JOURNAL OF CLINICAL MICROBIOLOGY, Mar. 2006, p. 1018–1028 Vol. 44, No. 3
0095-1137/06/$08.00+0 doi:10.1128/JCM.44.3.1018–1028.2006

Analysis of 525 Samples To Determine the Usefulness of PCR Amplification and Sequencing of the 16S rRNA Gene for Diagnosis of Bone and Joint Infections

Florence Fenollar,[1,2] Véronique Roux,[1,2] Andréas Stein,[1] Michel Drancourt,[1,2] and Didier Raoult[1,2]*

Unité des Rickettsies, CNRS UMR 6020, IFR 48, Faculté de Médecine, Université de la Méditerranée, Marseille, France,[1] and Fédération de Microbiologie Clinique, Assistance Publique-Hôpitaux de Marseille, Hôpital de la Timone, Marseille, France[2]

Received 23 August 2005/Returned for modification 5 October 2005/Accepted 22 November 2005

The 16S rRNA gene PCR in the diagnosis of bone and joint infections has not been systematically tested. Five hundred twenty-five bone and joint samples collected from 525 patients were cultured and submitted to 16S rRNA gene PCR detection of bacteria in parallel. The amplicons with mixed sequences were also cloned. When discordant results were observed, culture and PCR were performed once again. Bacteria were detected in 139 of 525 samples. Culture and 16S rRNA gene PCR yielded identical documentation in 475 samples. Discrepancies were linked to 13 false-positive culture results, 5 false-positive PCR results, 9 false-negative PCR results, 16 false-negative culture results, and 7 mixed infections. Cloning and sequencing of 16S rRNA gene amplicons in 6 of 8 patients with mixed infections identified 2 to 8 bacteria per sample. Rarely described human pathogens such as *Alcaligenes faecalis*, *Comamonas terrigena*, and 21 anaerobes were characterized. We also detected, by 16S rRNA gene PCR, four previously identified bacteria never reported in human infection, *Alkanindiges illinoisensis*, dehydroabietic acid-degrading bacterium DhA-73, unidentified Hailaer soda lake bacterium, and uncultured bacterium clone HuCa4. Seven organisms representing new potential species were also detected. PCR followed by cloning and sequencing may help to identify new pathogens involved in mixed bone infection.

The accurate diagnosis of bone and joint infections, including arthritis, osteomyelitis, and infections on orthopedic implants, has long been confounded by the difficulty of retrieval and detection of microorganisms (33, 52, 66). Gram-staining and routine culturing assays led to incomplete microbiological diagnosis (66). Treatment was therefore performed on an empirical basis with a poor prognosis (33, 52, 66). PCR amplification of the 16S rRNA gene is now an established technique for the detection of bacteria in clinical samples in cases of endophthalmitis (26), meningitis (41), and endocarditis (5).

As for the use of 16S rRNA gene-based detection of bacteria in bone and joint infections, several protocols have been proposed: (i) 16S rRNA gene amplification without identification by sequencing of the pathogen (60); (ii) 16S rRNA gene amplification followed by Southern hybridization (37); (iii) 16S rRNA gene amplification followed, when a positive PCR is obtained, by a multiplex PCR using mixtures of species-specific primer sets for species identification (36). All of these strategies present a similar problem because primer-dependent, amplification-based methods or postamplification probe detection strategies are not able to provide pathogen species identification of rare microorganisms for which there is limited sequence information.

16S rRNA gene PCR and sequencing has been applied only sporadically to obtain diagnoses of bone or joint infections caused by *Kingella kingae*, *Mycobacterium* spp., or *Helicobacter* spp. (20, 24, 40, 56, 58, 61) or to investigate pathogens associated with destabilized hip prostheses (27). This approach has been successfully applied, mainly on cardiac valves, for the diagnosis of endocarditis, allowing the detection of bacteria despite antibiotic therapy and for negative cardiac valve culture (5, 19, 50) due to fastidious bacteria such as *Mycoplasma* spp. or *Tropheryma whipplei* (15, 17). The use of 16S rRNA gene PCR has also been successfully applied to the microbiological diagnosis of salpingitis, leading to the identification of novel microorganisms (22).

In our laboratory, this technique is routinely applied to various samples for the diagnosis of bacterial infection. Here, we report the use of our strategy of 16S rRNA gene PCR amplification followed by sequencing of all amplified products for diagnostic purposes on a large series of 525 bone and joint samples. Few differences were observed in our studies between culture and PCR. This approach led mainly to the detection of previously unknown pathogens, some of them potential new bacterial species, in mixed infections.

MATERIALS AND METHODS

Patients. This study included 525 bone and joint samples collected from 525 patients. They were prospectively analyzed at Marseilles University Hospital from November 2003 to September 2004. All of the samples were taken for diagnostic purposes. In each case, a physician requested the PCR assays and standard culture.

Clinical sample collection. Specimens collected by needle aspiration or by surgical biopsy were placed into sterile tubes. They were shipped to the laboratory within 4 h after collection and divided into 3 parts: for culture, for PCR, and for storage at −80°C.

Bacterial isolation and identification. Direct microscopic examination of the pus after gram-staining was performed to note the presence of polymorphonuclear leukocytes and bacteria. The specimen was also tested for antibiotic activity in the laboratory as previously described rather than reviewing the records to assess if the patient was given antibiotic therapy (65). Briefly, the antimicrobial activity of the sample was detected by estimating the inhibition of growth of 2

* Corresponding author. Mailing address: Unité des Rickettsies, CNRS UMR 6020, IFR 48, Faculté de Médecine, Université de la Méditerranée, 27 Boulevard Jean Moulin, 13385 Marseille cedex 05, France. Phone: 33 4 91 32 43 75. Fax: 33 4 91 38 77 72. E-mail: Didier.Raoult@medecine.univ-mrs.fr.

