Survey of US wastewater for carbapenem-resistant *Enterobacteriaceae*

Jill Hoelle, James R. Johnson, Brian D. Johnston, Brian Kinkle, Laura Boczek, Hodon Ryu and Sam Hayes

ABSTRACT

A survey for antibiotic-resistant (AR) *Escherichia coli* in wastewater was undertaken by collecting samples from primary clarifiers and secondary effluents from seven geographically dispersed US wastewater treatment plants (WWTPs). Samples were collected at each WWTP in cool and summer months and cultured using selective media. The resulting isolates were characterized for resistance to imipenem, ciprofloxacin, cefotaxime, and ceftazidime, presence of carbapenemase and extended-spectrum beta-lactamase (ESBL) genes, and phylogroups and sequence types (STs). In total, 322 AR *E. coli* isolates were identified, of which 65 were imipenem-resistant. Of the 65 carbapenem-resistant *E. coli* (CREC) isolates, 62% were positive for more than one and 31% were positive for two or more of carbapenemase and ESBL genes targeted. The most commonly detected carbapenemase gene was bla_{VIM} ($n = 36$), followed by bla_{KPC} ($n = 2$). A widespread dispersal of carbapenem-resistant STs and other clinically significant AR STs observed in the present study suggested the plausible release of these strains into the environment. The occurrence of CREC in wastewater is a potential concern because this matrix may serve as a reservoir for gene exchange and thereby increase the risk of AR bacteria (including CR) being disseminated into the environment and thence back to humans.

Key words | carbapenem-resistant *E. coli*, carbapenemases, ESBL, wastewater

Jill Hoelle (corresponding author)
Laura Boczek
Hodon Ryu
Sam Hayes
National Risk Management Research Laboratory,
Office of Research and Development,
United States Environmental Protection Agency,
Cincinnati, OH 45268,
USA
E-mail: *hoelle.jill@epa.gov*

James R. Johnson
Brian D. Johnston
Infectious Diseases Section, VA Medical Center
and Department of Medicine,
University of Minnesota,
Minneapolis, MN 55417,
USA

Jill Hoelle
Brian Kinkle
Department of Biological Sciences,
University of Cincinnati,
Cincinnati, OH 45221,
USA

This article has been made Open Access thanks to the generous support of a global network of libraries as part of the Knowledge Unlatched Select initiative.

INTRODUCTION

Escherichia coli, a member of the family *Enterobacteriaceae*, is the predominant enterobacterial gut resident in humans and other mammals and is commonly used as an indicator of fecal pollution in source, drinking, and recreational waters (Elmund *et al.* 1999; Edberg *et al.* 2000; Odonkor & Ampofo 2013). Antibiotic resistance, including to extended-spectrum cephalosporins (ESCs) and carbapenems, is increasingly prevalent among *Enterobacteriaceae*. According to the Centers for Disease Control and Prevention (CDC), prior to 2002 less than 1% of *E. coli* clinical isolates were ESC-resistant or produced extended-spectrum β-lactamases (ESBLs), whereas in a recent report approximately 22% of clinical *E. coli* isolates were ESC-resistant (Weiner *et al.* 2016). Prior to 2010, reports of carbapenem-resistant *Enterobacteriaceae* (CRE) infections were uncommon in the USA; however, according to the CDC's National Healthcare Surveillance Network, the percentage of CRE isolates identified from hospital acquired infections ranged from 3.3 to 10.9%, depending on the site of infection (Weiner *et al.* 2016).

Historically, carbapenem antibiotics have been effective against multi-drug-resistant Gram-negative bacilli and a mainstay of therapy for infections due to such organisms. Carbapenem-resistant *E. coli* (CREC) is increasingly prevalent, mainly due to the emergence of novel carbapenemases.

doi: 10.2166/wh.2019.165

Currently, the three carbapenemases categorized as being most important are *Klebsiella pneumoniae* carbapenemase (KPC; Ambler class A), certain metalloenzymes (VIM, NDM; Ambler class B), and OXA-type enzymes (e.g., OXA-48; Ambler class D) (Nordmann *et al.* 2012). The associated carbapenemase genes are frequently found on mobile genetic elements and have the potential to spread to other Gram-negative bacteria (Nordmann *et al.* 2012).

