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Molecular characterization of *Enterobacteriaceae* isolates from
environment in Thailand

（タイの環境水より分離された腸内細菌科細菌の分子性状解析）

< abstract >

Antimicrobial resistance (AMR) is a major issue posing a serious threat to global health. In Thailand, infectious diseases with AMR have been responsible for the deaths of around 38,000 adults per year. The lives of the people in Southeastern Asian countries, including Thailand are closely linked to the water environment and, as a result, they are at high risk of exposure to antimicrobial resistant bacteria (ARB) and antimicrobial resistance genes (ARGs). Therefore, it is necessary to investigate AMR in the environment especially in those areas. ARB and ARGs are transmitted between human, animal and environments. However, the information on prevalence of AMR in bacteria derived from environmental water is limited. To control further spread of AMR in environments, monitoring the prevalence of AMR and appropriate countermeasures are urgently required. In the present studies, water samples were collected from 21 sites including livestock farm wastewater, city canal, wastewater treatment plant, rural river and estuary in Bangkok, Ratchaburi and Trang in Thailand.

In CHAPTER I, our objective was to understand the situation of AMR in aquatic environment using coliforms and *Aeromonas* spp. as indicator bacteria. In total, 61 coliforms and 69 *Aeromonas* spp. were isolated and these bacteria showed various AMR patterns in each environmental site. In pig farm wastewater in Ratchaburi, all coliforms were resistant to ampicillin and cefazoline (CEZ), and more than 70% and 60% of coliforms were resistance to sulfamethoxazole and tetracycline (TET), respectively. Prevalence of resistant coliforms to each antimicrobial was higher in pig farm in Ratchaburi than those in Trang, and city canal in Bangkok than river in Trang. Multidrug resistant coliforms were frequently observed in

livestock farm wastewater and city canal, however not found in river and estuary at all. The characteristics of resistance in each environmental water could be affected by antimicrobial use in humans and animals and discharge into environments. Our findings suggested coliforms and *Aeromonas* spp. were practical indicators for monitoring of ARB in environments.

In CHAPTER II, our objective was to gain insight into genetic information of AMR in environmental water in Thailand. Coliforms were isolated on DHL agar, DHL agar with ciprofloxacin, Chromocult Coliform agar and Chromocult Coliform agar with cefotaxime (CTX). Then, ARGs, integrons, and replicon types were analyzed. And furthermore, plasmids carrying ARGs were identified by S1-PFGE analysis and transmissibility was confirmed by transconjugation experiments. In 130 coliforms isolated, 89 were resistant to CEZ while 41 isolates were susceptible. CEZ-resistant coliforms were found to be significantly resistant to CTX and TET as compared to susceptible isolates. *bla*_{TEM} and *tetM* correlating with β -lactam antibiotics and tetracyclines resistance, respectively, were found to co-localize on the IncFrepB plasmids in isolates from pig farms' wastewater by S1-PFGE analysis. Moreover, transmissibility of the plasmids was confirmed. Results obtained in this study suggested that ARGs in coliforms may have been spreading on the farm via IncFrepB plasmids. Hence, appropriate use of antimicrobials and good hygiene management on the farm is required to prevent the spread of ARGs.