

Summary of Doctoral Thesis

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Agricultural and Environmental Engineering

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Title	Multidrug-resistant <i>Escherichia coli</i> in municipal wastewater as an indicator of its prevalence and a source of farm contamination in Southeast Asia
<p>With the wide use of antibiotics for human medical treatment, livestock farming, aquaculture and agriculture, increasing antibiotic resistance is a major public health threat that has raised global concerns. Although a variety of antibiotics to kill pathogenic bacteria in different mechanisms have been developed from penicillin in 1920s, we have repeatedly experienced occurrence of bacteria resistant to the newly developed antibiotics. Bacteria with resistance to a wide range of antibiotics, which are called multidrug-resistant (MDR) bacteria, can cause a serious problem since we have limited number of antibiotics effective for them. Actually, nosocomial infections with MDR bacteria, such as MDR <i>Pseudomonas aeruginosa</i>, MDR <i>Acinetobacter</i> sp., extended beta-lactamase (ESBL) and carbapenemase (CP) producing <i>Enterobacteriaceae</i>, have been frequently reported in recent years. Due to their low pathogenicity, these bacteria are suspected to circulate among healthy population with asymptomatic infections. Nevertheless, less attentions have been paid to such community-acquired infections associated with MDR strains.</p> <p>Monitoring MDR bacteria in municipal wastewater is a powerful tool to detect the community-acquired infections, especially for ESBL and CP producing <i>Enterobacteriaceae</i>, since it should contain all enteric bacteria excreted from the whole population regardless of their health status. The main objective of this study was to apply the wastewater monitoring to Bangkok, Thailand, with the aim to investigate the prevalence, pathogenicity and resistance mechanisms of ESBL and CP producing <i>Escherichia coli</i> (ESBL-Ec and CP-Ec, respectively) as the representative of <i>Enterobacteriaceae</i> which are circulating there. Raw wastewater samples were collected from Bangsue and Jatujak wastewater treatment plants located in Bangkok, Thailand bimonthly for four months and assessed for the of ESBL-Ec and CP-Ec abundance. The isolates were then processed and examined to determine their antibiotic resistance profiles, antibiotic resistance genes, and the pathotypes followed by multi-locus sequence typing (MLST) of the pathogenic isolates and whole-genome sequencing (WGS) and the isolates harboring both ESBL and CP producing genes. MLST was used to determine clone diversity while WGS explored the genetic material to provide detailed genetic information. From the results, in wastewater samples, ESBL-Ec were more abundant than CP-Ec isolates accounting for 54% and 10%, respectively of the 139 <i>E. coli</i> isolates recovered during the study. ESBL-Ec were recovered from the two sites while CP-Ec were only recovered from the Jatujak wastewater treatment plant. The relatively high abundance of ESBL-Ec results from its high prevalence and carriage among the general population; CP-Ec is associated with clinical care usually resulting from its use in treatments. The isolates had an extensive antibiotic resistance profile with 80% of ESBL-Ec and all the CP-Ec conforming to MDR traits. The CP-Ec isolates were highly resistant (>60% resistance) to fifteen antibiotic agents but were</p>	

susceptible to amikacin and fosfomycin. The ESBL-Ec isolates were also highly resistant exhibiting more than 50% resistance to 10 antibiotics including 100% resistance to ampicillin, cefuroxime and cefotaxime. Low-level resistance (<10%) to seven antibiotics was observed in ESBL-Ec including fosfomycin but no amikacin. Antibiotic resistance of ESBL-Ec isolates from Bangkok was higher than isolates recovered from a wastewater treatment plant in Japan, specifically, in the antibiotics with low resistance rates (<60%). Both ESBL-Ec and CP-Ec isolates harbored *bla*_{CTX-M}, *bla*_{OXA-1}, *bla*_{OXA-4}, *bla*_{OXA-30} and *bla*_{OXA-48like}, and the *bla*_{CTX-M} was the most detected gene. In addition, other ESBL genes (*bla*_{TEM} and *bla*_{SHV}) were detected in ESBL-Ec. Pathogenicity was low in both isolates at 13% and 20% for ESBL-Ec and CP-Ec respectively, and CP-Ec exhibited enteropathogenic *E. coli* (EPEC) virulence while ESBL-Ec did both EPEC and enterotoxigenic *E. coli* (ETEC) virulence. The clone diversity of isolates from Jatujak accounting for 92.3% of the pathogenic isolates consisted of 10 clones and clone sequence type (ST) 4450 encoded both ESBL and CP productions. WGS revealed three plasmids harboring more genes associated with antibiotic resistance and virulence.

