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Genetic Diversity and Antimicrobial Resistance Determinants of
Clinical *Salmonella* Enteritidis in Thailand

（タイで患者から分離された *Salmonella* Enteritidis の
遺伝学多様性と抗菌薬耐性決定因子）

Salmonella Enteritidis (SE) is a predominant nontyphoidal *Salmonella* causing foodborne disease worldwide. In Thailand, SE is a major cause of invasive salmonellosis and significant increase of antimicrobial resistance rate was observed among SE isolated from chicken meat and clinical samples, since 1994. The genetic characteristics of SE strains distributed in Thailand are required to understand the epidemiological features and important for efficient monitoring and control measures.

This study revealed the genetic diversity and antimicrobial resistance determinants among clinical SE isolates from throughout Thailand during 2004-2007. As SE was claimed to be highly clonal bacteria, multilocus variable number tandem repeat (MLVA) typing classified by five polymorphic loci of variable number tandem repeat (VNTR) has been used to discriminate the strains. To elucidate quinolone resistance determinants among SE isolates, point mutation in topoisomerase genes in the quinolone resistance-determining region (*gyrA* and *gyrB*) and plasmid mediated quinolone resistance (*qnr* gene) were analysed.

In chapter I, the retrospective study displayed the nationwide features of MLVA types, antimicrobial resistance and virulence determinants of clinical SE isolates in 2004 - 2007. Twenty MLVA types were identified in SE strains circulating in Thailand. Of these, three closely related MLVA types predominantly shared SE populations in Thailand. Over half of the isolates were resistant to nalidixic acid (83.2%), ciprofloxacin (51.1%) and ampicillin (50.5%). Multidrug resistance was observed in 25.5% of the isolates. All SE isolates carried *Salmonella* difference fragment (*sdfI*), which has been proposed as specific DNA marker for SE identification. Moreover, SE strains were considered as potentially invasive by the possession of prophage-encoded virulence-associated genes (*sodC1* and *sopE*) and virulence plasmid as *spvA*. The fact that a high incidence of strains resistant to certain antimicrobials correlated with dominant MLVA types may indicate the spread of

these strains under selection pressures. The usage of antimicrobials in human medicine and farming might act as selective pressures for SE that causes human infections.

In chapter II, the quinolone resistance determinants, including point mutations in *gyrA/gyrB* and the presence of *qnr* genes were investigated among the quinolone resistant SE strains in Thailand. The quinolone resistance was found to be mediated predominantly by *gyrA* mutations that caused amino acid substitutions Asp87Tyr and Ser83Tyr as well as a new amino acid substitution, Ser83Ile. A strong correlation between mutations and specific MLVA types suggested a possible clonal expansion nationwide in Thailand. In addition, the presence of a plasmid-mediated quinolone-resistant gene, *qnrS1*, raises concerns about a broad dissemination of resistant strains.

These findings raise concern about potential antimicrobial resistance of SE circulating in Thailand. Based on our data, it is recommended to restrict the usage of quinolone for therapeutic and farming purposes in order to effectively control the emergence and spread of fluoroquinolone resistant SE. The molecular epidemiological data found in this study were baseline information for monitoring the SE strains in Thailand. Therefore, SE isolates collected in more recent years are needed to be analysed. Moreover, further investigation for the other antimicrobial resistance determinants has also been recommended.