應用PCR-RFLP方法快速鑑定感染非結核枝桿菌之研究

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摘 要

非結核分枝桿菌(non-tuberculous mycobacteria, NTM)普遍存在於自然環境中，包括環境中的水或土壤。非結核分枝桿菌是伺機性感染的病原體，可引發許多不同的疾病，常見的有肺部、皮膚及導管有關的感染、骨髓炎，甚至是全身散播性感染。本研究目的在應用分子檢測方法快速鑑定非結核分枝桿菌感染，並探討台灣雲嘉南地區非結核分枝桿菌與結核分枝桿菌感染之流行情形與比較。本研究是以嘉義長庚醫院中，疑似結核分枝桿菌感染而做細菌培養之病患檢體為對象，採用分枝桿菌傳統培養與檢驗，結合分子診斷技術—聚合?鏈鎖反應-核酸限制?截切片段長度多型性(Polymerase Chain Reaction Coupled Restriction Fragment Length Polymorphism, PCR-RFLP)分析法進行檢測。細菌培養陽性的臨床檢體，以抗酸性染色確認，再進行次培養，並持續觀察細菌生長速度和型態。待菌落長出後，先以結核分枝桿菌群(Mycobacterium tuberculosis complexes, MTBC)特有的IS6110序列進行PCR，擴增其中的123-bp DNA片段，以DNA電泳分析判定是否為結核分枝桿菌；若非，再以聚合?鏈鎖反應進行增幅65kDa heat shock protein標的基因(hsp65)中的一段439-bp DNA片段，再以BstEII、HaeIII兩種核酸限制?分別切割後，進行DNA電泳分析，判定各所得DNA片段長度，進行資料庫比對並鑑定出特定的非結核分枝桿菌的菌種。

結果發現疑似結核分枝桿菌感染的所有檢體中，分枝桿菌的陽性率為10.7?s，其中結核分枝桿菌群只佔5.2?s；非結核分枝桿菌反而略多，佔5.5?s。以PCR-RFLP分析鑑定臨床檢體中所分離出來的非結核分枝桿菌，其中最常出現的前六項菌種依序為M. intracellulare (41.5%)、M. gordonae (12.7%)、M. absccessus (7.6%)、Slow-growing NTM (7.3%)、M. avium (6.6%)，及M. lentiflavum (4.1%)，此六菌種就佔了所有感染之非結核分枝桿菌的79.8%，且發現M. intracellulare廣泛地感染不同科室病人。非結核分枝桿菌感染的檢體中，以肺部感染檢體為最多，且研究結果顯示痰液檢體培養陽性的件數最多。在非結核分枝桿菌肺部感染的主要菌種依次為M. intracellulare (41.6%)，其次為M. gordonae (13.0%)和M . abscessus (7.5%)。由此可知，在雲嘉南地區非結核分枝桿菌感染的分佈中，M. intracellulare是最常出現的菌種，其次是M. gordonae。

近來新種非結核分枝桿菌陸續被發現，我們使用的PCR-RFLP鑑定方法可快速鑑別四十幾種常見的非結核分枝桿菌，我們的研究也發現少見的非結核分枝桿菌種別，無法用PCR-RFLP方法鑑定，且有二例是結核與非結核分枝桿菌混合感染。結核分枝桿菌與非結核分枝桿菌感染所用的治療藥物截然不同，非結核分枝桿菌感染治療所使用之抗生素多樣化，確定非結核分枝桿菌感染、快速菌種鑑定與藥物敏感性試驗結果才能確立治療藥物。非結核分枝桿菌流行病學的研究與不同分枝桿菌藥物敏感性試驗資料的建立，是未來漸趨頻繁的非結核分枝桿菌感染之研究方向。

關鍵詞 : 細菌感染、分枝桿菌、非結核分枝桿菌、分子診斷、聚合?鏈鎖反應-核酸限制?截切片段長度多型性分析、流行病學

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