Although CREC have been recognized primarily in health care settings, investigators at the US Environmental Protection Agency (EPA) proposed that antimicrobial resistance surveillance involving sewage isolates could be informative as to the occurrence and dissemination of specific clonal groups or sequence types within a given community or population (Boczek *et al.* 2007). wastewater treatment plants (WWTPs) typically use a multi-stage treatment approach, consisting of preliminary, primary, and secondary treatment with effluent disinfection. Primary treatment occurs when wastewater received by the treatment plant is collected into settling basins, where solids and grease are removed from the liquid portion using screens and gravity. Such processes are successful at removing approximately 60% of suspended solids from the wastewater. This primary effluent is then subjected to some type of secondary treatment, typically involving biological treatment to further breakdown the organic material in the wastewater. Following secondary treatment, the effluent is disinfected either with a chemical oxidizing agent, such as chlorine, or a physical treatment such as exposure to UV radiation (*Manual of Practice 8; Design of Wastewater Treatment Plants* 1998; Boczek *et al.* 2010).

This study is follow-on research to preliminarily survey wastewater, specifically primary and secondary effluents, from seven locations in the USA for antibiotic-resistant (AR) *E. coli* to ESCs and carbapenem, to characterize the resulting isolates for the presence of carbapenemase and ESBL genes and identify sequence types that have been associated with extraintestinal infections in humans.

MATERIALS AND METHODS

Study sites, sample collection and titer determination

Seven geographically dispersed WWTPs in the continental US were selected opportunistically for sampling. Permission was granted by WWTP operators on the condition of plant anonymity. The WWTPs were located in New Jersey, Maryland, Ohio, Texas, Colorado, northern California, and southern California, and served four urban and three rural/suburban areas. All participating plants used conventional activated sludge for secondary treatment of primary clarified effluents.

Samples were collected at each WWTP once during cooler months (November 2012 or April 2013) and once during the summer (July 2013 or August 2013), for a total of 28 samples, four per WWTP. Each sample consisted of either 1 liter from the primary clarifier effluent or an effluent from secondary treatment after disinfection. The samples were collected in sterile polypropylene bottles, shipped overnight on ice to the US EPA, Cincinnati, OH, and analyzed within 24 hours of collection.

E. coli titers were determined for all samples using membrane filtration method 9222 I (*Standard Methods for the Examination of Water and Wastewater* 2017). *E. coli* identification was confirmed using BBL Crystal Kits and Crystal Mind Software with the Autoreader (BD Bioscience, Sparks, MD). Confirmed *E. coli* isolates were then assigned a unique identification number and stored in 10% glycerol at −80 °C until further analysis.

Isolation of antibiotic-resistant *E. coli* and antimicrobial susceptibility testing

AR *E. coli* were isolated using a membrane filtration procedure, using mFC agar (Becton Dickinson, Franklin Lakes, NJ) supplemented with 1 mg/L imipenem, 4 mg/L ciprofloxacin, 4 mg/L cefotaxime, or 16 mg/L ceftazidime (Sigma-Aldrich, St. Louis, MO), reflecting the respective resistance breakpoints specified by the Clinical and Laboratory Standards Institute (CLSI 2012). Briefly, serial dilutions ranging from 10 mL to 0.01 mL of sample were filtered through 0.45 μm polycarbonate filters and transferred to mFC plates supplemented with antibiotics and incubated at 44.5 °C. Filters that contained countable colonies (<100) were transferred to mFC supplemented with 4-methylumbelliferyl-β-D-glucuronide (MUG) to obtain AR *E. coli* titers and isolates. All MUG positive isolates were chosen for testing.

The four antibiotics were selected because of the 2015 Centers for Disease Control and Prevention's definition for

CRE, i.e., full resistance to two third generation cephalosporins and intermediate to full resistance to a carbapenem. Ciprofloxacin was chosen to ensure coverage for the *H*30R subclone within STc131. This subclone is known for its characteristic resistance to ciprofloxacin and for causing most multi-drug-resistant *E. coli* infections in the USA (Johnson *et al.* 2010).