Wastewater treatment plants are important routes through which antibiotic resistance bacteria are disseminated from humans to the environment. The wastewater is mainly treated with a biological process based on the high bacterial abundance and nutrient rich environments which allow the development and growth of antibiotic resistant bacteria. During raw wastewater sampling, samples were also collected from the activated sludge process to assess the dynamic changes in antibiotic resistance and pathogenicity. 52.4% and 4.8% of the isolates from the sludge samples (n=84) were ESBL-Ec and CP-Ec, respectively, showing a slight decrease compared to the isolates from raw wastewater. The CP-Ec were still highly resistant but susceptible to tigecycline and gained resistance to amikacin. The ESBL-Ec isolates, however, had reduced resistance to the antibiotics with low-level resistance in the influent. But the isolates still presented high resistance (60%) to more than six antibiotics. Regardless of the lower abundance compared to the influent isolates, the sludge isolates were highly pathogenic with 86% carrying ETEC, EPEC, enterohaemorrhagic and enteroaggregative *E. coli* pathotypes. The extensive antibiotic resistance, the diverse genes encoding both ESBLs and CP, and widespread pathogenic isolates found in urban wastewater reflect its high prevalence and the underlying potential health risk from ESBL-Ec and CR-Ec. Bangkok has a high antibiotic consumption rate which is believed to fuel increasing antibiotic resistance. The current level of ESBL-Ec circulating in the community as revealed by the raw wastewater results, and observations in activated sludge characterized by the acquisition of resistance and pathogenic genes by bacteria call for stringent measures to tackle bacterial spread in communities and through wastewater treatment plants.

ESBL-Ec and CR-Ec frequently detected in municipal wastewater raises a serious concern about the contamination of downstream environment since wastewater treatment plants (WWTPs) are still unavailable in Southeast Asia except big cities. For this concern, the study further examined the spread of *E. coli* from urban areas to downstream agricultural fields in Hue city, Vietnam lacking in WWTP. After *E. coli* concentration was determined in irrigation water (IRW), MWW, soil, vegetables (VEG), and manure, its dispersion from MWW was tracked using MLST and phylogenetic analyses during the wet and dry seasons. IRW was severely contaminated; 94% of the samples were positive with *E. coli* exceeding the stipulated standards, while VEG contamination was very low in both seasons. The total number of confirmed isolates of *E. coli* was comparable

between the seasons; however, results from MLST and phylogenetic clustering demonstrated more links between the sites and samples to MWW during the wet season. The wet season had four mixed clusters of *E. coli* isolates from multiple locations and samples linked to MWW, while only one mixed cluster also linking MWW to IRW was observed during the dry season. The most prevalent ST complex 10 and two others (40 and 155) have been associated with disease outbreaks, while other STs have links to major pathotypes. Irrigation canals were significant routes for *E. coli* dispersion through direct links to the urban drainage-infested river. The numerous links between the samples and sites revealed MWW discharge as the source of *E. coli* contamination in the downstream environment that was enhanced by flooding, although MDR *E. coli* was not detected.

In summary, this study successfully detected and characterized ESBL-Ec and CP-Ec, which are clinically important MDR bacteria, circulating in Bangkok, Thailand by monitoring them in municipal wastewater. Monitoring of pathogens in municipal wastewater for surveillance of infectious diseases, called wastewater-based epidemiology (WBE), is now intensively studied for COVID-19 worldwide to detect the epidemic including asymptomatic cases. The WBE has been rarely applied to antibiotic-resistant bacteria (ARB) with no reports from developing countries which are believed as the hot spots. Therefore, the findings of this study, such as high prevalence of ESBL-Ec and CP-Ec in Thailand, can support both local and global efforts to fight ARB. And in the one health approach frequently used for those efforts, proper treatment of municipal wastewater is highly recommended for reducing MDR bacteria dispersed from urban areas to the downstream environment, as revealed in Hue city, Vietnam.

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