Occurrence of carbapenem-resistant *Escherichia coli* from wastewater in the United States: A retrospective analysis of isolates from 2005

Laura Wessels, Laura Boczek, Jill Hoelle, Mark Rodgers, and Hodon Ryu
U.S. EPA, ORD, NRMRL, Cincinnati, OH 45268, USA

It has been well documented that wastewater effluents are one source of antibiotic resistant bacteria and antibiotic resistant genes (ARGs) found in surface waters. We have recently reported the occurrence of an emerging carbapenem-resistant *Enterobacteriaceae* (CRE) in wastewater collected from seven geographically dispersed wastewater treatment plants (WWTPs) in the United States (U.S.) between 2012 and 2013. Carbapenem antibiotics are typically held in reserve for multidrug resistant (MDR) infections, but with the rise of these types of infections, their use is dramatically increasing in healthcare facilities in the U.S. As a result, it was hypothesized that CRE would be detected rarely in environmental samples prior to the widespread usage of carbapenem antibiotics used to treat MDR infections. In this study, we investigated archived *E. coli* isolates from studies completed before the carbapenem era.

E. coli isolates from primary and secondary effluents collected from seven WWTPs between 2003 and 2004 were recovered and then screened using one of four antibiotics (trimethoprim-sulfamethoxazole, ampicillin, tetracycline, and trimethoprim). We now report on the testing of a subset of these isolates to determine whether they met the Centers for Disease Control and Prevention (CDC) 2012 CRE definition (intermediate or full resistance to one or more carbapenem antibiotics (imipenem) and resistant to at least two extended-spectrum cephalosporins (cefotaxime, ceftazidime)) or the updated CDC 2015 definition (resistant to a carbapenem antibiotic or producing a carbapenemase). Based on minimum inhibitory concentrations (MICs), isolates classified as nonsusceptible to imipenem or resistant to the two cephalosporin antibiotics or resistant to a fluoroquinolone (ciprofloxacin) were used for PCR assays targeting nine carbapenemase and extended-spectrum β-lactamase (ESBL) genes.

Of the 500 antibiotic-resistant *E. coli* isolates tested, the most prevalent resistance was to cefotaxime (3.6%), followed by ciprofloxacin (2.6%), ceftazidime (2.2%) and imipenem (1.8%). Six (1.2%) isolates were nonsusceptible to imipenem, and resistant to cefotaxime and ceftazidime, meeting the CDC 2012 CRE definition. According to the CDC's updated definition, eight (1.6%) isolates were CRE with full resistance to imipenem; only two of these eight isolates were also determined to be CRE according to the CDC 2012 CRE definition. While none of these isolates were positive using the modified Hodge's test, all 12 CRE isolates determined by both of the CDC definitions showed either ESBL production or having at least two ESBL genes. These results suggested that the production of ESBLs conferred resistance to carbapenem antibiotics, but we have no evidence of carbapenem specific hydrolyzing enzymes. In contrast, seven of 85 CRE *E. coli* isolates recovered in 2015 showed the production of carbapenemase as

well as ESBL. Additionally, from the present study, 32 isolates, including the 12 CRE isolates, were selected based on the aforementioned MICs for further PCR assays. While 9% of 32 isolates were negative for all target genes, 78% and 16% were positive for more than 2 and 4 genes respectively, indicating multiple mechanisms of antibiotic resistance. This study demonstrates the occurrence of CRE *E. coli* in wastewater collected before the widespread use of carbapenem antibiotics in healthcare settings in the U.S. and provides additional information about their potential multiple mechanisms of antibiotic resistance.