**Streptococcus pneumoniae** Endocarditis: Persistence of DNA on Heart Valve Material 7 Years after Infectious Episode

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We amplified by PCR and sequenced *Streptococcus pneumoniae rpoB* from DNA of the cardiac valve of a man who had presented with pneumococcal endocarditis 7 years earlier. Histopathologically, the valve did not show evidence of endocarditis. This case raises the question of persistence of DNA without any evidence of infection.

The use of a broad-range bacterial PCR followed by direct sequencing has been successful in the detection of bacterial DNA in excised cardiac tissues of patients with infective endocarditis (IE) (2, 10). 16S rRNA gene amplification by PCR and sequencing were performed on two valves sampled at 7-year intervals in a case of *Streptococcus pneumoniae* endocarditis. The first valve was obtained after an episode of Pneumococcus IE, and the second was obtained after replacement of the bioprosthetic valve for hemodynamic reasons.

**Case report.** A 48-year-old alcoholic man with a bicuspid aortic valve was admitted in 1994 for endocarditis with meningitis and a cerebral abscess. An aortic vegetation was found, and all three of the blood cultures performed yielded *S. pneumoniae*. He was treated with ceftriaxone (4 g/day) and thiophenicol, which was replaced with gentamicin (240 mg/day) after 1 week. Worsening aortic insufficiency and persistent fever led to replacement of the aortic valve by pulmonary homograft 15 days later. Macroscopic examination showed a big aortic vegetation (4 cm) with valve destruction and a septal abscess. Microscopic examination showed features typical of IE. Bacterial cultures of the valve were negative.

Ceftriaxone (4 g daily) was prescribed for 1 month and then changed to oral amoxicillin (6 g/day) for 1 month. He was monitored as an outpatient with cardiac echography and blood tests 3 and 6 months later and was considered cured. In February 2002, the aortic bioprosthesis was replaced with a mechanical prosthesis because of progressive heart failure. The patient was not febrile, and his white blood cell counts, erythrocyte sedimentation rate, and C-reactive protein levels were normal. Three blood cultures were negative. Macroscopic examination of the valve showed aortic homograft degeneration and calcifications, but no inflammatory cells were found. Gram staining and culture were negative. The two valves were tested at 7-year intervals for bacterial DNA by means of a universal PCR targeting eubacterial 16S rRNA genes. DNA was extracted with Qiagen columns (QIAamp tissue kit; QIAGEN, Hilden, Germany) in accordance with the manufacturer’s instructions. PCR amplification with broad-range 16S rRNA gene primers (24) (Table 1), sequencing, and purification of PCR products were performed as previously described (15). After amplification and sequencing of the PCR products, the sequences were compared with those in DNA databases with the BLAST 2.0 program (National Center for Biotechnology Information). The 1995 cardiac valve amplification product showed a nucleotide sequence 100% identical to the 16S rRNA gene sequence of *S. pneumoniae* (556 of 556 basepairs [bp]; GenBank accession no. AE008552), 99% identical to the sequence of *S. mitis* (553 of 556 bp; GenBank accession no. AF479580), and 99% identical to the sequence of *S. oralis* (553 of 556 bp; GenBank accession no. AF003932). The 2002 cardiac valve amplification product showed a nucleotide sequence 95% identical to that of *S. mitis* (426 of 448 bp; GenBank accession no. AF543291), 95% identical to that of *S. oralis* (427 of 449 bp; GenBank accession no. AF003932), and 94% identical to that of *S. pneumoniae* (424 of 449 bp; GenBank accession no. AE008552). Alignment of the amplified 16S rRNA sequences from the valves showed just five different sites. Partial amplification of rpoB performed on the two valve specimens then yielded an 807-bp nucleotide fragment for the valve removed in 1995 and an 811-bp nucleotide fragment for the valve removed in 2002; both were 95% identical to the GenBank *S. pneumoniae* sequence (722 of 724 bp; GenBank accession no. AE074786), and these sequences were 99.9% similar (just one site was different). These sequences showed 93 and 94% (1995 and 2002 valves, respectively) homology with the *S. mitis rpoB* sequence (GenBank accession no. AF194518) and 92% homology with the *S. oralis rpoB* sequence (GenBank accession no. AF194520). This was confirmed with other primers that amplify the *gki* gene (7) (Table 1), which has 99% homology to the sequence of the *S. pneumoniae gki* sequence from the 2002 valve (394 of 397 bp; GenBank accession no. AJ232335).

Pneumococcal endocarditis has become a rare illness in the antibiotic era, causing 1 to 3% of all cases of native-valve endocarditis (1), and 0.8 to 3.4% of pneumococcal bacteremia is estimated to result in endocarditis (3, 4). Despite the availability of penicillin, the mortality rate associated with this disease remains high, with case fatality rates ranging from 28 to 60%.

