CM-O1

應用rep-PCR作為院內感染MRSA菌株快速分型比對之研究

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MRSA – A Global Threat

- MRSA在院內傳播是全球性的危機
- 早期發現感染源(Identifying Source)是遏止院內傳播的有效方法
- 傳統感染源的鑑定 –脈衝場凝膠電泳(PFGE)
- PFGE的缺點
  - 比對結果要好幾天 – 緩不濟急
  - 需要高超的技術 - 時間與金錢的損失
  - ??

PFGE的缺點

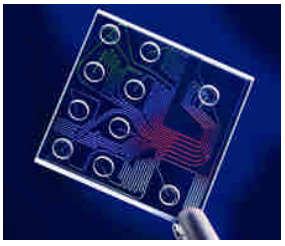
- 比對結果要好幾天 – 緩不濟急
- 需要高超的技術
- 前後批次無法建檔比對，需要重新在同一批中比對

rep-PCR

- 利用細菌基因上短小,高度保守且反覆的序列做為為 PCR 增幅的模板，PCR 後產生一些 DNA 片段的樣式，根據這些樣式的組合來斷定細菌間的親緣關係
- Rep-PCR簡單操作、省時省錢，準確度高，鑑別菌種之多樣性比16S rDNA多。

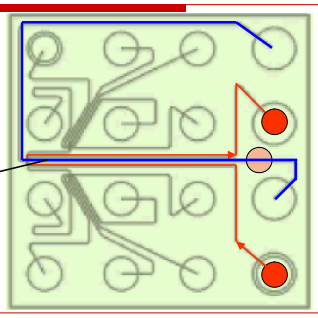
毛細電泳 (Capillary Electrophoresis)

- Lab-on-a-Chip – 生物晶片自動化操作

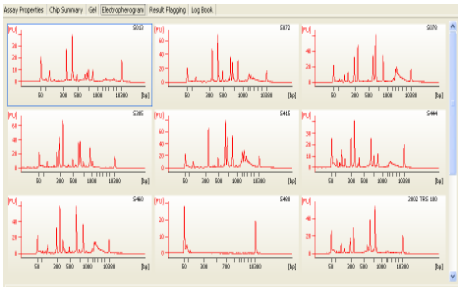


毛細電泳

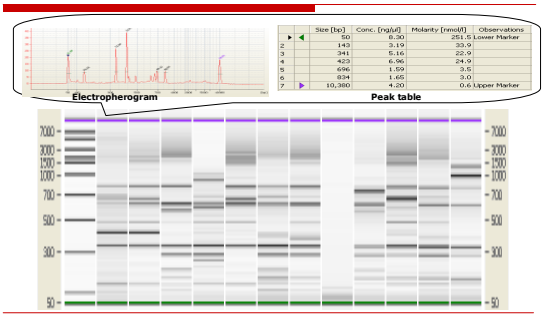
Loading
(ladder)
1.5 min



數位化輸出



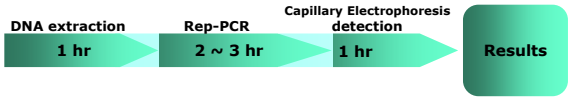
數位結果(Digital)轉類比(Analog)



Gel-like View

rep-PCR + Capillary Electrophoresis + Lab-on-a-Chip

- 所需時間短



- 操作程序標準化，結果再現性高
- 數位化結果輸出，前後批次菌株可比對

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Clinical Evaluation of the DiversiLab Microbial Typing System Using Repetitive-Sequence-Based PCR for Characterization of *Staphylococcus aureus* Strains

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The DiversiLab System, which includes microfluidics-based detection, reagent kits, and software for data processing and analysis, is an automated method using repetitive sequence-based PCR (rep-PCR) for microbial strain typing. To assess the reliability of the DiversiLab System for strain characterization of *Staphylococcus aureus*, we tested clinical isolates sent to ARUP Laboratories for typing and compared results to those of pulsed field electrophoresis (PFGE) aided by the cluster analysis provided by BioNumerics software. *spa* typing was performed when the results of these two methods for an outbreak were not concordant. The study included 89 *S. aureus* isolates (65 *mec*A positive, 24 *mec*A negative) from 19 outbreaks (2 to 11 isolates/outbreak). The DiversiLab and PFGE-BioNumerics results were concordant for 15 of the 19 outbreaks. For the remaining four outbreaks, there was partial concordance between the two methods. *spa* typing results were the same as or more similar to rep-PCR results for three of those outbreaks and were more similar to PFGE results for one. With regard to performance, the DiversiLab system was considerably less labor intensive than PFGE and provided results in less than 24 h, compared with 2 to 3 days for PFGE. Additionally, the Web-based DiversiLab software provides standardized comparisons among isolates almost instantaneously and generates user-friendly, customized reports.