Minimum inhibitory concentrations (MICs) for ciprofloxacin, imipenem, ceftazidime, and cefotaxime were determined using E-Test™ Strips (bioMerieux, Marcy'Etoile, France). Isolates were classified as resistant, intermediate, or susceptible according to specified breakpoints (CLSI 2012). For data analysis, intermediate isolates were regarded as resistant.

Real-time PCR for carbapenemase and ESBL genes

CREC isolates were screened by real-time polymerase chain reaction (PCR) for four types of ESBL-encoding genes and five types of carbapanemase-encoding genes (Table 1). PCR was performed in a QuantStudio 6 Flex instrument (Applied Biosystems, Foster City, CA) as previously described (Ryu *et al.* 2012). Each PCR plate included controls to check for sample cross-contamination and positive controls. Dissociation curves were examined for evidence of potential primer-dimers and other non-specific reaction products.

Phylotyping and sequence typing

Phylogenetic groups of the AR *E. coli* isolates were defined by the quadraplex PCR method of Clermont *et al.* (2012). Clonal lineages were identified using 2-locus sequence analysis of *fumC* and *fimH* (CH typing) (Weissman *et al.* 2012). Each allele combination (i.e., CH type) was assigned to a putative sequence type (ST) based on the known association of CH types with STs (Weissman *et al.* 2012). In ambiguous situations, additional housekeeping gene loci were sequenced to clarify the most likely associated ST, according to Enterobase (http://enterobase.warwick.ac.uk).

RESULTS AND DISCUSSION

Titers of total *E. coli*, AR *E. coli*, and CREC were calculated for the WWTP samples to assess relative percentages of resistant organisms. Table 2 presents a summary of total *E. coli* titers along with the number of AR *E. coli* and CREC isolates obtained from each WWTP. Only results

Table 1 | Sequence and reference information for qPCR analysis, with target genes, primer sequences, amplicon sizes, and annealing temperatures

Target gene	Primer sequence (5′–3′)	Amplicon size (bp)	Annealing temperature	References	Control strains
bla_{TEM}	TEM_F: AAGATGCTGAAGATCA; TEM_R: TTGGTATGGCTTCATTC	425	44 °C	Speldooren *et al.* (1998)	*E. coli* T5
bla_{SHV}	SHV_F: GCGAAAGCCAGCTGTCGGGC; SHV_R: GATTGGCGGCGCTGTTATCGC	304	62 °C	Henriques *et al.* (2006a)	*E. coli* 52
$bla_{CTX\text{-}M}$	CTX_F:58 SCVATGTGCAGYACCAGTAA; CTX_R: G55CTGCCGGTYTTATCVCC	652	55 °C	Lu *et al.* (2010)	*E. coli* 85.01
bla_{IMP}	IMP_F: GAATAGAGTGGCTTAATTGTC; IMP_R: GGTTTAAYAAAACAACCACC	232	55 °C	Henriques *et al.* (2006b)	NTU 92/99 (IMP – 1) and *P. aeruginosa*
bla_{VIM}	VIM_F: GATGGTGTTTGGTCGCATATCG; VIM_R: GCCACGTTCCCCGCAGACG	475	58 °C	Henriques *et al.* (2006a)	*P. putida*
bla_{KPC}	KPC_F: CATTCAAGGGCTTTCTTGCTGC; KPC_R: ACGACGGCATAGTCATTT	538	55 °C	Dallenne *et al.* (2010)	*E. coli* USVAST – 0600
bla_{GES}	GES_F: AGTCGGCTAGACCGGAAAG; GES_R: TTTGTCCGTGCTCAGGAT	399	57 °C	Dallenne *et al.* (2010)	*E. coli* GES
bla_{NDM}	NDM_F: GGGCAGTCGCTTCCAACGGT; NDM_R: GTAGTGCTCAGTGTCGGCAT	405	60 °C	Manchanda *et al.* (2011)	*E. coli* MH01

Table 2 | Summary of WWTP locations, *Escherichia coli* loads in primary clarifier effluents and antibiotic-resistant *E. coli* isolates obtained from each site