Molecular techniques using broad-range primers provide a sensitive diagnostic test. However, false-positive results can...
sometimes be generated. This can occur by amplification of environmental contaminants or previously amplified products (amplicon carryover) that may be present in a laboratory in which PCRs are frequently performed. No other PCRs of S. pneumoniae sequences were conducted in our laboratory for several months, thus excluding amplicon contamination, and all negative controls (negative valve tissue) were negative. Moreover, we tested three different genes for S. pneumoniae that were positive in the two valves and the sequences were very similar to each other. On the basis of 16S rRNA gene sequence analysis, species of the genus Streptococcus were separated into six major clusters or species groups (15). Within these clusters, the S. mitis group contained several clinically important species, such as S. pneumoniae, S. mitis, S. oralis, and S. sanguinis. However, S. oralis and S. mitis are most closely related on the basis of 16S rRNA and shared more than 99% sequence identity with S. pneumoniae, although DNA-DNA similarity values for the entire chromosome are estimated to be less than 60% (17). It often seems difficult to identify precisely some strains in this group. However, S. pneumoniae was identified by blood culture in 1995 in a patient with a clinical presentation compatible with an invasive S. pneumoniae infection. The sequence amplified from the 2002 valve was very similar to the sequence amplified from the 1995 valve, which showed 100% homology to the S. pneumoniae 16S rRNA sequence. The rpoB sequences obtained from the two valves were strongly similar and yielded the best identity with S. pneumoniae. We also tested the gki gene because it was considered likely to be one of the most polymorphic within the pneumococcal population (7). This result suggests that the DNAs amplified from the two valves are very close and that the best identification obtained with these three genes is S. pneumoniae, within the limits of our knowledge and the sequences available in the GenBank database.

It has been suggested that molecular diagnosis should be included as an additional major criterion in the Duke classification scheme (20). However, the period during which it is possible to detect the causative agent after IE is not known and this could impair the predictive value of these methods for IE.

Gauduchon et al. amplified S. mutans 16S rRNA genes from the valve of a patient with IE and blood cultures positive for Escherichia coli. Thirty-one months before, he had experienced an episode of IE with a blood culture positive for S. mutans (9). He was cured after a course of antibiotics. The discordance between the presence of microorganisms in blood cultures and the results of PCR tests raises the question of the significance of PCR results; thus, the role of S. mutans in the second episode must be questioned.

Our case raises the question of whether this observation indicates recurrence of the patient’s prior S. pneumoniae infection or the presence of DNA without other significance.

To our knowledge, only three cases of recurrent pneumococcal endocarditis have been published, all within 2 years (5, 11, 18). However, in our case, IE was excluded. We therefore believe that the DNA detected here does not predict the occurrence of IE. One disadvantage of DNA-based methods is that they do not distinguish between living and dead organisms (16), limiting their use for monitoring purposes. Josephson et al. (13) performed PCR amplification of DNA from cells killed by boiling or exposure to UV light, while Masters et al. (19) showed that there was no relationship between viability and PCR detection of DNA targets in Listeria monocytogenes or E. coli that had been exposed to heat, acid, hydrogen peroxide, drying, or starvation. In the latter study, even autoclaved cells provided positive PCR signals. PCR methods can produce result that can sometimes be difficult to interpret through the amplification of target DNA or rRNA gene sequences from nonviable bacteria (22). The half-life of DNA in dead bacterial cells may vary greatly and is highly dependent on environmental conditions. DNA persists in tissues, as described for Borrelia burgdorferi DNA detected in the muscle of four of eight patients with chronic myalgia (8). However, positive PCR assays represent detection of genetic sequences of DNA that may or may not be part of living, dormant, or latent organisms (21, 23). In one study in which blood samples from healthy subjects were examined by PCR, 17% were positive for S. pneumoniae DNA (from children), suggesting an invasive infection without any clinical correlation (6). Molecular methods, which provide a direct measurement of bacterial metabolism, can potentially circumvent this problem. The use of mRNA species as a marker for bacterial viability seems to be interesting. Prokaryotic mRNA, in contrast to DNA and rRNA, is rapidly degraded, with a typical half-life of 3 min. As a result, an mRNA-based amplification assay is likely to detect only viable

## TABLE 1. Target genes and primers used for PCR analysis of excised valves

<table>
<thead>
<tr>
<th>Gene (reference)</th>
<th>Primer (5′-3′ sequence)*</th>
<th>Location of primer (S. pneumoniae TIGR4 genome)</th>
<th>Fragment size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>16S rRNA (24)</td>
<td>fD1 (AGAGTTTGATCCTGGCTCAG) rP2 (ACGGCTACCTTGTTACGACTT)</td>
<td>1975719–1975700 1974211–1974231 1913703–1913684 1912195–1912215 15355–15374 16863–16843</td>
<td>1,496 1,496 1,496</td>
</tr>
<tr>
<td>rpoBb</td>
<td>StrpF-Ecoq (TGIArTTTrTCATCAACCATGTG) StrpR-Ecoq (AARYTGGMCTGAAGAATAA)</td>
<td>4279–4296 5013–4994</td>
<td>590</td>
</tr>
<tr>
<td>gki gene (7)</td>
<td>gki-up (GGCATCTGGAAATGGGATGACC) gki-dn (TCTCCCCGCAGCTGACAC)</td>
<td>9497–9516 10122–10106</td>
<td>698</td>
</tr>
</tbody>
</table>

* i, inosinate; r, A or G; Y, C or T; M, A or C.

b Primers were chosen after comparison of sequences from Staphylococcus aureus, Listeria monocytogenes, Enterococcus faecalis, and Streptococcus pyogenes.
organisms and be a good indicator of susceptibility to antimicrobial drugs and/or chemotherapeutic efficacy, as studies of Mycobacterium tuberculosis have suggested (12, 14).

In conclusion, DNA from the causative agent of IE may persist in the valves of patients for years without any evidence of infection.

REFERENCES