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Identification to the Species Level and Differentiation between Strains of *Aspergillus* Clinical Isolates by Automated Repetitive-Sequence-Based PCR

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A commercially available repetitive-sequence-based PCR (rep-PCR) DNA fingerprinting assay adapted to an automated format, the DiversiLab system, enables rapid microbial identification and strain typing. We explored the performance of the DiversiLab system as a molecular typing tool for 69 *Aspergillus* isolates (38 *A. fumigatus*, 15 *A. flavus*, and 16 *A. terreus* isolates) had been previously characterized by morphological analysis. Initially, 27 *Aspergillus* isolates (10 *A. fumigatus*, 9 *A. flavus*, and 8 *A. terreus* isolates) were used as controls to create a rep-PCR-based DNA fingerprint library with the DiversiLab software. Then, 42 blinded *Aspergillus* isolates were typed using the system. The rep-PCR-based profile revealed 98% concordance with morphology-based identification. rep-PCR-based DNA fingerprints were reproducible and were consistent for DNA from both hyphae and conidia. DiversiLab dendrogram reports correctly identified all *A. fumigatus* ($n = 28$), *A. terreus* ($n = 8$), and *A. flavus* ($n = 6$) isolates in the 42 blinded *Aspergillus* isolates. rep-PCR-based identification of all isolates was 100% in agreement with the contiguous internal transcribed spacer (ITS) region (ITS1-5.8S-ITS2) sequence-based identification of the respective isolates. Additionally, the DiversiLab system could demonstrate strain-level differentiation of *A. flavus* and *A. terreus*. Automated rep-PCR may be a time-efficient, effective, easy-to-use, novel genotyping tool for identifying and determining the strain relatedness of fungi. This system may be useful for epidemiological studies, molecular typing, and surveillance of *Aspergillus* species.

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Identification of *Histoplasma capsulatum*, *Blastomyces dermatitidis*, and *Coccidioides* Species by Repetitive-Sequence-Based PCR

June I. Pounder,[1]* Dewey Hansen,[2] and Gail L. Woods[3]

Associated Regional and University Pathologists, Inc., Institute for Clinical and Experimental Pathology, Salt Lake City, Utah[1]; ARUP Microbiology Laboratory, Salt Lake City, Utah[2]; and Department of Pathology and Laboratory Services, University of Arkansas for Medical Sciences, Little Rock, Arkansas[3]

Received 31 March 2006/Returned for modification 15 May 2006/Accepted 31 May 2006

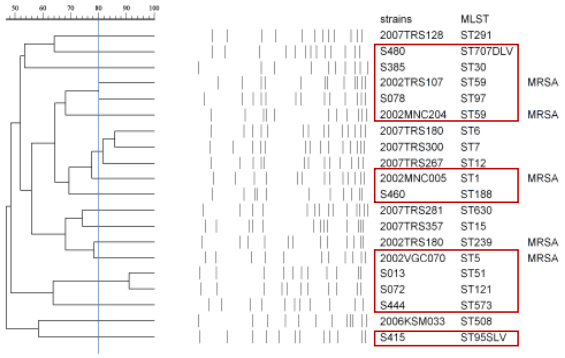
The performance of repetitive-sequence-based PCR (rep-PCR) using the DiversiLab system for identification of *Coccidioides* species, *Blastomyces dermatitidis*, and *Histoplasma capsulatum* was assessed by comparing data obtained to colony morphology and microscopic characteristics and to nucleic acid probe results. DNA from cultures of 23 *Coccidioides*, 24 *B. dermatitidis*, 24 *H. capsulatum*, 3 *Arthrographis*, and 2 *Malbranchea* isolates was extracted using a microbial DNA isolation kit as recommended by Bacterial Barcodes, Inc. Rep-PCR and probe results agreed for 97.2% of the dimorphic fungi when ≥85% similarity was used as the criterion for identification. Two *H. capsulatum* isolates were not identified, but no isolates were misidentified. From 43 of those cultures (15 *Coccidioides*, 14 *B. dermatitidis*, 14 *H. capsulatum*, 3 *Arthrographis*, and 2 *Malbranchea*), DNA also was extracted using an IDI lysis kit, a simpler method. Rep-PCR and probe results agreed for 97.7% of the dimorphic fungi when a criterion of ≥90% similarity was used for identification. One *H. capsulatum* isolate could not be identified; no isolates were misidentified. Using ≥85% similarity for identification resulted in one misidentification. These data suggest that the DiversiLab system can be used to identify *Coccidioides* and *B. dermatitidis* and, possibly, *H. capsulatum* isolates.

