Detection of tet(K)and tet(M)in staphylococcus aureus of Asian countries by the polymerase chain reaction

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論文内容の要旨

Abstract

This study describes the use of the polymerase chain reaction (PCR) to detect the *tet* (K) and *tet*(M) tetracycline resistance genes in *Staphylococcus aureus*. Primers based on the DNA sequence of the *tet*(K) and *tet*(M) genes from *S. aureus* were used in the PCR assay to detect the presence of the genes. Results obtained with 215 isolates of *S. aureus* from Asian countries such as Japan, Indonesia, China, Korea and Thailand have revealed that *tet*(K) specifies resistance to tetracycline but not to minocycline, and that *tet*(M) specifies resistance to both tetracycline and minocycline. We observed two different types of clinical isolates of *S. aureus* strains resistant to minocycline and tetracycline: the first carried only the *tet*(M) gene, while the second carried both the *tet*(M) and the *tet* (K)genes. Almost all of the clinical isolates of S. aureus resistant to minocycline and tetracycline obtained in Indonesia, China and Thailand carried both *tet*(M) and *tet*(K) genes, while most of clinical isolates of *S. aureus* obtained in Japan and Korea carried only the *tet*(M) gene.

Introduction

In our previous epidemiological study on *Staphylococcus aureus* obtained in Indonesia during the period between 1986 and 1993, we found that the isolation frequency of tetracycline-resistant strains among clinical isolates was more than 60% and that of minocycline-resistant strains was 30%, and that almost all methicillin-resistant *S. aureus* (MRSA) strains were resistant to minocycline and tetracycline. In contrast, *S. aureus* strains isolated in Japan in 1986 and 1989 were resistant to minocycline and tetracycline at frequencies of only 2.6% and 6.5%, respectively.

Bacteria can become resistant to tetracycline by several approaches: limited access of tetracycline to the ribosome by efflux: the ribosome may be altered to prevent the effective binding of tetracycline, or enzymes may be produced to inactivate tetracycline. The tetracycline-resistance(TCr) determinants in staphylococci were assigned to the classes K, L, M and O. In *S. aureus*, TCr determinants of the classes K and M have been studied in detail. The *tet*(M) determinant is known to protect ribosomes from inhibitory effects of tetracycline, whereas *tet*(K) and *tet*(L) determinants specify membrane-associated efflux systems. The *tet*(M) gene is chromosomal or plasmid-borne, and its gene product mediates resistance to tetracycline and minocycline. The *tet*(K) gene is plasmid-borne and mediates inducible resistance only to tetracycline but not to minocycline. The polymerase chain reaction has been used to determine tetracycline resistance genes in Gram-negative and -positive microorganisms.

The objective of this study was to define the pattern of tetracycline resistance determinant genes *tet*(K) and *tet*(M) in clinical isolates of *S. aureus* obtained in Asian countries.

Materials and Methods

A total of 215 clinical isolates of *S. aureus* obtained in Asian countries were evaluated: 136 strains from Japan, 60 strains from Indonesia, 6 strains from China, 5 strains from Korea and 8 strains from Thailand. Susceptibility and minimal inhibitory concentrations (MICs) were determined in Sensitivity Test agar by the agar dilution method.

Two 18-mer oligonucleotide primers were synthesized based on the DNA sequence of pT181, a tetracycline-resistance plasmid *tet*(K) from S. aureus. Primer K1, 5'-CAGCAGATCCTACTCCTT-3', corresponded to nucleotides 531 to 548 numbering of Khan et. al., and primer K2, 5'-TCGATAGGAACAGCAGTA-3', was complementary to nucleotides 682 to 699. These primers, located within the *tet*(K), were separated by 168 bp. Also two 20-mer oligonucleotide primers for *tet*(M) were synthesized based on the *tet*(M) sequence from *S. aureus* 101: primer M1, including nucleotides 563 to 582 numbering of Mirjana Nesin et al., with the sequence 5'-GTGGACAAAGGTACAACGAG-3', and primer M2 which was complementary to nucleotides 949 to 968 with the sequence 5'-CGGTAAAGTTCGTCACACAC-3'. The *tet*(M) primers produced a predicted fragment of 405 bp. These primers were prepared and designed to be used together in PCR assay.

DNA extracted from *S. aureus* was used directly for the PCR assay. Finally, 10 μl of PCR product was analyzed by 1% agarose gel electrophoresis in Tris borate buffer and stained with ethidium bromide. Following electrophoresis, the band of amplified DNA was visualized under UV light.