WWTP site	Average facility capacity	Dates of collection	Total *E. coli* (CFU/100 mL)	AR *E. coli* isolates	CR *E. coli* isolates	Raw wastewater composition
Ohio	13 MGD	July 2013 Sept. 2013	9.00×10^6 2.70×10^6	51	8	Domestic, urban
New Jersey	45 MGD	July 2013 Nov. 2012	3.50×10^6 4.50×10^6	84	22	Domestic, urban
N. California	33 MGD	July 2013 April 2013	1.00×10^6 TNTC	8	1	Agriculture, domestic, suburban
Texas	7.0 MGD	July 2013 April 2013	1.50×10^6 2.00×10^7	14	1	Agriculture, domestic, rural
Maryland	5.0 MGD	July 2013 April 2013	3.00×10^7 5.50×10^7	32	8	Domestic, industrial, suburban
Colorado	135 MGD	July 2013 April 2013	7.50×10^6 3.00×10^7	29	10	Agriculture, domestic, industrial, urban
S. California	275 MGD	July 2013 Sept. 2013	1.70×10^7 1.00×10^7	104	15	Industrial, agriculture, domestic, urban

from the primary clarifiers are presented, as no AR *E. coli* isolates were obtained from the secondary effluents. Overall, 322 isolates were obtained that were resistant to $\geq$1 of the four antibiotics tested. Among the 322 AR *E. coli* isolates, the prevalence of resistance to individual agents declined sequentially as follows: cefotaxime (74%), ciprofloxacin (72%), ceftazidime (68%), and imipenem (20%). Additionally, 235 (73%) isolates qualified as multidrug-resistant, defined here as having resistance to $\geq$2 antibiotics tested. Notably, based on biochemical reactions, antibiotic resistance patterns, and sequence typing (results for the latter two are discussed below), and because no enrichment media were used, the AR isolates seemed unlikely to include replicates of the same clone from a given sample in the isolate collection.

To estimate the overall prevalence of AR *E. coli* within the WWTP-associated *E. coli* population, colony counts for all 14 primary effluent samples were summed and an average was calculated for total *E. coli* and for *E. coli* resistant to imipenem, ciprofloxacin, cefotaxime, and ceftazidime. The average AR fraction within the total *E. coli* population, by agent, was 0.0022% for imipenem, 0.38% for cefotaxime, 0.031% for ceftazidime, and 3.1% for ciprofloxacin (Table S1, available with the online version of this paper). Comparable percentages were reported previously for raw wastewater from Poland, although no carbapenem-resistant organisms were found in that study (Łuczkiewwicz *et al.* 2010). It is difficult to compare the percentages of AR *E. coli* seen in wastewater to clinical studies, but percentages of CREC ranged from 0.7 to 1.9% in a survey of clinical infections, depending on the site of infection (Weiner *et al.* 2016). These higher carbapenem resistance percentages, as compared to the present findings in wastewater, would be expected because clinical settings will naturally concentrate AR isolates.

Antibiotic resistance in *E. coli* is more concerning when it occurs in strains capable of causing clinical infections, due to the potential for dissemination and the resulting morbidity and mortality. Phylogenetic analyses have shown consistently that most clinical *E. coli* isolates from human extraintestinal infections are derived from phylogroups B2 and D (Clermont *et al.* 2012). However, transfer of antibiotic resistance genetic elements from commensal to pathogenic strains is suspected to occur in reservoirs where these groups co-mingle, including WWTPs (Marshall *et al.* 2009; Bailey *et al.* 2010). Table 3 presents the phylotypes and associated resistance profiles of the present 322 AR *E. coli* isolates. Phylotypes B2 and D accounted collectively for nearly half ($n = 181$, 45%) of the sewage-source AR isolates (Table 3). Adding potentially pathogenic phylogroup F (Clermont *et al.* 2012) increased this to 56% of the total.

