論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

近年、地球規模で問題となっている薬剤耐性菌、特にカルバペネム耐性腸内細菌科細菌（CRE；carbapenem resistant Enterobacteriaceae）は、その薬剤耐性責任分子であるカルバペネム分解酵素遺伝子をプラスミド上にコードしていることから、他菌種への薬剤耐性因子の伝達が容易であり、菌種同定に留まらない迅速な遺伝子検出系の開発が感染制御対策上、診療上必要とされている。また、途上国におけるCREのまん延は重大な問題であり、途上国においても活用可能となるために安価であることも検査法開発の条件となる。

申請者は、上記の目的を解決するために複数種のカルバペネマーゼ遺伝子を同時に検出する新規迅速検出法であるPCR-dipstick chromatography法の開発に成功した。さらに申請者は、本法を用いて、日本国内およびタイ王国においてCREのサーベイランスを便検体を用いて行い、従来法以上の感度・特異度でCREを検出可能であることを示し、実臨床の場における実用性においても問題がないことを示した。本検査法は、すでに国内において商品化がなされている。以上のことから、本論文は、開発途上国を含めた医療現場や環境、食品分野で使用可能であり、先見性、社会のニーズ、実用性等、今後の薬剤耐性菌感染対策を進める上で必要な多くの要件に対して1つの解決策を示したものであり、博士（医学）の学位授与に値すると考えられる。

論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	Shanmuga Kani Rathina Kumar
論文題名 Title	PCR-Dipstick Chromatography for Differential Detection of Carbapenemase Genes Directly in Stool Specimens (PCR-Dipstickクロマトグラフィーを用いた便検体直接カルバペネマーゼ遺伝子検出同定法)

論文内容の要旨

〔目　的(Purpose)〕

Healthcare-associated infections caused by multidrug-resistant organisms pose a serious threat to health sectors not only because of rapid spreading but also due to the limited treatment options. Since carbapenems are regarded as the last line of defense for several multidrug-resistant bacterial infections, carbapenem-resistant organisms impede the effective treatment options and increase the mortality rate of afflicted patients. Amongst different carbapenem-resistant organisms, carbapenemase-producing *Enterobacteriaceae* (CPE) is gaining much attention due to their unique feature of transferring the carbapenemase genes through mobile genetic elements to naïve *Enterobacteriaceae* which then will become the CPE by expressing the carbapenemases. Due to this transfer mechanism, CPE has spread worldwide with a varied epidemiology of different carbapenemases. Thus, it is vital to diagnose CPE at the earliest to undertake relevant preventive measures against their infection/transmission. Here, we attempted to develop PCR-dipstick, a simple, rapid and cost-effective detection system for CPE directly from clinical specimens. Further, we evaluated the efficacy of PCR-dipstick by comparing with the conventional method for CPE detection.

〔方法(Methods)〕

We choose the four major epidemiologically significant carbapenemase genes - bla_{NDM}, bla_{KPC}, bla_{IMP}, and bla_{OXA-48} to target the CPE. The construction of PCR-dipstick commences with the optimization of multiplex PCR conditions using the primers labelled with biotin and distinct probe for each targets. The PCR-product was then subjected to dipstick development and the reaction conditions for dipstick development without any non-specificity were determined. After the establishment of PCR-dipstick for isolated genomic DNA, it was examined for its utility for the DNA isolated from boiled clinical isolates. Then, the efficacy of PCR-dipstick in detecting the carbapenemase genes in direct stool specimens was examined by comparing with a reference comparator which is the conventional PCR for the detection of carbapenemase genes from the CPE isolated from stool specimens.

〔成績(Results)〕

The optimal experimental conditions for the detection of CPE by PCR-dipstick were determined. PCR-dipstick showed 100% sensitivity and specificity in detecting the carbapenemase genes in CPE isolates in comparison with reference comparator. For CPE detection directly from stool specimens, it showed a very good sensitivity and specificity of 93.3% (28/30) and 99.1% (121/122) respectively. Further, PCR-dipstick is 10 times more sensitive than agarose gel electrophoresis in detecting the PCR products.

〔総　括(Conclusion)〕

PCR-dipstick, a new detection system for the identification of CPE directly from clinical specimens was established. PCR-dipstick was found to possess several advantageous characteristics such as flexibility, specificity, sensitivity, multiplicity, simplicity, rapidity, clinical sample compatibility, and visual interpretation of results. The results of our current study shows that PCR-dipstick serve as an efficient tool for surveillance of CPE to undertake appropriate infection control measures to control their dissemination.