TABLE E-1 Characteristics of Oligonucleotide Primers and Probes Used in the PCR-RLB

Oligonucleotide Name	Sequence	Target	Pmol of Oligonucleotide per Lane on Membrane	Nucleotide Position in the 16S rRNA Gene
Primers				
16S8-27F	AGAGTTTGATCMTGGCTCAG	Eubacteria		8-27
B-16S509-525R*	GCTGCTGGCACGDAGTT	Eubacteria		509-525
Probes**				
A-16S A339	TCCTACGGGAGGC(AT)GCA	Eubacteria	25 & 50	339-355
A-16S CAMPY113	GTGAGTAAGGTATAGTTAATC	Campylobacter genus	200	113-133
A-16S CORYN451	CGAAGCTTTTGTGACGG	Corynebacterium genus	20	451-467
A-16S Eaero474	GGCGATTGACGTTACTC	*Enterobacter aerogenes*	100	474-491
A-16S Eaggl471	CCTGTCGATTGACGTTACC	*Enterobacter agglomerans*	200	471-489
A-16S ECEAE136	CTGATGGAGGGGGATAA	Enterobacteriaceae group	1500	136-153
A-16S Ecoli451	AAGGGAGTAAAGTTAATACCT	*Escherichia coli*	50	451-471
A-16S Efaec220	TTTCGGGTGTCGCTGAT	*Enterococcus faecalis/faecium*	100	220-236
A-16S ENTER448	AGGAAGGTGTTGTGGTTAA	Enterobacter genus	50	448-467
A-16S Fmagn210	CAAAGATTTATCGGTCATAGA	*Finegoldia magna (Peptostreptococcus magnus)*	1500	210-230
A-16S Hpylo82	CTTGCTAGAGTGCTGATTA	*Helicobacter pylori*	1500	82-100
A-16S Kpneu73	CACAGAGAGCTTGCTCT	*Klebsiella pneumoniae*	1500	73-90
A-16S Mpneu75	TAGTAATACTTTAGAGGCGAA	*Mycoplasma pneumoniae*	1000	75-95
A-16S Mtube211	CTTTAGCGGTGTGGGAT	*Mycobacterium tuberculosis*	800	211-227
A-16S MYCOB72	AAAGGTCTCTTCGGAGATA	Mycobacterium genus	800	72-90
A-16S NEISS120	AACATATCGGAACGTACC	Neisseria genus	400	120-137
A-16S Ngono129	GAACGTACCGGGTAGC	*Neisseria gonorrhoeae*	200	129-144
A-16S Nmeni185	TCTTGAGAGAGAAAGCAGG	*Neisseria meningitidis*	50	185-201
A-16S Pacne69	GAAAGGCCCTGCTTTTG	*Propionibacterium acnes*	1000	69-85
A-16S Pmira461	GGTTAATACCCTTATCAATTGA	*Proteus mirabilis*	200	461-482
A-16S PSEUD141	GGATAACGTCCGGAAAC	Pseudomonas genus	800	141-157
A-16S Pvulg463	TTAATACTCTTAGCAATTGACG	*Proteus vulgaris*	2	463-484
A-16S SALMO452	GGTGTTGTGGTTAATAACC	Salmonella genus	400	452-470
A-16S Saure195	CATGGTTCAAAAGTGAAAGAC	*Staphylococcus aureus*	400	195-215
A-16S Spyog85	TAACGCATGTTAGTAATTTAAAAG	*Streptococcus pyogenes*	50	85-108
A-16S STAPH129	AACCTACCTATAAGACTGG	Staphylococcus genus	400	129-147
A-16S STREP142	GCGGGGGATAACTATTG	Streptococcus genus	750	142-158
A-16S TpD	CCGCTTCTATGTGACAG	Spike DNA	25 & 100	
Hybridization control				
B-A339/Salg/Ngon101	GCTACCCGGTACGTTCCAATAGTTATCCCCCGCTGC			
Spike oligonucleotide				
TpD spike	AGAGTTTGATCATGGCTCAGCCGCTTCTATGTGACAGAACTTCGTGCCAGCAGC			

*Reversed primer is labeled with a 5'-biotin label.

**All oligonucleotide probes are labeled with a 5'-C6-aminolinker.

TABLE E-2 Species Identification by Culture, PCR-RLB, and DNA Sequencing of PCR Products in 76 Patients