Overall, 65/322 (20%) isolates were imipenem-resistant. CREC ($n = 65$) were concentrated in virulence-associated phylogroups B2, D and F (38/181 (21%)), as compared with the other phylogroups combined (27/141 (19%)). By

Table 3 | Phylotyping of AR *Escherichia coli* with associated resistance profiles

Phylotype	Number of isolates	Imipenem	Ciprofloxacin	Cefotaxime	Ceftazidime
A	64	6	51	45	41
B1	44	9	30	38	36
B2	62	10	47	37	33
C	30	12	27	25	25
D	84	25	42	67	60
E	3	0	3	2	2
F	35	3	32	25	23
Totals	322	65 (20)	232 (72)	239 (74)	220 (68)

Percentage of total number of isolates resistant to specific antibiotic in parentheses.

contrast, all phylogroups had a high prevalence of multi-resistant isolates, including resistance specifically to ciprofloxacin and ESCs. On a percentage basis, phylogroup C had the highest percentages of total AR *E. coli*.

The proportions of CRE per phylogroup are as follows (# CRE/# in phylogroup, %): A (6/64, 9%), B1 (9/44, 20%), B2 (10/62, 16%), C (12/30, 40%), D (25/84, 30%), and F (3/35, 9%). Thus, most CREC isolates ($38/65 = 58\%$) were from phylogroups B2, D, or F and could represent extraintestinal pathogenic *E. coli* (ExPEC) which have an enhanced potential for causing disease in humans.

To further characterize the AR *E. coli* isolates, STs were determined to identify lineages associated with extraintestinal infections (Table 4). CH typing identified several ExPEC-associated clonal complexes (Johnson *et al.* 2010, 2017; Pitout 2012; Xia *et al.* 2017), namely (number of isolates, % of 322), STc131 (28, 8.7%), STc648 (17, 5.3%), ST1193 (25, 7.8%), and STc405 (12, 3.7%). Regarding the temporal and geographical distribution of these AR isolates from ExPEC-associated STs, carbapenem-resistant STc131 was identified in three separate samples from two locations (NJ, both summer and cooler temperatures; southern CA, summer only). Likewise, STc648 was identified in five locations, including two carbapenem-resistant isolates from two separate samples from one location (NJ, cooler and summer months); ST1193 was identified in five locations, including three CREC isolates from one sample in one location (NJ, summer); and STc405 was identified in five locations, including two CREC isolates from one sample in one location (MD, summer). These data indicate a widespread dispersal of these clinically significant AR STs with respect to locale and seasonality.

Table 4 | Number of sequence types complexes found among AR *E. coli* known to be associated with clinically significant infections and their associated antibiotic resistance

Antibiotic resistance	B2-ST131-O25b and B2-ST131-O16	D-ST_c 405	ST_c 648	ST 1193
	($n = 28$)	($n = 12$)	($n = 18$)	($n = 27$)
Imipenem	4 (14)	2 (17)	2 (11)	3 (11)
Ciprofloxacin	21 (75)	11 (92)	17 (94)	22 (81)
Ceftazidime	15 (54)	9 (75)	9 (50)	10 (37)
Cefotaxime	18 (64)	11 (92)	12 (67)	12 (44)

Cell number represents number of isolates resistant to antibiotic, with percent in parentheses.

Determining the mechanisms that impart carbapenem resistance is imperative for epidemiological reasons. The major reason for carbapenem resistance is the presence of a carbapenemase-encoding gene. Many such genes are located on mobile genetic elements (Tait 1993), and tracking their dissemination can provide important information to health care professionals. Screening of our 65 CREC isolates by PCR for five carbapenemase genes and four ESBL genes (Table 5) showed that 45 (62%) of the isolates contained ≥ 1, and 20 (31%) contained ≥ 2, of the nine studied carbapenemase and ESBL genes, mainly VIM (36, 55%) and TEM (24, 37%), but also GES, SHV, IMP, OXA, KPC, CTX, and NDM. Few of these isolates harbored bla_{KPC} ($n = 2$), and none carried bla_{NDM} or bla_{OXA}, which are the focus of multiple clinical case studies. The 20 (31%) remaining CREC isolates with no detected target gene conceivably could harbor antibiotic resistance genes not targeted in the

Table 5 | Gene panel profiles of carbapenem and ESBL-resistant *E. coli* isolates from wastewater

