

利用聚合?連鎖反應偵測臨床葡萄球菌菌株之評估

Evaluation on the detection of clinical *Staphylococcus* isolates using polymerase chain reaction

中文摘要

開業醫師最常使用的廣效性抗生素 cephalosporin 是否可被使用於葡萄球菌之感染，該問題長久以來一直是令人關心的問題，傳統上一般實驗室通常是使用表現型(phenotyping)傳統方法亦即使用 oxacillin 紙錠來鑑定 MRS (methicillin resistant *Staphylococcus*)，進一步以做為監測 cephalosporin 使用的適當性，也因為有的菌株並未被正確的鑑定出來而有一些爭議。

MRS 誤鑑定為 MSS (methicillin susceptible *Staphylococcus*)，結果將會造成治療上的失敗，因而造成 MRS 菌株的擴散；相反地，當 MSS 誤鑑定為 MRS 時也會造成健康者被隔離而造成的一些不必要花費；因此，正確的鑑定出 MRS 是實驗室所必須的。

在本論文中，我們採用 2004 年的 NCCLS 規範，亦即建議使用 ceftazidime 及 oxacillin 來鑑定 MRSA(methicillin resistant *Staphylococcus aureus*)以及 MRCNS(methicillin resistant coagulase negative *Staphylococcus*)菌株。

另外，利用 PCR 測試兩種不同引子(primers)的 *mecA* 基因當作依據 (gold standard)；同時也測定 *clfA* 和 *nucA* 基因來鑑定 *S. aureus* 菌株之正確性，並且利用 Eubacterial 16S rRNA 及 *Staphylococcal* 16S rRNA 都具有之保留區段 (conserved regions)的 house-keeping gene 來確保細菌 DNA 的抽取成功；實驗偵測六個相關性基因直接或間接以得知 Cephalosporin 類藥物是否可有效的使用於治療該菌株的感染。由實驗得知鑑定 *S. aureus* 若能同時使用 Staph Latex Kit 試驗及 DNase 試驗其靈敏度及有效性可由單獨使用 Staph Latex Kit 試驗(分別為 99.2%，99.4%) 提昇到均為 100%；鑑定 MRSA 或 MRCNS 時單獨使用 ceftazidime 紙錠之靈敏度及特異性(分別為 96.1%，100%)優於單獨使用 oxacillin 紙錠(分別為 94.7%，97.8%)，若兩種紙錠同時使用則靈敏度更可提高至 98.7%，特異性維持在 97.8%，精確度提昇至 98.2%。

英文摘要

Whether the widely-effective antibiotics can be used to cure the infection of *Staphylococcus* has been a long-concerned question. Traditionally, investigators in microbiology laboratories use oxacillin to inspect the fitness of using cephalosporin and general laboratories adopt traditional phenotyping to identify MRS (methicillin resistant *Staphylococcus*). But some strains are not correctly identified causing a little confusion.

When MRS is wrongly identified as MSS (methicillin susceptible *Staphylococcus*), it

will lead to the failure of treatment. Furthermore, if there is no efficient infection control, it will cause the spread of MRS strains. When MSS is wrongly identified as MRS, it will cause money waste resulting from unnecessary isolation of healthy people. Therefore, it's necessary to correctly identify MRS.

In this study, we adopt NCCLS guideline of 2004, with which cefoxitin and oxacillin are both used to identify MRSA (methicillin resistant *Staphylococcus aureus*) and MRCNS (methicillin resistant coagulase negative *Staphylococcus*) strains. Besides, Then compare with using PCR technique to identify *mecA* gene as gold standard by two different primer pairs. At the same time, detect *clfA* and *nucA* gene (also by PCR) to monitor the accuracy of *S. aureus* identification, and using the house-keeping gene in the conserved regions of Eubacterial 16S rRNA and *Staphylococcal* 16S rRNA to be a positive control. We can evaluate whether cephalosporin is a efficient treatment of *S. aureus* directly or indirectly by assaying these six related gene. In this report, bother Staph Latex kit and DNase test were evaluated for their sensitivity and specificity on the identification of *S. aureus* by using it separately or combined together. The results showed that the sensitivity and accuracy are 99.2% and 99.4% respectively and could be improved to 100% by combining two tests. For identifying of MRSA or MRCNS, both cefoxitin and oxacillin were tested. The results indicated that in cefoxitin alone, the sensitivity and specificity are 96.1% and 100% respectively and in oxacillin alone the sensitivity and specificity are 94.7% and 97.8% respectively. When two tests were combined, the sensitivity and specificity could be improved to 98.7% and 97.8% respectively and the accuracy could be increased to 98.2%.