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论文

KPC型碳青霉烯酶基因荧光定量PCR快速鉴定

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摘要：

目的 建立一种准确地鉴定肺炎克雷伯菌碳青霉烯酶(KPC)基因的方法。方法 比对11种KPC型碳青霉烯酶基因,在保守区设计引物和探针,优化PCR反应体系,评价特异性、灵敏度和重复性,对样本分离株进行检测,通过测序、药敏实验等验证该方法的正确性。结果 该方法可准确、特异地鉴定KPC型碳青霉烯酶基因,其他不携带该基因的菌株均无阳性结果;阳性质粒、纯培养细菌和模拟样本的灵敏度分别为10 copies/μL、10 CFU/mL和10^2 CFU/mL;检测489株临床痰液样本分离菌,产KPC型碳青霉烯酶的肺炎克雷伯菌阳性7例,大肠埃希菌阳性11例、枸橼酸杆菌阳性1例,与测序结果完全一致。结论 荧光定量PCR法能准确、特异、快速地鉴定KPC型碳青霉烯酶基因,操作简便,可用于产KPC型碳青霉烯酶菌株的临床诊断及流行病学调查,同时,检测结果表明浙江地区目前以携带产KPC-2型碳青霉烯酶为主的菌株。

关键词： 荧光定量PCR KPC型碳青霉烯酶基因 鉴定

Rapid detection of KPC type carbapenemase gene with real time PCR

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Abstract:

Objective To develop a rapid,specific assay for detection of *Klebsiella pneumoniae* carbapenemase (KPC) type carbapenemase gene.Methods The sequences of KPC type carbapenemase gene from 11 kinds of genotypes were aligned,and then the primer pair and probe from conserved sequences were designed.The suitable PCR condition was obtained through systematical optimization of PCR reaction and thereafter the specificity,sensitivity and reproducibility were evaluated.Meanwhile,the assay was applied to detect the isolates from the clinical specimens,and then the validity of the assay was verified through direct sequencing and drug resistance experiment.Results The KPC type carbapenemase gene was identified by real time PCR accurately and quickly.Furthermore,when other strains not containing KPC type carbapenemase gene were detected,no positive results appeared.Consequently,the detection limit for control plasmid,pure culture,and mocked specimen of *Klebsiella pneumoniae* producing KPC type carbapenemase gene was 10 copies/μL,10 colony forming unit(CFU)/mL,and 10^2 CFU/mL,respcetively.When the assay was applied directly to identify the 489 isolates,the results showed that 7 were positive to *Klebsiella pneumoniae*,11 positive to *Escherichia coli* and one positive to *Citrobacter*.The results were the same to the results obtained from direct sequencing assay.Conclusion Real time PCR is a rapid,reliable and easy-to-perform assay for the detection of KPC type carbapenemase gene.and could be applied to clinical diagnosis and investigation of epidemic disease.Meanwhile,the results of direct sequencing indicate that the strains carrying KPC type carbapenemase-2 gene are the main bacteria in Zhejiang region.

Keywords: real time PCR KPC type carbapenemase gene detection

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