Letters to the Editor

Sporadic Cases of Staphylococcus aureus Organisms Negative for a Species-Specific 442-Base Pair Chromosomal Fragment

Staphylococcus aureus has been well known as a major pathogen for many years, causing a variety of nosocomial infections. The rapid and reliable detection of methicillin-resistant S. aureus (MRSA) organisms is of utmost importance to prevent the spread of infection. Species identification is primarily based on biochemical characteristics of cultured organisms, but to increase accuracy of identification and to obtain reliable results more rapidly, genotypic methods are increasingly used for species determination and detection of the methicillin resistance gene mecA. Since up to 70% of the coagulase-negative staphylococci are carrying the mecA gene, species confirmation is an essential step in the genotypic detection of MRSA.

From November 2001 to July 2002 we used a recently published rapid-cycle real-time PCR protocol (3, 4) to screen approximately 4,500 isolates of putative S. aureus organisms cultured from a variety of clinical specimens for the presence of a species-specific 442-bp chromosomal fragment (2) and the mecA gene. Bacterial colonies subjected to PCR were predominantly selected on morphological criteria and by applying the Staphaurex Plus system (Murex Diagnostica, Burgwedel, Germany). In the case of a negative PCR result for the S. aureus-specific target, the absence of S. aureus organisms was further confirmed by a series of biochemical tests including coagulase activity, Apistaph (bioMerieux, Nuertingen, Germany), and Vitek (bioMerieux). When species identification was still inconclusive, the complete sequence of the bacterial 16S ribosomal DNA (rDNA) gene of the respective isolate was determined and aligned with GenBank and an in-house database.

In the period of the study, we encountered two staphylococcal isolates which tested negative for the species-specific 442-bp chromosomal fragment but were coagulase positive and presented a pattern of biochemical reactions typical for S. aureus. For both isolates complete homology to S. aureus GenBank sequence entries was observed within the determined 1,490-bp region of the bacterial 16S rDNA. Positive PCR results for the gene encoding the thermostable nuclease (nuc) were further supportive of the presence of S. aureus organisms (1).

This observation is an example of the fact that the natural diversity of clinical isolates may also be reflected on the genomic level. The more clinical isolates that are tested for a given target sequence, the more the nucleotide mutations or deletions that may be encountered. This also has implications for the design of specificity panels in the course of assay development: the panel of deposited type strains does not necessarily cover the spectrum of variant isolates which may be found in clinical samples.

Despite the occurrence of two false-negative results, the sensitivity of the species-specific chromosomal fragment assay remained high at 99.9% (4,498/4,500). Incorporation of an internal control, such as the gene for thermostable nuclease, in assays as an additional quality control measure of the efficiency of the PCR should also be considered.

REFERENCES

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