TABLE 1. Primers used for broad-range 16S rRNA gene PCR and, according to species identified by sequencing, primers targeting a second gene for confirmation of positive 16S rRNA gene PCR results when discordant results where observed

Microorganism(s)	Targeted sequence	Forward primer (sequence)	Reverse primer (sequence)
Eubacteria	16S rRNA	536F (5′-CAGCAGCCGCGGTAATAC)[a]	rp2 (5′-ACGGCTACCTTGTTACGACTT)[a]
Eubacteria	16S rRNA	M13d (5′-GTAAAACGACGGCCAG)[b]	M13r (5′-CAGGAAACAGCTATGAC)[b]
Staphylococcus aureus	*RpoB*	Saur.Rpob.F (5′-GTTTGAATTGCATGGTAGCGT)	SaurRpob.R (5′-GAAGCAATGATATCTGCTGGTG)
Streptococcus group B	*RpoB*	SagaRpob.F (5′-CAATTGCAGAGCATATCGATGG)	SagaRpob.R (5′-TCCAACAATAGTAACAACACGG)
Streptococcus pneumoniae	*RpoB*	SpneuRpob.F (5′-GGTAGAAGCTGGTACGATTATGAC)	SpneuRpob.R (5′-GATCAGTTGGAGCAACAACCT)
Enterococcus spp.	*RpoB*	StrpF (5′-AARYTIGGMCCTGAAGAAAT)	StrpR (5′-TGIARTTTRTCATCAACCATGTG)
Granulicatella adiacens	*RpoB*	GadiaRpob.F (5′-TGTAACTCTAACACTTGTCCGA)	GadiaRpob.R (5′-GGACGTCACGGTAATAAAGGG)
Staphylococcus epidermidis	*RpoB*	SepiRpob.F (5′-GTGATACGTCCATGTAATCCA)	SepiRpob.R (5′-TTTGACAGCTGATGAAGAGGA)
Enterobacteriaceae	*RpoB*	CM7 (5′-AACCAGTTCCGCGTTGGCCTGG)	CM31b (5′-CCTGAACAACACGGCTCGGA)
Escherichia coli	*RpoB*	EcolRpob.F (5′-TTCACCAACGATCTGGATCAC)	EcolRpob.R (5′-GAAGAACAGGTTCTCGAACAG)
Pseudomonas aeruginosa	*RpoB*	PaerRpob.F (5′-TGTACACCAACGACATCGAC)	PaerRpob.R (5′-AAGAACAGGTTGCCGAACAG)
Clostridium perfringens	Eabc[c]	CloPerF (5′-GACACATTAGTTGGGAAGTG)	CloPerR (5′-GTTGTTAACCAATGAGCAGC)
Propionibacterium acnes	16S-23S rRNA	PacnF (5′-CTAAGGAGTTTTTGTGAGTGG)	PacnR (5′-CTTTGCACAACACCACGTC)
Prevotella spp.	CfxA2-like β-lactamase	PrevoF (5′-GGATAAACTTGACCCAAAGAC)	PrevoR (5′-GATGTATAGTTAGAGTAAGCC)

[a] The primers were used for both 16S rRNA gene PCR.
[b] The primers were used only for 16S rRNA gene sequencing.
[c] Blood group A- and B-cleaving endo-beta-galactosidase gene.

different bacteria (*Escherichia coli* and *Micrococcus luteus*) inoculated on agar in the presence of the specimen. These 2 bacteria were chosen because of their susceptibility to most antibiotics. Besides, this test allows confirmation that antibiotics penetrate joints or bones. The sample was inoculated onto 5% sheep blood Columbia and chocolate agar (bio-Mérieux, Marcy L'Etoile, France), incubated at 37°C in parallel in a 5% CO_2 atmosphere and an anaerobic atmosphere for 10 days. Pure bacterial cultures, obtained by picking isolated colonies were identified with commercially available biochemical assays and, when needed, with molecular identification methods (14).

Molecular detection. To perform the first PCR assay, bone and joint samples were incubated overnight using proteinase K and lysis buffer at 55°C, and DNA was extracted with a MagNAPure LC instrument and the MagNAPure LC DNA isolation kit II (Roche Molecular Biochemicals, Mannheim, Germany) according to the manufacturer's instruction. If it was necessary to perform a second PCR, the new assay was performed on freshly extracted DNA using an alternative protocol, boiling in 20% Chelex 100 resin for 30 min (Bio-Rad, Hercules, CA) according to the manufacturer's instructions, followed by centrifugation. Finally, DNA was extracted from the supernatant using the MagNAPure LC DNA isolation kit II (Roche) according to the manufacturer's instructions.

Extracted DNA was PCR amplified with the 536F and rp2 (Eurogentec, Seraing, Belgium) primer pair (Table 1) targeting the 16S rRNA gene as previously described (30). A PCR-positive sample collected from a case of culture-proven *S. aureus* infection was used as a positive control, and a mixture of all reagents used for DNA extraction and DNA extracted from normal bone tissue were processed as negative controls. One negative control was included for every 5 samples. PCR and sequencing were performed as previously described (30), using the primers mentioned in Table 1. The procedure was considered unreliable and was done again if amplification of any negative control occurred. All PCR products were sequenced in both directions using the Big-Dye Terminator, version 1.1, cycle sequencing kit (Perkin-Elmer, Coignieres, France) as described by the manufacturer. Sequencing products were resolved using an ABI 3100 automated sequencer (Perkin Elmer). Multisequence alignment was made with CLUSTAL W software, version 1.81 (57). When mixed sequences were observed on the electropherogram (Fig. 1) or when culture and 16S rRNA bacterial identification were discordant, polymicrobial infection was suspected. PCR products were cloned in PGEM-T Easy vector (Promega, Charbonnières, France), as described by the manufacturer. Ten clones were cultivated in LB medium (USB, Cleveland, OH) overnight, and PCR and sequencing were performed as described above. Note, when we have obtained a polymicrobial electropherogram using 16S rRNA gene PCR, we have performed the cloning procedure directly because each time that we have previously performed a new 16S rRNA gene PCR, we have systematically obtained a polymicrobial electropherogram. In addition, we systematically performed the cloning procedure only once because the sequencing is time-consuming and most of the microorganisms that we have identified using this technique have not been previously amplified in our laboratory, reducing the risk of PCR contamination.

Finally, the identification of bacteria was determined by comparing our sequence with that of existing sequences in the GenBank database using the BLAST program available at the National Center for Biotechnology Information website (http://www.ncbi.nlm.nih.gov). A sequence similarity of <97% of the 16S rRNA gene was the criterion used to define a potentially new bacterial species (13, 14, 55).

Phylogenetic analysis of unidentified bacteria. For the isolates which were not identified as a validated species by 16S rRNA gene sequence analysis, taxonomic relationships were inferred from 16S rRNA gene sequence comparisons. Phylogenetic trees were elaborated using the neighbor joining method, as previously described (14).

Interpretation of discordant results. Positive 16S rRNA gene PCR results were considered certain when congruence was observed between the culture and the PCR assays. In the presence of a discordant result between culture and 16S rRNA gene PCR, additional PCR assays were performed using a different DNA extraction protocol, followed by a second 16S rRNA gene PCR and an additional PCR targeting another gene, usually the *rpoB* gene. The new PCR amplification and sequencing were performed using primers mentioned in Table 1. Positive PCR results were considered certain when congruence was observed between the different PCR assays.