Culture	No. of Patients (%)	RLB	No. of Patients (%)	Sequencing of RLB Samples	No. of Patients (%)
S. aureus	8 (11)	*S. aureus*	10 (13)	*S. aureus*	8 (11)
				Not determinable	2 (3)
Coagulase-negative staphylococci	5 (7)	Staphylococcus genus	4 (5)	*S. epidermidis*	2 (3)
S. lugdunensis	1 (1)			*S. lugdunensis*	1 (1)
No specification	4 (5)			Not determinable	1 (1)
Streptococcus spp.	5 (7)	Streptococcus genus	8 (11)	*S. dysgalactiae*	3 (4)
α-hemolytic streptococci	2 (3)			*S. mitis*	2 (3)
β-hemolytic streptococci	2 (3)			Not determinable	3 (4)
S. oralis	1 (1)				
E. faecalis	1 (1)	*E. faecalis/faecium*	1 (1)	*Haemophilus influenzae*	1 (1)
E. cloacae	1 (1)	*E. agglomerans*	2 (3)	*E. cloacae*	1 (1)
				Not determinable	1 (1)
P. canis	1 (1)	Eubacteria	2 (3)	*P. canis*	1 (1)
H. influenzae	1 (1)			*H. influenzae*	1 (1)
		Enterobacteriaceae	1 (1)	Not determinable	1 (1)
		Corynebacterium genus	1 (1)	*C. xerosis*	1 (1)
		P. acnes	1 (1)	*P. acnes*	1 (1)
Mixed culture	4 (5)	Mixed culture	5 (7)		
M. morganii & Enterococcus spp. & *P. aeruginosa*	1 (1)	*E. agglomerans* & *E. faecalis* & *P. aeruginosa*	1 (1)	*M. morganii*	1 (1)
E. coli & Enterococcus spp.	1 (1)	*E. coli* & *E. faecalis*	1 (1)	*E. coli*	1 (1)
M. tuberculosis & *S. aureus*	1 (1)	*M. tuberculosis* & *S. aureus*	1 (1)	Not determinable	1 (1)
α-hemolytic streptococci & *S. aureus*	1 (1)	Streptococcus genus & Staphylococcus genus	1 (1)	*S. dysgalactiae*	1 (1)
		E. faecalis/faecium & Staphylococcus genus	1 (1)	*S. epidermidis*	1 (1)
C. albicans	1 (1)				
Negative	49 (64)	Negative	41 (54)		

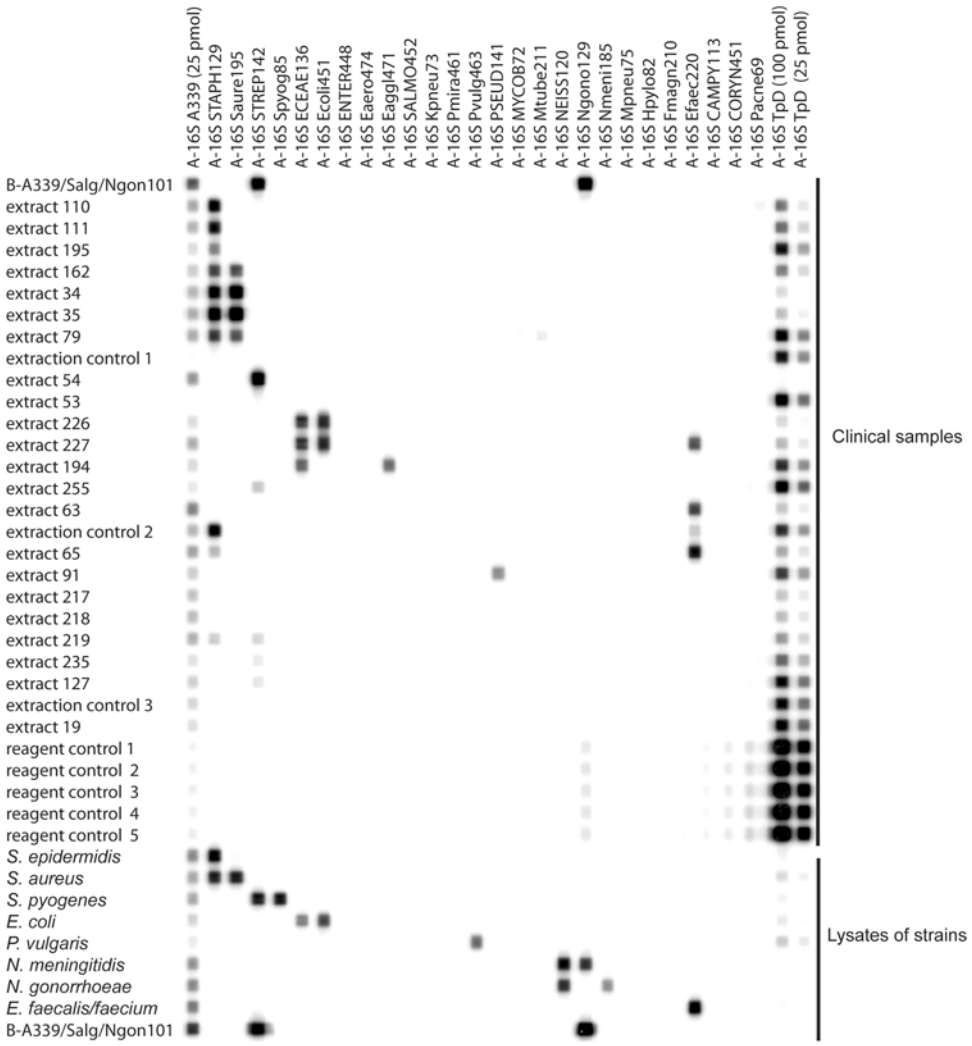

Fig. E-1

Reverse line blot analysis of 16S gene amplicons from patients in whom an orthopaedic infection was suspected on the basis of patient data. Polymerase-chain-reaction products obtained from patients and controls were applied in the rows and oligonucleotide probes, in the columns. The specificities of the oligonucleotide probes can be found in Table E-1. In the first and last row, a biotin-labeled oligonucleotide reacting with three different oligonucleotide probes was included as a positive hybridization control. The eight lanes at the bottom of the blot display results obtained with lysates of a number of bacterial species. Extraction control 2 exemplifies a false-positive extraction control. Extraction control 3 shows weak reactivity with the eubacterial probe, illustrating the normal background reactivity with this probe in the assay. Extract = polymerase chain reaction on extracts from clinical samples, extraction control = polymerase chain reaction on negative controls obtained during DNA extraction from clinical samples, and reagent control = negative control obtained by polymerase chain reaction on reagents only.