No. of genes positive	bla_{IMP}	bla_{KPC}	bla_{TEM}	$bla_{CTX\text{-}M}$	bla_{VIM}	No. of isolates
0	–	–	–	–	–	20
1	–	–	–	+	–	2
	–	–	–	–	+	16
	–	–	+	–	–	7
2	–	–	+	–	+	14
	–	+	–	–	+	1
3	–	+	–	+	+	1
	–	–	+	+	+	3
	+	–	+	–	+	1
	1	2	25	6	36	65

Samples were all negative for bla_{NDM}, bla_{SHV}, bla_{GES}, and bla_{OXA} genes.

study, or an alternate carbapenem resistance mechanism, such as loss of outer membrane proteins or efflux pumps/porin mutations, which have been demonstrated previously (Kong *et al.* 2018).

The 36 bla_{VIM}-containing CREC isolates were distributed by phylogroup as follows (% of 36): group D (19 isolates, 53%), B2 (6, 17%), C (5, 14%), B1 (3, 8%), and A (3, 8%). Thus, 70% of the bla_{VIM}-positive isolates belonged to extraintestinal virulence-associated phylogroups B2 and D. As for distribution by ST, bla_{VIM} occurred in three STc131 isolates (one of which also had bla_{KPC}), one STc405 isolate, and one ST1193 isolate. The high prevalence of bla_{VIM} in our study is notable considering the typical occurrence of this gene within integrons on broad-host-range plasmids. We are unaware of previously published data demonstrating such extensive prevalence of bla_{VIM} among clinical *E. coli* isolates. Metallo-β-lactamases such as VIM were reported previously to be common worldwide, but rare in *Enterobacteriaceae* in the USA (Gupta *et al.* 2011; Nordmann *et al.* 2011).

CONCLUSIONS

This one-year study of water samples from seven geographically dispersed WWTPs in the USA identified 65 CREC isolates, of which 38 were from ExPEC-associated phlyogroups, including 11 from ExPEC-associated STs. Carbapenem-resistant STc131 was identified in three separate samples from two locations, and other clinically important STs were identified in multiple samples from other locations, demonstrating the plausible release of these organisms into receiving bodies of water.

Antibiotic resistance, including resistance to carbapenems, was not limited to pathogenic *E. coli* phylotypes or STs, as evidenced by the commensal phylotypes (A and C) also harboring multi-drug resistance patterns. Conceivably, this could be indicative of ectotherms harboring AR *E. coli* in their intestinal tracts as part of their normal microbiota.

The occurrence of CREC and other AR *E. coli* with potentially mobile resistance genes in wastewater is concerning because wastewater may serve as a reservoir for gene exchange. This implies a risk of AR *E. coli* and CREC being disseminated into the environment and thence back to humans, with routes of exposure potentially including contaminated recreational water and land application of biosolids. Our ability to understand the extent of this risk will depend, in part, on more detailed information concerning the fate of AR *E. coli*, including CREC, introduced in indigenous microbial communities, the rate of horizontal gene transfer in these communities, and how best to define the relative risk associated with both introduced and indigenous AR bacteria (Pepper *et al.* 2018).

Future studies should address the impact of multiple factors (e.g., geographic location, season, types of wastewater, and treatment plant configuration) on the prevalence of AR in wastewater. Additionally, rapid and reliable methods are needed to allow enumeration and characterization of AR organisms with respect to phylogenetic and clonal background, pathogenic potential, and resistance mechanisms. Identification of genetic factors that impart antibiotic resistance is essential when utilizing wastewater as an epidemiological screening tool for detection of emerging resistance patterns in comparison with clinical isolates from a given geographical locale.

ACKNOWLEDGEMENTS

The US Environmental Protection Agency (EPA), through its Office of Research and Development, funded and managed the research described herein. This work has been subjected to the agency's administrative review and

has been approved for external publication. This work was also supported in part by Office of Research and Development, Department of Veterans Affairs (VA). Any opinions expressed in this paper are those of the authors and do not necessarily reflect the views of the EPA or VA; therefore, no official endorsement should be inferred. Any mention of trade names or commercial products does not constitute endorsement or recommendation for use. All the positive control strains for qPCR testing were graciously provided by Drs Johann Pitout and Gisele Pierano from the University at Calgary Medical School, Calgary, Alberta, Canada. We would also like to thank Connie Clabots and Steph Porter for their technical assistance.