Strategies for the reconciliation of culture and PCR assays, when discordant results were observed, are summarized in Fig. 2. The same microorganism had to be isolated at least two times to consider the culture a true positive. If a microorganism was isolated only once among the 3 assays and it was a potential skin contaminant, it was considered a contaminant and a false culture positive. In the case of a negative culture, the same microorganism had to be identified at least twice using two different PCR assays to be considered a true PCR positive. If only

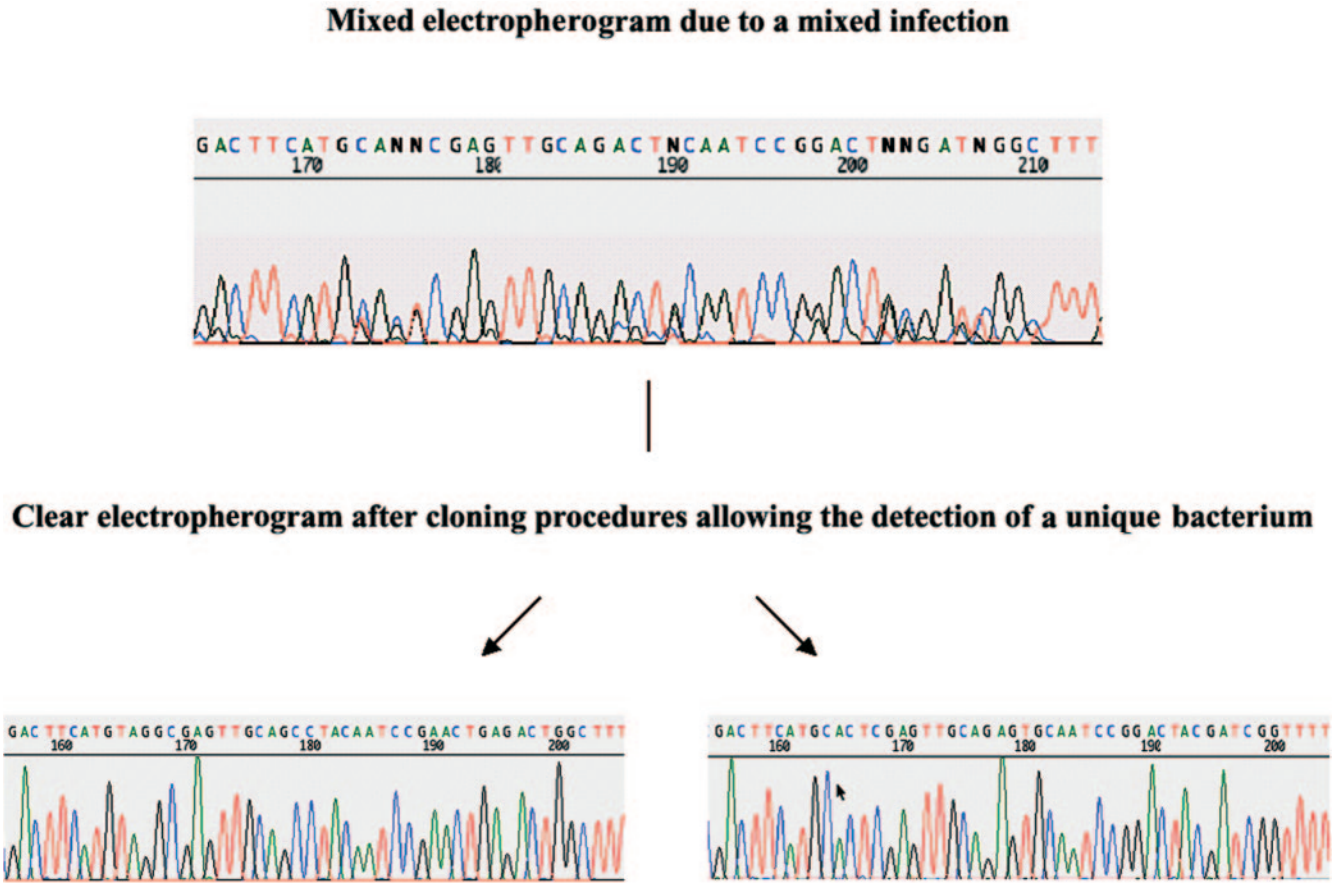

FIG. 1. Mixed electropherogram which indicates polymicrobial infection and should be followed by a systematic cloning step.

one 16S rRNA gene PCR was positive for a potential PCR contaminant, the result was considered a false PCR positive. If only the PCR targeting the second gene was positive and the identified microorganism was the same as that isolated using culture, the PCR assay was considered positive. The efficiency of DNA extraction and the possible presence of inhibitors in the samples which were positive by culture and negative by PCR were tested using primers RS42 (5′-GCT CACTCAGTGTGGCAAAG-3′) and Km (5′-GGTTGGCCAATCTACTCCCA GG-3′) targeting a fragment of the human β-globin gene.

Bibliographic search. To find the reported associations between bacterial species and the human infections they cause, we searched for published cases with the names of the bacteria and infection as key words with PubMed (http://www.ncbi.nlm.nih.gov). The bacteria were classified as relatively common (>10 published cases), rare (≤10 published cases), or unique (no published clinical cases) in joint and bone infection.

Statistical analysis. Data were compared with the chi-square test using Epi Info, version 6.04a (Centers for Disease Control and Prevention, Atlanta, Ga.). *P* values of <0.05 were considered statistically significant.

RESULTS

Among the 525 samples, 255 corresponded to needle aspirations and 270 corresponded to tissue biopsies. Samples were from 196 hips (including 108 patients with a hip prosthesis), 176 knees (including 47 patients with a knee prosthesis), 44 femurs, 36 tibias, 18 vertebral column samples, 14 elbows, 7 shoulders, 7 hands, 6 ankles, 5 humeri, 4 calcanei, 3 clavicles, 3 feet, 2 fibulas, 2 radii, 1 maxilla, and 1 mandible. The comparison of the data obtained using culture and 16S rRNA gene PCR followed by sequencing are summarized in Fig. 3 and Table 2.