研究目的

- 使用rep-PCR評估*S. aureus*之親源比對結果

研究方法

- *Staphylococcus aureus*菌株之DNA – 高醫盧柏樑主任與NHRI陳逢叡博士提供
- 採用菌株編號 - S013, S072, S078, S385, S415, S444, S460, S480, 2002 TRS 180, 2002 MNC 204, 2002 MNC 005, 2002 VGC 070

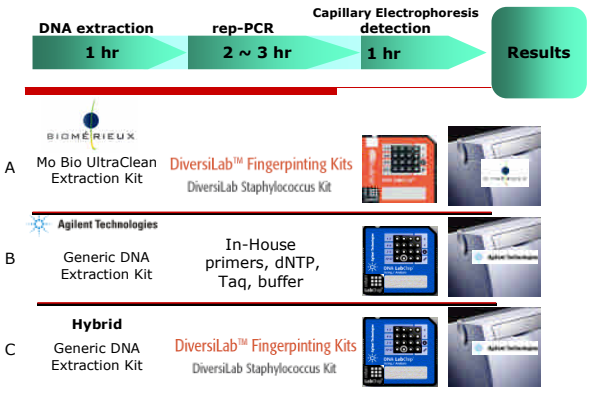


實驗設備

- bioMerieux DiversiLab

研究方法

- 使用bioMerieux DiversiLab系統進行*S. aureus* 菌株親源比對
- 使用Agilent BioAnalyzer 系統進行*S. aureus* 菌株親源比對



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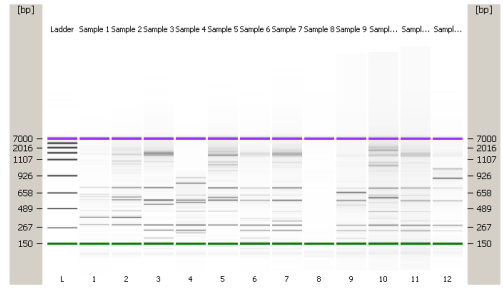
研究結果 – bioMerieux System (A)



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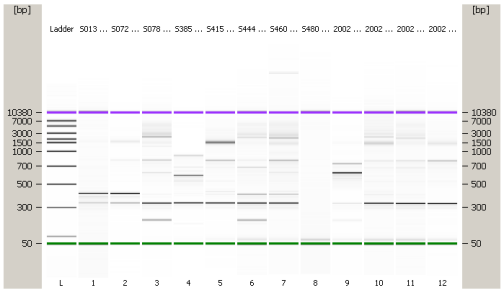
研究結果 – Agilent System (B)



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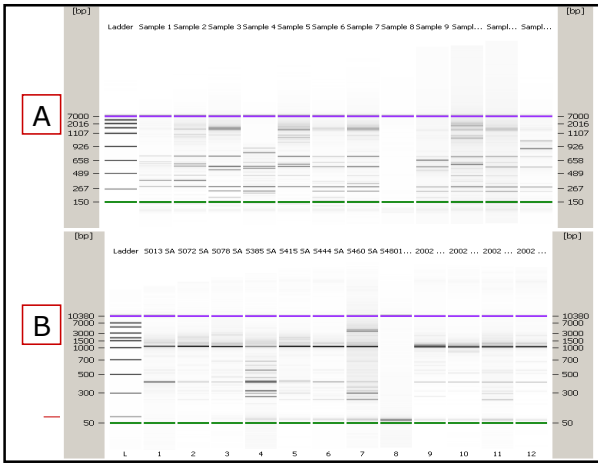
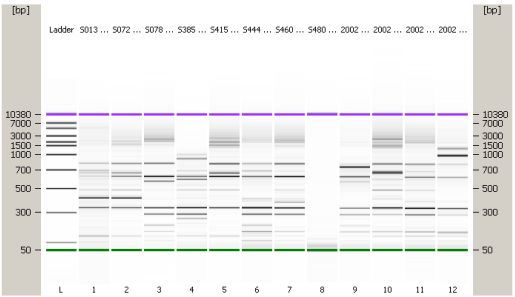