Results and Discussion

It was found that 39.1% of *S. aureus* resistant to tetracycline and minocycline isolated from Indonesia carried both the *tet*(K) and *tet*(M) genes. All the *S. aureus* clinical isolates from China and 50% of isolates from Thailand that were resistant to tetracycline

and minocycline carried both the *tet*(K) and *tet*(M) genes. On the other hand, 99.2% of clinical isolates of *S. aureus* strains resistant to tetracycline and minocycline obtained in Japan and all the isolates from Korea harbored only the *tet*(M) gene. A combination of specific primers was designed to detect *tet*(K) and *tet*(M) determinants from *S. aureus* in a single reaction. The pair of primers, K1 and K2, worked only for the *tet*(K) determinant and another pair of primers, M1 and M2, worked only for the *tet*(M) determinant. These combination primers gave an appropriate PCR product for the *tet*(M) determinant with 215 different clinical isolates of *S. aureus* examined. We found that these primers were highly specific to detect the *tet*(K) and *tet*(M) genes in *S. aureus*. There was an excellent correlation between the resistant phenotypes of the strains studied, as determined by MICs, and the genotypes inferred from PCR experiments using this combination of specific primers. *S. aureus* strains harboring *tet*(K) were resistant to tetracycline and susceptible to minocycline, and those harboring *tet*(M) were resistant to both antibiotics. There was a high MIC of tetracycline against *S. aureus* harboring both *tet*(M) and *tet*(K). In studying clinical isolates of *S. aureus* that were resistant to minocycline and tetracycline obtained in various countries in Asia, it was found that isolates from Indonesia, China and Thailand carried both the *tet*(K) and *tet*(M) genes, while those from Japan and Korea carried only the *tet*(M) gene. The approach using combination primers for *tet*(K) and *tet*(M) in PCR assay has been proven to be useful for epidemiological study to trace strains harboring tetracycline-resistance determinants.

論文審査の結果の要旨

近年，多剤耐性（メチシリン耐性）黄色ブドウ球菌（MRSA）が医学的及び社会的に大きな問題になっており，黄色ブドウ球菌の薬剤耐性機構の詳細な解明の必要性及び広範な疫学調査の必要性が指摘されている。テトラサイクリンあるいはミノサイクリンに対する薬剤耐性を示す黄色ブドウ球菌は，日本に比べて，インドネシアでは非常に高率に検出される。ブドウ球菌のテトラサイクリン耐性決定因子にはK，L，M，Oの４種類があり，黄色ブドウ球菌ではKとMがよく解析されている。それらをコードする遺伝子も同定されており，*tet*(M)遺伝子はテトラサイクリンとミノサイクリンの両者に対する耐性を，また，*tet*(K)遺伝子はテトラサイクリンのみに対する耐性を担うことが知られている。本研究は，アジア諸国で分離された黄色ブドウ球菌における*tet*(M)遺伝子および*tet*(K)遺伝子の保有状況について，PCR法を用いて解析したものである。

申請者は，まず，*tet*(M)遺伝子および*tet*(K)遺伝子のプライマーを作製し，それぞれの遺伝子を特異的に検出するPCR法を確立した。PCR産物の大きさ，制限酵素切断断片多型性（RFLP）解析及びハイブリダイゼーション法による成績に基づいて，本法が鋭敏かつ特異的であることを証明し，さらに，テトラサイクリンおよびミノサイクリンに対する従来の感受性試験の成績と非常に良く相関することを示した。これらの成績は本PCR法の正当性を裏づけるものであり，疫学調査における本法の使用に理論的根拠を与えるものである。

以上のようにして確立されたPCR法を用いて，インドネシア，タイ，中国，韓国及び日本で分離された黄色ブドウ球菌の*tet*(M)遺伝子及び*tet*(K)遺伝子の保有状況について調べたところ，日本や

韓国ではテトラサイクリンとミノサイクリンに耐性の黄色ブドウ球菌のうちほとんどのものが*tet*(M)遺伝子のみを有するのに対して，インドネシア，タイ，中国では*tet*(M)遺伝子と*tet*(K)遺伝子の両方をあわせ持つものが多くみられることがわかった。*tet*(K)遺伝子のみを有する菌株は，いずれの国においても全くみられなかった。また，上記の*tet*(M)遺伝子及び*tet*(K)遺伝子保有状況による黄色ブドウ球菌の分類は，コアグラーゼ型別による分類とも良く相関していた。

以上，本研究は，インドネシア，タイ，中国，韓国及び日本における黄色ブドウ球菌の*tet*(M)遺伝子及び*tet*(K)遺伝子の保有状況について解析したものであるが，従来ほとんど行われていなかった東南アジア地域の黄色ブドウ球菌の薬剤耐性遺伝子の動向について重要な知見を得たものとして価値ある集積であると認める。よって，本研究者は，博士（医学）の学位を得る資格があると認める。