Congruent results between culture and PCR. Four hundred seventy-five samples yielded congruent results between culture and 16S rRNA gene PCR. Eighty-nine patients presented positive results, with the following organisms retrieved using both techniques (numbers of isolates are indicated): 35 *Staphylococcus aureus*, 9 *Staphylococcus epidermidis*, 6 *Staphylococcus lugdunensis*, 3 *Staphylococcus capitis*, 11 *Streptococcus* spp. and related genera (4 *Streptococcus* group B, 2 *Streptococcus* group A, 2 *Enterococcus faecalis*, 1 *Streptococcus pasteuranius*, 2 *Streptococcus pneumoniae*), 11 enterobacteria (6 *Escherichia coli*, 3 *Enterobacter cloacae*, 1 *Enterobacter aerogenes*, 1 *Morganella morganii*), 5 *Pseudomonas aeruginosa*, 3 anaerobes (1 *Peptostreptococcus micros*, 1 *Bacteroides fragilis*, 1 *Fusobacterium nucleatum*), 3 HACEK group bacteria (1 *Haemophilus influenzae*, 1 *Kingella kingae*, and 1 *Eikenella corrodens*), 1 *Mycobacterium tuberculosis*, 1 *Alcaligenes xylosoxidans*, and 1 *Corynebacterium striatum*. Three hundred eighty-six patients presented negative results. Among them, 104 (49%) were receiving antibiotic therapy for a previously diagnosed infection at the time of sampling during the scheduled surgical removal or replacement of the orthopedic device. One hundred two patients presented with a final diagnosis other than infection: 45 had a diagnosis of inflammatory rheumatism, 29 had a

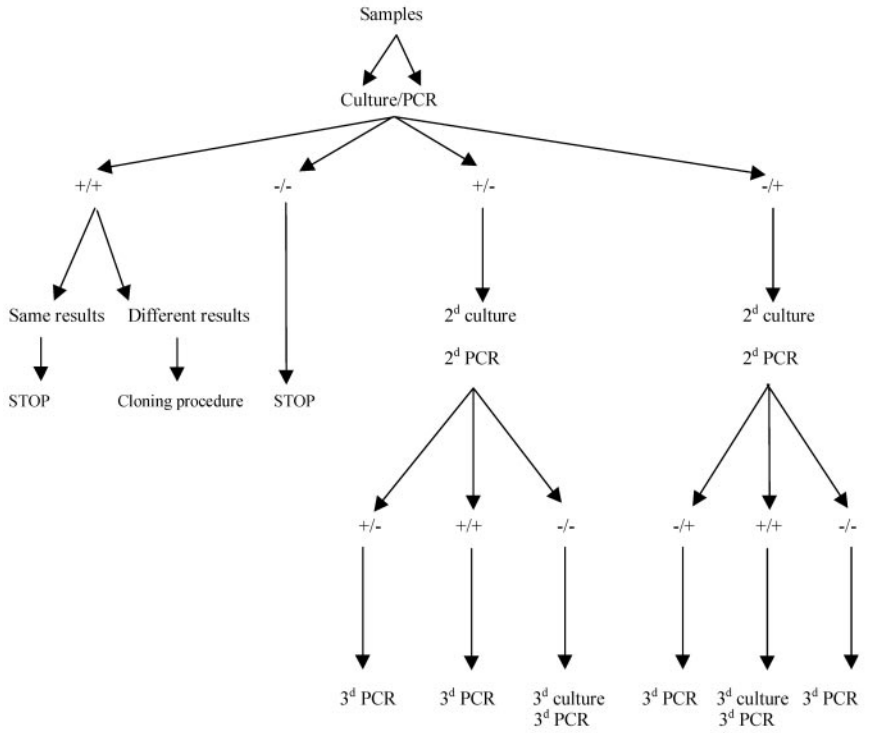

FIG. 2. Schematic strategy for the reconciliation of conventional culture and PCR assays.

diagnosis of benign or malignant tumors, 10 had a diagnosis of chondrocalcinosis, 8 had a diagnosis of gout, 5 had a diagnosis of algodystrophy, 3 had a diagnosis of hygroma, and 2 had a diagnosis of osteonecrosis. Eighty-six patients were sampled prior to a prosthesis implantation.

Discordant results between culture and PCR. Fifty results were discrepant, including a positive culture and negative PCR assay, in 22 cases (Table 3). Based on our scheme of interpretation of discordant results, they included 13 culture contaminants and 9 false-negative PCR results due to a lack of sensitivity of 16S rRNA gene PCR. Interestingly, in 4 of these 9 cases, *rpoB* amplification was successful, and in 3 others, PCR inhibitors were detected, as the β-globin gene was not amplified.

PCR only was positive for 21 patients. This was determined to be due to PCR contamination in 5 cases and a lack of sensitivity of culture in 16 cases. Antibiotic activity was found in 7 of the 16 cases (44%), and 2 of the 16 cases contained fastidious bacteria (*Granulicatella adiacens* for one sample and 2 anaerobes for another), as demonstrated by PCR assays followed by cloning procedures in the case of mixed infection (Tables 3 and 4). The 2 identified anaerobes were *Veillonella parvula* and a potentially novel bacterial species "*Candidatus* Prevotella conceptionensis."

Discordant positive results between culture and PCR. Seven samples containing different microorganisms between culture and PCR were all linked to mixed polymicrobial infections (Tables 3 and 4). Cloning could not be performed for 2 samples, as no material remained. Patient 44 was a 70-year-old man with an open femoral fracture who developed osteitis. *Clostridium perfringens* was isolated from blood culture at the time of diagnosis, but two different cultures of a femur biopsy retrieved only *S. aureus*. Two 16S rRNA gene PCR assays performed on the same sample detected *C. perfringens*, and a specific PCR confirmed these results. Specific *S. aureus* PCR detected the microorganism on the same sample. The patient was treated with antibiotics active against both identified microorganisms, and he is cured. Patient 45 was an 82-year-old man who received a knee prosthesis implantation in 1999 and removal in 2002. Two years later, a fistula appeared, and *S. aureus* and *Leclercia adecarboxylata* were isolated from a knee biopsy sample. One 16S rRNA gene PCR assay detected *Peptoniphilus harei*. The patient was lost in follow-up. Patient 46, a 26-year-old woman, developed a femoral osteitis following an open fracture after a motorcycle accident. Cloning procedures detected 6 bacteria, including the only one detected by culture, *Acinetobacter lwoffii* and 5 noncultured bacteria, *Comamonas terrigena*, *Stenotrophomonas maltophilia*, *Staphylococcus* spp., a potential novel bacterial species, "*Candidatus*" "Anaerococcus massiliensis," and *Alkanindiges illinoisensis*, a bacterium never reported in a human. Patient 47, a 33-year-old man, developed tibial osteitis following an open fracture after a motorcycle accident. Cloning procedures detected 6 bacteria, including the only one cultured, *Alcaligenes faecalis*. The noncultured bacteria were 5 anaerobes: *Peptinophilus lacrimalis*, *Anaerococcus vaginalis*, *Peptostreptococcus micros*, *Porphyromonas asaccharolytica*, and *Prevotella* spp. oral. Patient 48, a 24-year-old man who had been paraplegic since 1999 presented with right decubital pressure ulcers of several months duration and had consequently developed arthritis of the right hip. Cloning procedures detected 7 bacteria, including the only one cultured, *Staphylococcus aureus*. Noncultured bacteria in-