REFERENCES

APHA/AWWA/WEF 2017 *Standard Methods for the Examination of Water and Wastewater*, 23rd edn. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC, USA.

Bailey, J. K., Pinyon, J. L., Anantham, S. & Hall, R. M. 2010 Commensal *Escherichia coli* of healthy humans: a reservoir for antibiotic-resistance determinants. *J. Med. Microbiol.* **59**, 1331–1339.

Boczek, L., Rice, E. W., Johnston, B. & Johnson, J. R. 2007 Occurrence of antibiotic-resistant uropathogenic *Escherichia coli* Clonal Group A in wastewater effluents. *Appl. Environ. Microbiol.* **73** (13), 4180–4184.

Boczek, L., Johnson, C. & Meckes, M. 2010 Chlorine disinfection of blended municipal wastewater effluents. *Water Environ. Res.* **82** (12), 2373–2379.

Clermont, O., Christenson, J. K., Denamur, E. & Gordon, D. M. 2012 The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. *Environ. Microbiol. Reports* **5** (1), 58–65.

CLSI 2012 *Performance Standards for Antimicrobial Susceptibility Testing: Twenty Second Informational Supplement*. CLSI document M100-S22. Clinical and Laboratory Standards Institute, Wayne, PA, USA.

Dallenne, C., Da Costa, A., Decré, D., Favier, C. & Arlet, G. 2010 Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in *Enterobacteriaceae*. *J. Antimicrob. Chemother.* **65** (3), 490–495.

Edberg, S., Rice, E. W., Karlin, R. J. & Allen, M. J. 2000 *Escherichia coli*: the best biological drinking water indicator for public health protection. *J. Appl. Microbiol.* **88**, 106S–116S.

Elmund, G. K., Allen, M. & Rice, E. 1999 Comparison of *Escherichia coli*, total coliform, and fecal coliform populations as indicators of wastewater treatment efficiency. *Water Environ. Res.* **71** (3), 332–339.

Gupta, N., Limbago, B. M., Patel, J. B. & Kallen, A. J. 2011 Carbapenem-resistant *Enterobacteriaceae*: epidemiology and prevention. *Healthcare Epidemiol.* **53** (1), 60–67.

Henriques, I., Fonseca, F., Alves, A., Saavedra, M. & Correia, A. 2006a Occurrence and diversity of integrons and β-lactamase genes among ampicillin-resistant isolates from estuarine waters. *Res. Microbiol.* **157** (10), 938–947.

Henriques, I., Moura, A., Alves, A., Saavedra, M. & Correia, A. 2006b Analysing diversity among β-lactamase encoding genes in aquatic environments. *FEMS Microbiol. Ecol.* **56** (3), 418–429.

Johnson, J. R., Johnston, B., Clabots, C., Kuskowski, M. A. & Castanheira, M. 2010 *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. *Clin. Infect. Dis.* **51** (3), 286–294.

Johnson, J. R., Johnston, B. D. & Gordon, D. M. 2017 Rapid and specific detection of the *Escherichia coli* sequence type ST 648 complex within phylogroup F. *J. Clin. Microbiol.* **55** (4), 1116–1121.

Kong, H., Pan, Q., Lo, W., Liu, X., Law, C., Chan, T., Ho, P. & Lau, T. 2018 Fine-tuning carbapenem resistance by reducing porin permeability of bacteria activated in the selection process of conjugation. *Sci. Rep.* **8**, 15248.

Lu, S., Zhang, Y., Geng, S., Li, T., Ye, Z., Zhang, D., Zou, F. & Zhou, H. 2010 High diversity of extended-spectrum beta-lactamase-producing bacteria in an urban river sediment habitat. *Appl. Environ. Microbiol.* **76** (17), 5972–5976.

