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Use of 16S ribosomal (rRNA) genes and immunofluorescence microscopy to detect prosthetic hip infection

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Although aseptic mechanical loosening is reported to be the most common cause of prosthetic joint failure (Fitzgerald 1992), we have recently shown that the incidence of prosthetic hip infection is greatly underestimated (Tunney et al 1998). In our study, the use of mild ultrasonication to dislodge bacteria growing within adherent biofilms on the surface of prostheses resulted in bacteria being cultured from 22% of retrieved prostheses (26/120 implants). Review of the notes from 18 of these 26 individuals revealed that infection prior to revision was suspected in only 6 cases and that in only 2 of these cases were bacteria cultured from the pre-operative aspirates or tissue removed at the time of surgery. Parallel examination of the inflammatory response in associated tissues showed that inflammatory cells indicative of infection were present in all of the patients from whom bacteria were cultured. Examination of associated tissue samples taken from culture-negative patients revealed a similar inflammatory response in 87% of patients. This implies that the detection of infection by culture may still be an underestimate of the real infection rate. The aim of the present study was, therefore, to determine if the detection of prosthetic hip infection could be improved by using 16S rRNA gene probes and immuno-labelling in conjunction with fluorescence microscopy.

Bacterial DNA was extracted from sonicates which had been stored at -70°C following ultrasonication of the retrieved implants. The 16S rRNA gene was detected by electrophoresis following polymerase chain reaction (PCR) using oligonucleotide primers specific for the eubacterial domain. In parallel, samples were screened by immuno-labelling and fluorescence microscopy (Patrick et al 1995) using monoclonal antibody hybridoma secreting cell lines which are specific for *Propionibacterium acnes* surface antigens and polyclonal antisera specific for *P. acnes*, *Staphylococcus aureus* and *S. epidermidis*.

Bacterial 16S rRNA genes were detected in all 26 culture-positive implants and in 64 of the 94 (68%) culture negative implants (Table 1). The PCR results related well to the presence of inflammatory cells in associated tissue with 55 of the 73 tissue samples positive for the presence of inflammatory cells having corresponding implants which were PCR positive. Bacteria were detected in both culture-positive and culture-negative samples using immunolabelling and fluorescence microscopy.

Table 1. Detection of 16S rRNA genes

		Culture	
		positive	negative
PCR	positive	26	64
	negative	0	30

In this study, the detection of bacteria in sonicate from patients whose implants were culture-negative using immunological and genetic probes suggests that these implants may have been infected by bacteria which were not isolated previously by microbiological techniques. The use of these techniques in association with culture techniques to detect prosthetic hip infection in clinical diagnostic practice is feasible and, therefore, warrants further evaluation.

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