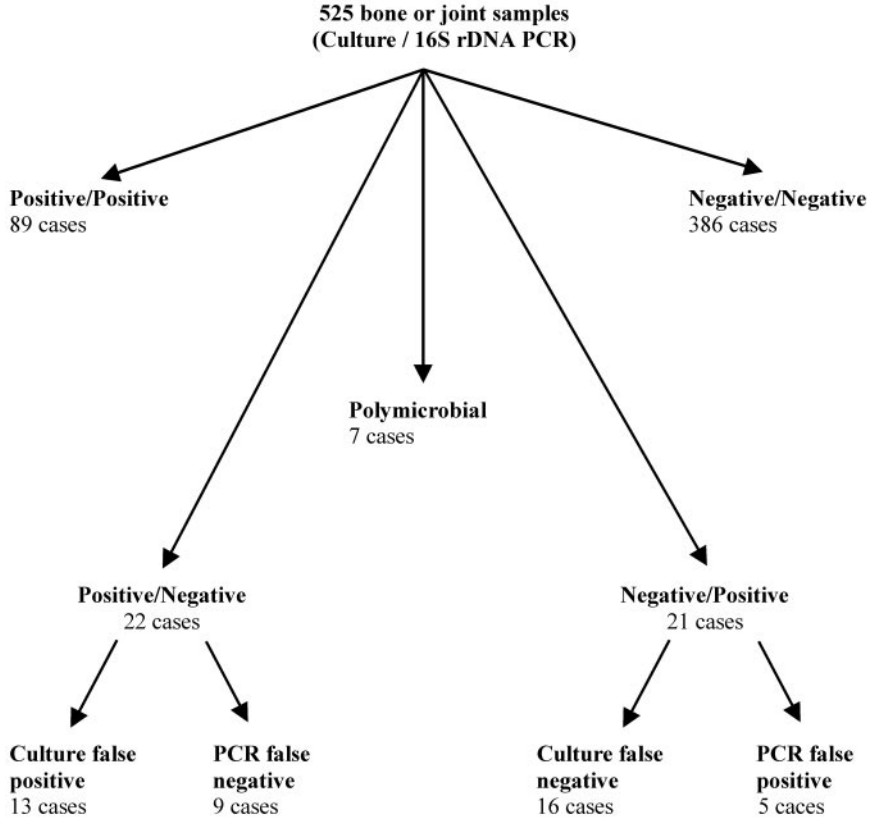

FIG. 3. Comparison of the data obtained for the analysis of 525 bone or joint samples using conventional culture and 16S rRNA gene PCR followed by sequencing.

cluded *Streptococcus anginosus* and 5 anaerobes: 1 *Prevotella bivia* isolate, 1 *Anaerococcus vaginalis* isolate, 1 *Peptoniphilus harei* isolate, and 2 anaerobes corresponding to potential novel species ("*Candidatus* Anaerococcus timonensis" and "*Candidatus* Peptoniphilus massiliensis"). Patient 49, a 77-year-old woman presented with a hip fracture. Her medical history included obesity, diabetes, and the presence of a pacemaker. Because of treatment with an oral vitamin K antagonist, surgery was delayed for 1 week. During this period, decubital pressure ulcers appeared. An infection developed 1 week after the surgery. Cloning procedures detected 7 bacteria, whereas the culture detected only 2, *S. aureus* and *Proteus mirabilis*. Using cloning procedures, *Propionibacterium acnes*, *Sphingobacterium mizutaii*, and *Veillonella atypica* were also amplified, and two bacteria, never described as human pathogens, were retrieved: a β-proteobacterium (dehydroabietic acid-degrading bacterium) and an unidentified Hailaer soda lake bacterium. Patient 50 was a 29-year-old man who had been paraplegic since 1997. Left decubital pressure ulcers were present for several months when the patient developed arthritis of the left hip. Cloning procedures detected 8 bacteria, whereas the culture detected only 1 bacterium, *E. coli*. Among the noncultured bacteria, 6 anaerobes were detected: *Prevotella buccalis*, *Peptostreptococcus anaerobius*, a newly described bacterium, *Porphyromonas somerae* (56a), and three potential novel species of anaerobes ("*Candidatus* Anaerococcus phoceensis," "*Candi-*

TABLE 2. Comparison of sensitivity, specificity, positive predictive value, and negative value between culture and 16S rRNA gene PCR followed by sequencing

Test	Result	No. of samples with definite conclusion		% Sensitivity[a]	% Specificity[b]	% Positive predictive value	% Negative predictive value
		Positive	Negative				
Culture	Positive	105	13	86.7	89	89	96
	Negative	16	391				
PCR	Positive	112	5	92.5	95.7	95.7	97.7
	Negative	9	389				
Total		121	424				

[a] $P = 0.13$ (chi-square test).
[b] $P = 0.06$ (chi-square test).

TABLE 3. Discrepant results between conventional culture and PCR followed by sequencing observed for 50 patients

