

Molecular epidemiology of *Clostridium difficile* isolated from piglets

ABSTRACT

Information on the epidemiology of *C. difficile* infection (CDI) in South-East Asian countries is limited, as is data on possible animal reservoirs of *C. difficile* in the region. We investigated the prevalence and molecular epidemiology of *C. difficile* in piglets and the piggery environment in Thailand and Malaysia. Piglet rectal swabs ($n = 224$) and piggery environmental specimens ($n = 23$) were collected between 2015 and 2016 from 11 farms located in Thailand and Malaysia. All specimens were tested for the presence of *C. difficile* with toxigenic culture. PCR assays were performed on isolates to determine the ribotype (RT), and the presence of toxin genes. Whole genome sequencing was used on a subset of isolates to determine the evolutionary relatedness of RT038 (the most prevalent RT identified) common to pigs and humans from Thailand and Indonesia. *C. difficile* was recovered from 35% (58/165) and 92% (54/59) of the piglets, and 89% (8/9) and 93% (13/14) of the environmental specimens from Thailand and Malaysia, respectively. All strains from Thailand, and 30 strains from Malaysia (23 piglet and 7 environmental isolates) were non-toxigenic. To our knowledge, this is the first and only report with a complete lack of toxigenic *C. difficile* among piglets, a feature which could have a protective effect on the host. The most common strain belonged to RT038 (ST48), accounting for 88% (51/58) of piglet and 78% (7/9) of environmental isolates from Thailand, and all 30 isolates tested from Malaysia. Piglet RT038 isolates from Thailand and Malaysia differed by only 18 core-genome single nucleotide variants (cgSNVs) and both were, on average, 30 cgSNVs different from the human strains from Thailand and Indonesia, indicating a common ancestor in the last two decades.

Keyword: *Clostridium difficile*; Environment; Epidemiology; Malaysia; Piglet; Thailand