研究結果 – Hybrid (C)



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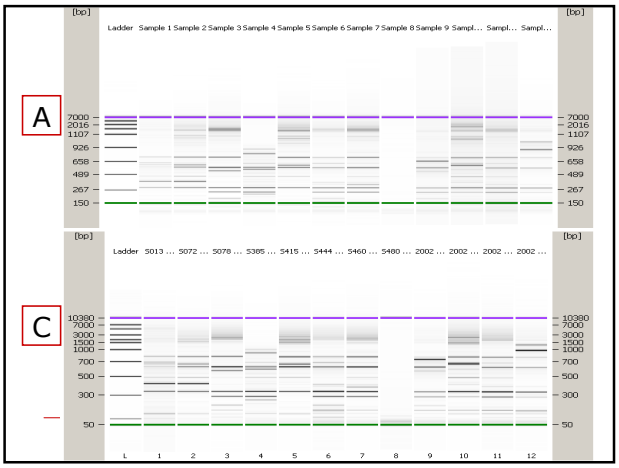
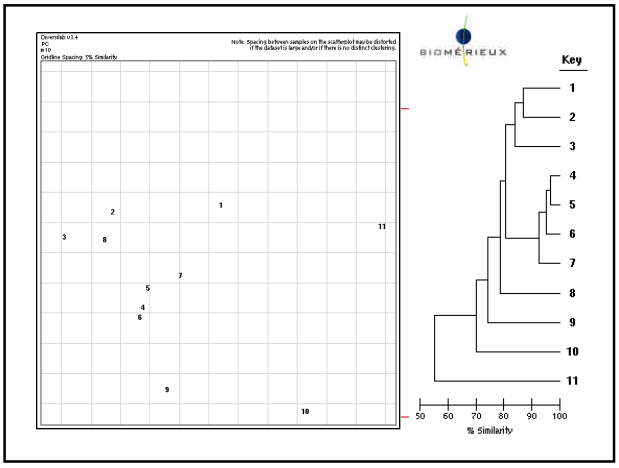


研究結果

- 三種方法皆可將序列片段分出
- 序列片段之比較
  - bioMerieux (A)序列片段較為清楚與豐富
  - Agilent (B) 與in-House rep-PCR reagent 產生之序列片段豐富度較差
  - Hybrid (C) 序列片段與bioMerieux (A)序列片段有些微差距，但豐富度相似



成本分析

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identification of the commonly encountered *Aspergillus* species (5), *Candida* species (2), dermatophytes (10), and *Fusarium* species (4), and, potentially, identification of some mycobacteria (G. Hecox and G. Woods, Abstr. ASM Gen. Meet., abstr. C-026, 2005). For these laboratories, use of the DiversiLab system would be both effective and efficient with respect to cost and time compared to commercial DNA probe methods. The cost (list price) for fungal identification using the mold kit with the DiversiLab system is $27.98 per sample, assuming that a full chip of 13 wells is analyzed. The cost (list price) of the Accuprobe assay, assuming one patient sample and positive and negative controls are tested, is $97.50. The time to a result is slightly longer for rep-PCR: approximately 3.5 h postextraction, including analysis of data, for rep-PCR versus 2.5 h for the DNA probe method.

平均每個檢體之成本分析

國外文獻	國內代理商售價	國內售價 (In House Kit)
美金$27.98 台幣$1,000	台幣$1,000 x N	台幣$600

討論

- rep-PCR+毛細電泳，可以快速地做出*S. aureus*的親源鑑定
  - 院內自行鑑定 – 上午純菌培養出之後，開始進行實驗，下午就可得知親源比對結果
  - 委外鑑定 – 下午用宅配寄出純菌，第二天中午過後就可以知道親源比對結果
- 平均檢體成本如為NT$600 ~ 1,000，與PFGE相當，但時間節省許多，實驗成功率也提高

本次報告pdf下載與更多資訊

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