Patient no. and reason for discrepancy (*n*)	Sample source	Result for:						
		Culture no. 1	Culture no. 2	Culture no. 3	16S PCR no. 1	16S PCR no. 2	PCR of second gene	Antibiotics[e]
Culture contamination (13)[a]								
1	Tibia	*S. epidermidis*	−	−	−	−	−	−
2	Clavicle	*S. epidermidis*	−	−	−	−	−	−
3	Hip	*S. epidermidis*	−	−	−	−	−	−
4	Knee	*S. epidermidis*	−	−	−	−	−	−
5	Tibia	*S. epidermidis*	−	−	−	−	−	−
6	Elbow	*S. epidermidis*	−	−	−	−	−	−
7	Knee	*S. epidermidis*	−	−	−	−	−	−
8	Hip	*S. epidermidis*	−	−	−	−	−	+
9	Knee	*S. epidermidis*	−	−	−	−	−	−
10	Clavicle	*P. acnes*	−	−	−	−	−	−
11	Rachis	*P. acnes*	−	−	−	−	−	−
12	Femur	*S. aureus*	−	−	−	−	−	+
13	Hip	Polymicrobial	−	−	−	−	−	−
PCR contamination (5)[b]								
14	Knee	−	−	NP[f]	*P. acnes*	−	−	−
15	Hip	−	−	NP	*S. epidermidis*	−	−	−
16	Hip	−	−	NP	*S. epidermidis*	−	−	+
17	Knee	−	−	NP	*S. epidermidis*	−	−	−
18	Knee	−	−	NP	*P. aeruginosa*	−	−	−
Lack of sensitivity of culture (16)[c]								
19	Elbow	−	−	NP	*S. aureus*	*S. aureus*	*S. aureus*	+
20	Hip	−	−	NP	*S. aureus*	−	*S. aureus*	+
21	Knee	−	−	NP	*S. aureus*	*S. aureus*	*S. aureus*	+
22	Knee	−	−	NP	*S. aureus*	−	*S. aureus*	−
23	Knee	−	−	NP	*S. pneumoniae*	−	*S. pneumoniae*	+
24	Knee	−	−	NP	*S. pneumoniae*	*S. pneumoniae*	*S. pneumoniae*	−
25	Hand	*C. striatum*	−	−	*E. faecalis*	*E. faecalis*	*E. faecalis*	−
26	Hip	−	−	NP	*G. adiacens*	*G. adiacens*	*G. adiacens*	−
27	Tibia	−	−	NP	*Prevotella* sp. (95% homology)	Polymicrobial	NP	−
28	Knee	−	−	NP	*S. agalactiae*	*S. agalactiae*	*S. agalactiae*	+
29	Knee	−	−	NP	*S. agalactiae*	*S. agalactiae*	*S. agalactiae*	−
30	Tibia	−	−	NP	*E. aerogenes*	*E. aerogenes*	*E. aerogenes*	−
31	Knee	−	−	NP	*S. agalactiae*	−	*S. agalactiae*	+
32	Femur	−	−	NP	*E. faecalis*	−	*E. faecalis*	−
33	Femur	−	−	NP	*E. cloacae*	*E. cloacae*	*E. cloacae*	+
34	Knee	−	−	NP	*E. faecalis*	−	*E. faecalis*	−
Lack of sensitivity of 16S rRNA gene PCR (4)								
35	Wrist	*S. aureus*	*S. aureus*	NP	−	−	*S. aureus*	−
36	Tibia	*S. aureus*	*S. aureus*	NP	−	−	*S. aureus*	−
37	Hip	*S. aureus*	*S. aureus*	NP	−	−	*S. aureus*	−
38	Knee	*S. aureus*	*S. aureus*	NP	−	−	*S. aureus*	−
Lack of sensitivity of PCR (5)[d]								
39	Tibia	*S. aureus*	*S. aureus*	NP	−	−	−	−
40	Hip	*S. aureus*	*S. aureus*	NP	−	−	−	−
41	Humerus	*S. epidermidis*	*S. epidermidis*	NP	−	−	−	−
42	Fibula	*S. epidermidis*	*S. epidermidis*	NP	−	−	−	−
43	Hip	*E. coli*	*E. coli*	NP	−	−	−	+
Difference between PCR and positive culture (7)								
44	Femur	*S. aureus*	*S. aureus*	NP	*C. perfringens*	*C. perfringens*	*C. perfringens*	−
45	Knee	*S. aureus* + *L. adecarboxylata*	NP	NP	*Peptoniphilus harei*	NP	NP	−
46	Hip	*Acinetobacter lwoffii*	*Acinetobacter lwoffii*	NP	Polymicrobial	NP	NP	−
47	Tibia	*A. faecalis*	*A. faecalis*	NP	Polymicrobial	NP	NP	−
48	Hip	*S. aureus*	*S. aureus*	NP	Polymicrobial	NP	NP	−
49	Hip	*S. aureus*	*S. aureus*	NP	Polymicrobial	NP	NP	−
50	Hip	*S. aureus* + *P. mirabilis*	*S. aureus* + *P. mirabilis*	NP	Polymicrobial	NP	NP	−

[a] The culture contamination group corresponds to microorganisms isolated only once among the 3 assays associated with negative PCR assay.
[b] The PCR contamination group corresponds to only one positive 16S rRNA gene PCR assay associated with negative culture.
[c] The lack of sensitivity of culture group corresponds to negative culture associated with the same microorganism identified at least twice using two different PCR assays.
[d] The lack of sensitivity of PCR group corresponds to negative 16S rRNA gene PCR associated with the same microorganism identified at least twice using two different PCR assays.
[e] Presence of antibiotic activity in the sample. +, positive; −, negative.
[f] NP, not performed.

TABLE 4. Results of cloning following by PCR and sequencing performed for 6 samples for which mixed sequences were obtained after 16S rRNA gene PCR

Patient no.	Sample source; context; treatment; follow-up	Microorganisms identified by 16S rRNA	% Homology[a]	GenBank accession no.	Final identification	Description in bone and joint infection
27	Tibia biopsy; osteitis following open fracture; specific treatment against anaerobes; cure	*Prevotella shahii*	**96**	AY995769	"*Candidatus* Prevotella conceptionensis"	Unique
		Veillonella parvula	99	AB108825	*Veillonella parvula*	Rare
46	Femur biopsy; osteitis following open fracture; lost in follow-up	*Acinetobacter lwoffii*	99	AY167273	*Acinetobacter lwoffii*	Unique
		Anaerococcus octavius	**96**	Y07841	"*Candidatus* Anaerococcus massiliensis"	Unique
		Stenotrophomas maltophilia	99	AY0404357	*Stenotrophomas maltophilia*	Rare
		Staphylococcus spp.	99	AJ276810	*Staphylococcus* spp.	Unique
		Comamonas terrigena	99	AJ43043	*Comamonas terrigena*	Unique
		Alkanindiges illinoisensis	99	AF513979	*Alkanindiges illinoisensis*	Unique
47	Tibia biopsy; ostcitis following open fracture; no specific treatment against anaerobes; no cure and amputation	*Alcaligenes faecalis*	99	AJ880765	*Alcaligenes faecalis*	Rare
		Peptoniphilus lacrimalis	100	AF542230	*Peptoniphilus lacrimalis*	Unique
		Anaerococcus vaginalis	99	AF542229	*Anaerococcus vaginalis*	Rare
		Prevotella spp. oral	99	L16474	*Prevotella* spp. oral	Rare
		Peptostreptococcus micros	99	AY435495	*Peptostreptococcus micros*	Common
		Porphyromonas asaccharolytica	99	AY360344	*Porphyromonas asaccharolytica*	Unique
48	Hip biopsy; hip arthritis associated with ulcers; lost in follow-up	*Staphylococcus aureus*	99	AP003136	*Staphylococcus aureus*	Common
		Prevotella bivia	99	L16475	*Prevotella bivia*	Rare
		Anaerococcus lactolyticus	**96**	AF542233	"*Candidatus* Anaerococcus timonensis"	Unique
		Anaerococcus vaginalis	98	AF542229	*Anaerococcus vaginalis*	Rare
		Peptoniphilus harei	98	Y07839	*Peptoniphilus harei*	Unique
		Streptococcus anginosus	99	AY691541	*Streptococcus anginosus*	Common
		Peptoniphilus ivorii	**95**	Y07839	"*Candidatus* Peptoniphilus massiliensis"	Unique
49	Hip biopsy; hip arthritis associated with ulcers; lost in follow-up	*Staphylococcus aureus*	99	AP003136	*Staphylococcus aureus*	Common
		Proteus mirabilis	99	AY820623	*Proteus mirabilis*	Common
		Dehydroabietic acid-degrading bacterium	99	AF125877	Dehydroabietic acid-degrading bacterium	Unique
		Veillonella atypica	99	AF201191	*Veillonella atypica*	Unique
		Unidentified Hailaer soda lake bacterium	99	AF275700	Unidentified Hailaer soda lake bacterium	Unique
		Shingobacterium mizutaii	98	AJ438175	*Shingobacterium mizutaii*	Unique
		Propionibacterium acnes	99	AB108482	*Propionibacterium acnes*	Common
50	Hip biopsy; hip arthritis associated with ulcers; specific treatment against anaerobic bacteria; cure	*Escherichia coli*	99	AB210981	*Escherichia coli*	Common
		Clostridium clostridioforme	**95**	M59089	"*Candidatus* Clostridium massiliensis"	Unique
		Anaerococcus lactolyticus	**96**	AF5422233	"*Candidatus* Anaerococcus phoceensis"	Unique
		Prevotella buccalis	99	L16476	*Prevotella buccalis*	Unique
		Peptostreptoccus anaerobius	98	AY326462	*Peptostreptoccus anaerobius*	Unique
		Porphyromonas "somerae"	99	AY968205	*Porphyromonas "somerae"*	Unique
		Uncultured bacterium clone HuCa4	99	AJ408960	Uncultured bacterium clone HuCa4	Unique
		Peptostreptococcus anaerobius	**96**	AY326462	"*Candidatus* Clostridium timonensis"	Unique