Łuczkiewicz, A., Jankowska, K., Fudala-Książek, S. & Olańczuk-Neyman, K. 2010 Antimicrobial resistance of fecal indicators in municipal wastewater treatment plant. *Water Res.* **44**, 5089–5097.

Manchanda, V., Rai, S., Gupta, S., Rautela, R. S., Chopra, R., Rawat, D. S., Verma, N., Singh, N. P., Kaur, I. R. & Bhalla, P. 2011 Development of taqMan real-time polymerase chain reaction for the detection of the newly emerging form of carbapenem resistance gene in clinical isolates of *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumanni*. *Indian J. Med. Microbiol.* **29** (3), 249–253.

Marshall, B. M., Ochieng, D. J. & Levy, S. B. 2009 Commensals: underappreciated reservoir of antibiotic resistance. *Microbe* **4** (5), 231–238.

Nordmann, P., Naas, T. & Poirel, L. 2011 Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg. Infect. Dis.* **17** (10), 1791–1798.

Nordmann, P., Dortet, L. & Poirel, L. 2012 Carbapenem resistance in *Enterobacteriaceae*: here is the storm! *Cell Press* **18**, 263–272.

Odonkor, S. & Ampofo, J. K. 2013 *Escherichia coli* as an indicator of bacteriological quality of water: an overview. *Microbiol. Res.* **4**, 5–11.

Pepper, I., Brooks, J. & Gerba, C. 2018 Antibiotic resistant bacteria in municipal wastes: is there reason for concern? *Environ. Sci. Technol.* **52**, 3949–3959.

Pitout, J. D. D. 2012 Extraintestinal pathogenic *Escherichia coli*: a combination of virulence with antibiotic resistance. *Front. Microbiol.* **3** (9), 1–7.

Ryu, H., Griffith, J. F., Khan, I. U. H., Hill, S., Edge, T. A., Toledo-Hernandez, C., Gonzalez-Nieves, J. & Santo Domingo, J. 2012 Comparison of gull feces-specific assays targeting the 16S rRNA genes of *Catellicoccus marimammalium* and *Streptococcus* spp. *Appl. Environ. Microbiol.* **78** (6), 1909–1916.

Speldooren, V., Heym, B., Labia, R. & Nicolas-Chanoine, M. 1998 Discriminatory detection of inhibitor-resistant β-Lactamases in *Escherichia coli* by single-strand conformation polymorphism-PCR. *Antimicrob. Agents Chemother.* **42** (4), 879–884.

Tait, S. 1993 Mobile genetic elements in antibiotic resistance. *J. Med. Microbiol.* **38**, 157–159.

WEF/ASCE/EWRI 1998 *Manual of Practice 8; Design of Municipal Wastewater Treatment Plants*, 4th edn. Water Environment Federation/American Society of Civil Engineers/Environmental and Water Resources Institute, McGraw-Hill, New York, USA.

Weiner, L. M., Webb, A. K., Limbago, B., Dudeck, M. A., Patel, J., Kallen, A. J., Edwards, J. R. & Sievert, D. M. 2016 Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the national healthcare safety network at the centers for disease control and prevention, 2011–2014. *Infect. Control Hosp. Epidemiol.* **37** (11), 1288–1301.

Weissman, S. J., Johnson, J. R., Tchesnokova, V., Billig, M., Dykhuizen, D., Riddell, K., Rogers, P., Qin, X., Butler-Wu, S., Cookson, B. T., Fang, F. C., Scholes, D., Chattopadhyay, S. & Sokurenko, E. 2012 High-resolution two-locus clonal typing of extraintestinal pathogenic *Escherichia coli*. *Appl. Environ. Microbiol.* **78** (5), 1353–1360.

Xia, L., Liu, Y., Xia, S., Kudinha, T., Xiao, S., Zhong, N., Ren, G. & Zhuo, C. 2017 Prevalence of ST1193 clone and inci1/ST16 plasmid in *E-coli* isolates carrying $bla_{\text{CTX-M-55}}$ gene from urinary tract infections patients in China. *Sci. Rep.* **7**, 44866.

First received 26 June 2018; accepted in revised form 28 November 2018. Available online 22 January 2019