插入序列應用於基因分型與抗藥基因鑑定

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摘 要

鮑氏不動桿菌 Acinetobacter baumannii 為一種伺機性的病原菌，在院內感染中，佔很大的比例，通常感染免疫力不足或較年長的病人。目前常用來治療 A. baumannii 的抗生素為 imipenem，但近年來發現 A. baumannii 對 imipenem 抗藥性愈來愈高，是由於其染色體中 OXA 類 β-lactamases 的上游帶有插入序列 (insertion sequence) ISAba1，ISAba1 插入到抗藥基因上游不僅可以增強基因的表現，還有可能形成一個轉位子 (transposon)，更易將抗藥基因散佈給未抗藥的細菌。本實驗從台灣北部二家醫院，包括林口長庚醫院與桃園聖保祿醫院，共收集從 2009 年到 2010 年六十二株的 A. baumannii，先使用脈衝式電泳 (Pulsed Field Gel Electrophoresis, PFGE) 將這群 A. baumannii 分成 41 種基因型 (genotype)。本研究所設計的 PCR typing 利用 2 組 ISAba1 特異性引子與隨機引子，以巢穴式 PCR (nested PCR)，可將六十二株菌分成 13 種分型，其中第一型佔 38 株 (62.3%)；第二型佔 10 株 (16.4%)；第三型 4 株 (6.6%)；第四型共 2 株 (3.3%)；第五到十三型共 9 株 (14.8%)，每型皆為 1 株。當 PCR 分型與 PFGE 分型比較，結果並沒有絕對相關。當 PCR typing 與菌株的抗藥性背景資料比對，發現第一型的菌株大部分都含有多重抗藥性基因或是抗藥基因與 ISAba1 形成的轉位子，主要包括 blaOXA-23、blaOXA-51-like 、ISAba1-blaOXA-23-ISAba1 (Tn2006)、ISAba1-blaOXA-23 (Tn2008)，第二型到第十三型，則是沒有很明顯的共同抗藥基因在內。另外利用 nested-PCR 的方法，尋找插入序列上下游的抗藥基因，將隨機引子擴增出大小不一的片段，並選擇 550 bp 以上的片段，將其純化後分析其 DNA 序列，以及在 NCBI 上的資料庫比對找出抗藥基因。選擇十二株 A. baumannii，與特異性引子擴增出的結果一致只有五株。在 A. baumannii genomic DNA 中，有許多 ISAba1 插在其上，最多發現有21個，導致利用 ISAba1 尋找其調控之抗藥基因的困難。另外在 Klebsiella pneumoniae、Escherichia coli (都能產生廣效性乙醯胺?Extended-spectrum β-lactamases, ESBLs) 中，各選取五株，共十株，利用插入序列 ISEcp1 尋找其所強化的抗藥基因。結果在K. pneumoniae 與 E. coli，與資料庫比對，十株中有七株與特異性引子尋找到的抗藥基因一致。希望本研究成果將有助於臨床的快速檢測中，提升臨床菌株抗藥基因之偵測。

關鍵詞 : 鮑氏不動桿菌、脈衝式電泳、插入序列

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