[a] Boldface type indicates a percentage of homology below 97% and a potentially new species.

datus Clostridium massiliensis," and "*Candidatus* Clostridium timonensis.") An unknown human pathogen, "uncultured bacterium clone HuCa4," was also detected. Phylogenetic relationships of potential novel species were inferred using the neighbor-joining method and are presented in Fig. 4.

DISCUSSION

The validity of the data reported here is based upon strict experimental procedures and controls. PCR assays were validated by the results of controls. In addition, any PCR-based identification had to be confirmed by the successful amplification of another gene and/or the PCR result was congruent with the results of culture. This is the first 16S rRNA gene-based detection study of bone and joint samples using such strict criteria. Overall, the distribution of microorganisms was as previously described (66). Twenty-three etiological diagnoses were performed solely by 16S rRNA gene PCR and 9 were performed using only culture. The 16S rRNA gene PCR and culture were not statistically different for sensitivity ($P = 0.13$) or specificity ($P = 0.06$).

False-positive culture results were observed in 13 cases due to contamination with cutaneous flora. False-positive PCR results, due to contaminated DNA, were detected in 5 cases. False-negative PCR results were observed in 9 cases and may have been due to inefficient DNA extraction, as suggested by the negative β-globin PCR in 3 of the samples. False-negative culture results were observed in 16 cases, including 7 (44%) cases of previous antibiotic therapy and 2 cases of fastidious pathogens, *Granulicatella adiacens* and a mixed infection caused by 2 anaerobes. In the 7 other cases, the most common microorganisms detected by PCR only were *Streptococcus* spp. and *Enterococcus* spp. Such a discrepancy was previously observed in a study comparing culture and 16S rRNA gene PCR in 382 pediatric specimens (21). The authors suggested that *S. pneumoniae* is a microorganism that may be difficult to grow because of its tendency to undergo autolysis, but in addition, it is usually covered in empirical antibiotic therapy. In addition, it is important to point out that *S. pneumoniae* DNA was detected in noninfected heart valve tissue 7 years after *S. pneumoniae* endocarditis, illustrating the persistence of DNA without any evidence of infection (9).

