Rapid Detection of Bacterial DNA in Mastoid Granulation Tissue with Nested-PCR Technique

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Abstract

Objective: To detect bacterial DNA in mastoid granulation tissue from patients with chronic suppurative otitis media (CSOM).

Material and Method: A two-step polymerase chain reaction (nested polymerase chain reaction) technique was employed. A 16s rRNA universal primer common to all bacteria was used as a bracket primer for the first step PCR reaction. Primers specific to P. aeruginosa and S. aureus were then used as nested primers for the second step PCR. Products of this process were identified by DNA sequencing.

Results: Among 15 clinical specimens collected, five showed positive bands specific to the species P. aeruginosa, and 11 showed bands specific to the genus Staphylococcus. DNA sequencing showed 99.7 to 100% accuracy for target organisms in clinical specimens with a positive signal. The average time taken to conduct the PCR procedure was about four hours

Conclusion: The nested PCR technique described worked well, even when the size of the mastoid granulation tissue was very small.

Keywords: Polymerase chain reaction, Bacteria, Chronic suppurative otitis media

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