台灣Acinetobacter baumannii臨床分離菌株攜帶Metailo-B-Lactamase基因及Integrons分子特性之探討

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摘 要

Acinetobacter baumannii 為醫院中造成院內感染常見的菌 株，由於臨床上抗生素的使用頻繁，形成了多重抗藥菌株，造成治療上的困難。根據台大醫院對此多重抗藥菌株研究的統計數據 顯示，從1998 年篩選到的0%到2000 年的6.5%，有逐年增加的 趨勢，可見此菌株抗藥性之快速傳播及其嚴重性。故本研究的目 的，就是希望從分子學上了解此菌株是攜帶何種抗藥基因和其散 播的主要機制。 在醫院臨床中受A. baumannii 感染後所使用之治療藥物以β - 內醯胺類之抗生素“ imipenem ”為主，故本研究主要探討對於 imipenem 具抗藥性的A. baumannii。研究之菌株來源，是由嘉義 長庚醫院所分離到188 株之A. baumannii，再以紙錠擴散感受性試 驗檢測其抗藥性，從中篩選到2 株具imipenem 抗藥性的菌株 (P-78 和P-210)，再加上由林口長庚醫院所篩選到6 株imipenem 抗藥菌株 (AB-394、AB-1756、AB-1757、AB-1758、AB-1759 和 AB-1760)，共8 株進行其imipenem 抗藥性比對和研究分析。並從 菌株中再挑出只對imipenem 不具抗藥性的菌株 (P-21 和P-23) 和 對所有抗生素都不具抗藥性的菌株 (P-2) 進行分析，以了解其抗 藥基因散播的情形。 為了解抗藥菌株是否存在常見對imipenem 抗藥之metallo- β -lactamase (例如blaIMP、blaVIM 與cfiA) 的基因，因此由已知的 blaIMP、blaVIM 與cfiA 序列設計出適當引子，再以PCR 的方法確 認出其中的AB-394 和P-78 兩株菌株具有blaIMP-1 的抗藥基因。相 對地，P-210 、AB-1756、AB-1757、AB-1758、AB-1759 和AB-1760 則不具有blaIMP、blaVIM 或cfiA 其中任一種之抗藥基因。此外，為了進一步研究抗藥基因的位置，以快速分離質體DNA (Kado and Liu) 之方法，分離出這8 株抗藥菌株之質體與染色體，並轉漬至 尼龍膜 (nylon membrane) 上，再以blaIMP-1 序列為探針做南方雜 交法實驗。結果發現AB-394 所攜帶的抗藥基因位於大質體 (>100 kb) 上，P-78 所攜帶的抗藥基因則位於約95-kb 的質體上。而 P-210、AB-1756、AB-1757、AB-1758 、AB-1759 和AB-1760 則 不帶有質體，因此若攜帶抗藥基因則很可能位於染色體中。同時 進一步分析菌株中是否帶有散播抗藥基因之“ integon ”結構，根據 臨床中最常被發現之integon I 序列設計引子，再以PCR 的方法確 認出P-21、P-23、P-78 、P-210、AB-394、AB-1756、AB-1757、 AB-1758、AB-1759 和AB-1760，共10 株菌株具有散播抗藥基因 之integron I 之結構。將PCR 產物經純化解序比對後發現，AB-394 與P-78 具有相同之integron I 之結構及序列，並且攜帶有imipenem 抗藥之blaIMP-1 基因。但在抗藥菌株P-210、AB-1756、AB-1757、 AB-1758、AB-1759 和AB-1760 之integron I 結構中未發現對 imipenem 抗藥之基因。至於這些菌株攜帶何種抗藥基因，或是其 它的抗藥機制，則需進一步研究。同時也藉由脈衝式膠體電泳 (pulsed-field gel electrophoresis, PFGE) 之分子分型方法，分析其 抗藥菌株與非抗藥菌株之間的遺傳差異與親源性。結果顯示多重 抗藥菌株AB-1758、AB-1759、AB-1760 和P-23 可能來自同一親 源，而P-21 和P-210 顯然具相同的親源，此結果也顯示近期從林 口長庚醫院所篩選到的臨床多重抗藥菌株，很可能源自同一親源 (same clone)。 目前對於台灣臨床菌株A. baumannii 之抗藥基因及integron 的探討才剛起步，本研究之結果將有助於台灣臨床A. baumannii 感染之控制及流行病學之了解。

關鍵詞 : Acinetobacter baumannii，抗藥基因，integron，PFGE

目錄

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