</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>20 (12.3)</td>
<td>NT</td>
</tr>
<tr>
<td>Di</td>
<td>0.956</td>
<td>0.970</td>
<td>0.947</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Number of isolates found**
<table>
<thead>
<tr>
<th>Location</th>
<th>No. of BPTs (no. of isolates in each type)</th>
<th>Total no. of BPTs (no. of isolates)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B1</td>
</tr>
<tr>
<td>STP 1</td>
<td>7 (7)</td>
<td>-</td>
</tr>
<tr>
<td>STP 2</td>
<td>3 (8)</td>
<td>-</td>
</tr>
<tr>
<td>STP 3</td>
<td>1 (1)</td>
<td>2 (11)</td>
</tr>
<tr>
<td>STP 4</td>
<td>4 (4)</td>
<td>1 (21)</td>
</tr>
<tr>
<td>Total</td>
<td>15 (20)</td>
<td>3 (32)</td>
</tr>
<tr>
<td>% of isolates</td>
<td>7.6</td>
<td>12.1</td>
</tr>
<tr>
<td>% BPT</td>
<td>16.5</td>
<td>3.2</td>
</tr>
</tbody>
</table>

* P < 0.0001 for number of isolates belonging to B2 versus both A and B1; and p < 0.0001 for D versus both A and B1
**TABLE 3.** Prevalence of virulence genes among *Escherichia coli* strains belonging to seven common (C) and persistent biochemical phenotypes (BPTs) found in all four sewage treatment plants (STPs). Some isolates were positive for multiple virulence genes.

<table>
<thead>
<tr>
<th>STP (no. of isolates in C-BPTs)</th>
<th><em>papAH</em> no. of isolates</th>
<th><em>papEF</em> no. of isolates</th>
<th><em>papC</em> no. of isolates</th>
<th><em>hlyA</em> no. of isolates</th>
<th><em>cnf1</em> no. of isolates</th>
<th><em>iroN</em> no. of isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td>STP 1 (68)</td>
<td>2 (2.9%)</td>
<td>12 (17.6%)</td>
<td>2 (2.9%)</td>
<td>52 (76.5%)</td>
<td>18 (26.5%)</td>
<td>44 (64.7%)</td>
</tr>
<tr>
<td>STP 2 (49)*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>26 (53.9%)</td>
<td>-</td>
<td>45 (91.8%)</td>
</tr>
<tr>
<td>STP 3 (13)</td>
<td>3 (23.1%)</td>
<td>3 (23.1%)</td>
<td>3 (23.1%)</td>
<td>13 (100%)</td>
<td>3 (23.1%)</td>
<td>13 (100%)</td>
</tr>
<tr>
<td>STP 4 (27)</td>
<td>3 (11.1%)</td>
<td>-</td>
<td>-</td>
<td>25 (92.6%)</td>
<td>-</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>Total (157)</td>
<td>8 (5.1%)</td>
<td>15 (9.5%)</td>
<td>5 (3.2%)</td>
<td>116 (73.9%)</td>
<td>21 (13.4%)</td>
<td>129 (82.2%)</td>
</tr>
</tbody>
</table>

*3 isolates were not tested