

他誌掲載論文 (2019年10月～2020年9月)

(1) Epidemiological Aspects of *Escherichia albertii* Outbreaks in Japan and Genetic Characteristics of the Causative Pathogen

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Zoonotic pathogen *Escherichia albertii* has been identified as the cause of several human disease outbreaks; however, factors such as the general symptoms and incubation period of *E. albertii* infection have yet to be defined. Therefore, we aimed to determine the unique aspects of *E. albertii* outbreaks in Japan and to examine the genetic characteristics of the causative pathogen. We studied all known *E. albertii* outbreaks that occurred in Japan up until 2015, which consisted of five confirmed outbreaks and one putative outbreak (Outbreaks 1-6). Outbreaks were re-examined based on personal communications between researchers in prefectural and municipal public health institutes, and through examination of any published study conducted at the time. Draft genome sequences of outbreak-associated *E. albertii* isolates were also generated. The most common symptom displayed by patients across the six episodes was watery diarrhea (>80%), followed by abdominal pain (50-84%) and fever (37.0-39.5 °C) (26-44%). The estimated average incubation period of *E. albertii* infection was 12-24 h. We assumed that most of the outbreaks were foodborne or waterborne, with restaurant foods, restaurant water, and boxed lunches being the suspected transmission vehicles. Three of the six outbreak-associated *E. albertii* isolates possessed intact ETT2 regions, while the remaining isolates contained disrupted ETT2-encoding genes. Virulence gene screening revealed that more than half (44/70) of the tested genes were present in all 5 strains examined, and that each of the strains contained more than 1 gene from 14 out of the 21 groups of virulence genes examined in this

study. The five *E. albertii* strains were classified into four of the five known phylogroups. Therefore, we determined that multiple *E. albertii* genotypes in Japan have the potential to cause outbreaks of diarrhea, abdominal pain, and/or fever following infection of a human host.

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(2) 特許技術！を活用した水質事故等の緊急時分析 (木村淳子, 水環境学会誌, 43, No. 2, 63-66, 2020)

迅速前処理カートリッジは、迅速かつ簡易な操作で水試料の前処理を行うことが可能であり、緊急時や頻繁なモニタリングに有効な技術である。短時間で前処理可能かつ電源を必要としないため、現場での前処理も可能である。環境水の調査に加えて、異常検知時の水道原水の迅速分析や排水のモニタリングなどの使い方も想定できる。AIQS-DBと組み合わせることで前処理から定量までの工程の大幅な時間短縮が可能となり、水質事故等緊急時の迅速分析への貢献が期待される。

本技術は、開発中の性能等の評価だけでなく、製品化後の技術普及に向けた研修の実施、環境調査への利用の検討など、地環研、国環研との繋がりに支えられてきた。今後も適用可能な物質の拡大や技術改善、情報の発信・共有などを推進し、ユーザーと一緒に本技術を育てていきたいと考えている。

(3) Single-Tube Multiplex Polymerase Chain Reaction for the Detection of Genes Encoding Enterobacteriaceae Carbapenemase

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A multiplex PCR assay in a single tube was developed for the detection of the carbapenemase genes of Enterobacteriaceae. Primers were designed to amplify the following six carbapenemase genes: *blaKPC*, *blaIMP*, *blaNDM*, *blaVIM*, *blaOXA-48-like*, and *blaGES*. Of 70 *blaIMP* variants, 67 subtypes were simulated to be PCR-positive based on in silico simulation and the primer-design strategy. After determining the optimal PCR conditions and performing in vitro assays, the performance of the PCR assay was evaluated using 51 and 91 clinical isolates with and without carbapenemase genes, respectively. In conclusion, the combination of multiplex PCR primers and QIAGEN Multiplex PCR Plus Kit was used to determine the best performance for the rapid and efficient screening of carbapenemase genes in Enterobacteriaceae. The assay had an overall sensitivity and specificity of 100%. This PCR assay compensates for the limitations of phenotypic testing, such as antimicrobial susceptibility testing and the modified carbapenem inactivation method, in clinical and public health settings.

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(4) 広島県で分離された腸管出血性大腸菌の病原因子保有状況調査 (2014-2018)

(平塚貴大, 増田加奈子, 秋田裕子, 重本直樹, 広島県獣医師会雑誌, No. 35, 97-101, 2020)

マルチプレックスPCR法を用いて, 広島県内で分離された腸管出血性大腸菌から病原因子の検出を行った. *stx1* 保有株が56.2%, *stx2* 保有株が21.9%, *stx1/stx2* 保有株が21.9%であった. その他の病原因子については, 99%の株が*hlyA*を, 94%の株が*eaeA*を, 18%の株が*astA*を, 1%の株がSTp遺伝子を保有していた. 病原因子の保有状況と保菌者の症状を比較すると, *stx2*及び*astA*の保有が病態の悪化に優位に影響していることが示唆された.