It has been claimed that PCR assays cannot be used to

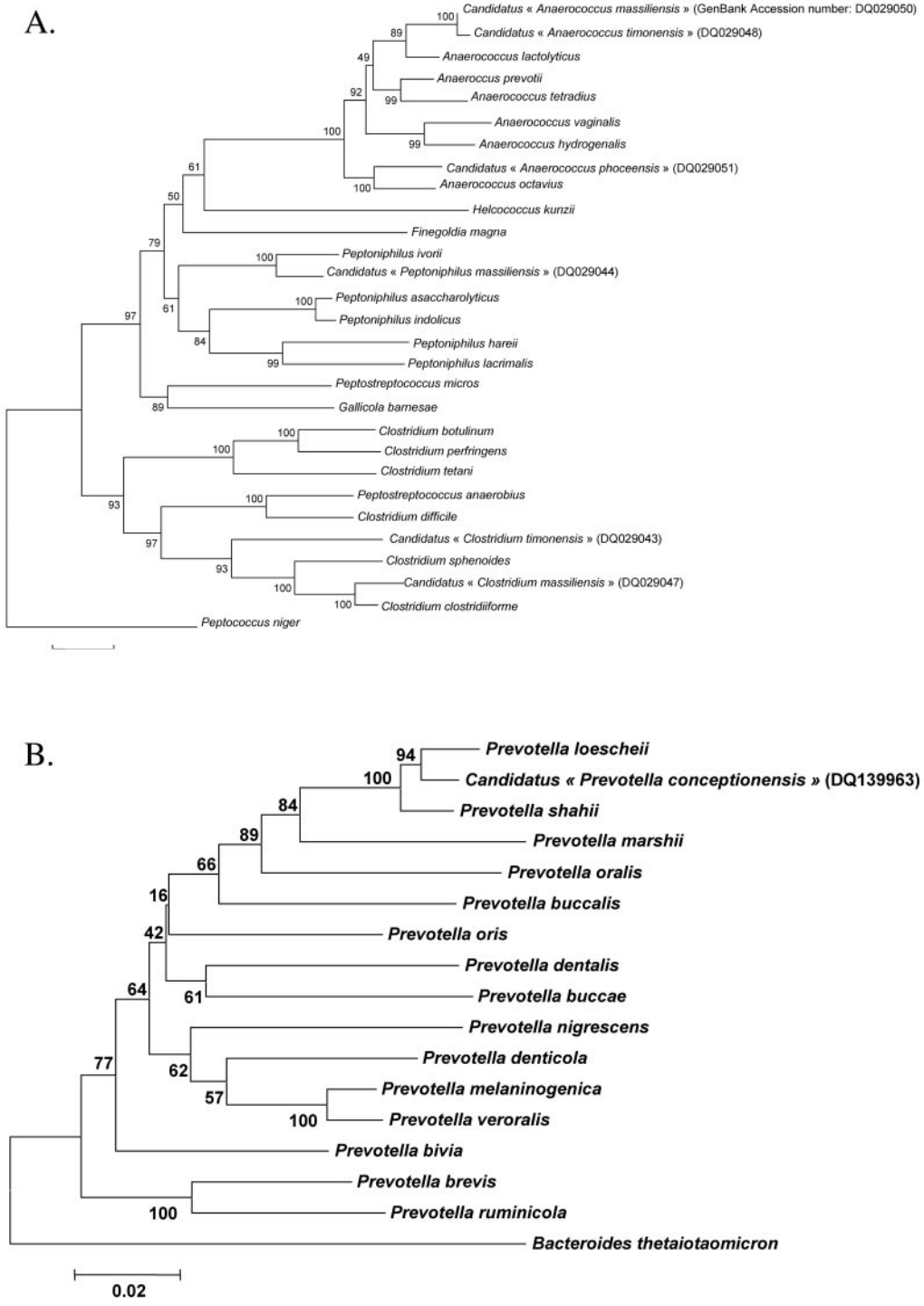

FIG. 4. (A) Phylogenetic tree of anaerobic gram-positive bacteria inferred from comparison of 16S rRNA gene sequences. Sequences (900 nucleotides) were aligned by using the multisequence alignment program Clustal X (version 1.8). Phylogenetic relationships were determined by using MEGA, version 2.1. Distance matrices were determined by using Kimura's parameters. These matrices were used to elaborate dendrograms by using the neighbor-joining method. Numbers at nodes are the proportion of 100 resamplings that support the topology shown. *Listeria monocytogenes* was used as the out-group. Bar, 0.02 nucleotide changes per nucleotide position. (B) Phylogenetic tree of *Prevotella* spp. inferred from comparison of 16S rRNA gene sequences. Sequences (900 nucleotides) were aligned by using the multisequence alignment program Clustal X (version 1.8). Phylogenetic relationships were determined by using MEGA, version 2.1. Distance matrices were determined by using Kimura's parameters. These matrices were used to elaborate dendrograms by using the neighbor-joining method. Number at nodes are the proportion of 100 resamplings that support the topology shown. *Bacteroides thetaiotaomicron* was used as the out-group. Bar, 0.02 nucleotide changes per nucleotide position.

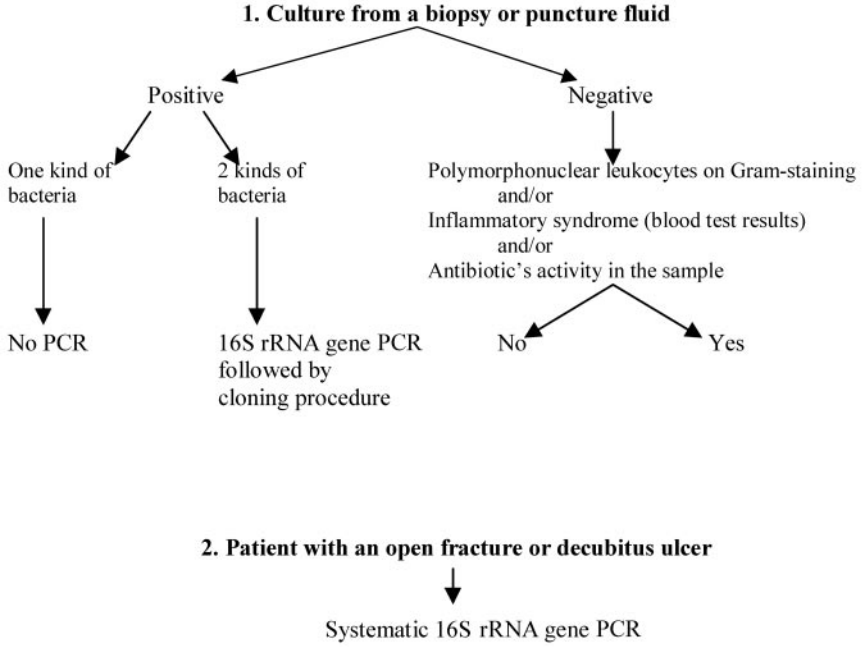

FIG. 5. Strategy for the use of 16S rRNA gene PCR for the diagnosis of bone or joint infections.

identify each pathogen in cases of mixed infection (66). Although the 16S rRNA gene PCR has been widely used to identify a single bacterial species infecting normally sterile sites, this technique has also been employed to define the complex bacterial flora from various environmental niches (1, 16, 18, 23, 28, 62), human caries, dental plaques, and human feces (8, 11, 25, 45). In our experience, mixed sequences on the electropherogram suggested the presence of polymicrobial flora. The detection of polymorphism using analysis of the peak pattern from the DNA electropherogram has already been described for the detection of mixed populations of human immunodeficiency virus type 1 variants (32, 39, 44). Here, the cloning of mixed amplicons allowed us to detect a high number of bacterial species in a single specimen, including pathogens rarely described in bone and joint infections, known human pathogens not previously described in bone and joint infections, unknown human pathogens, and new species.

Bacteria identified in these mixed infections comprised species rarely described in bone and joint infections. Indeed, *Stenotrophomonas maltophilia* bone and joint infections are uncommon (4, 12). *Alcaligenes faecalis*, commonly found in a watery environment, has been reported only once in fact, from tibial pus in a patient with a femoral fracture (6). *Streptococcus anginosus* forms part of the normal flora of the mouth, gastrointestinal tract, and genitourinary tract. In rare cases, it causes osteoarthritis, spondylodiscitis, or osteomyelitis (2, 38, 63). Finally, we retrieved three anaerobes, *Peptostreptococcus micros*, *Prevotella bivia*, and *Veillonella parvula*, rarely observed in bone and joint infections (3, 10, 29, 31, 43, 46, 48, 49).

Also unexpected was the detection of several human pathogens previously unreported in bone and joint infections, including *Peptoniphilus lacrimalis*, *Porphyromonas asaccharolytica*, *Prevotella buccalis*, *Peptoniphilus harei*, *Veillonella atypica*, and *Anaerococcus vaginalis*. *Comamonas terrigena* and *Acinetobacter lwoffii*, which are both environmental bacteria rarely causing human infection, were retrieved for the first time in bone and joint infections (51, 54). *Sphingobacterium mizutaii* has previously been reported in nosocomial infections but has never been found in a case of bone or joint infection (59). Finally, a bacterium corresponding to a newly deposited sequence, not yet published, *Porphyromonas* "*somerae*," was also detected.

Another finding of this study was the detection of 4 bacterial species which had previously not been associated with human infection. *Alkanindiges illinoisensis* was first isolated in 2003 from chronically crude oil-contaminated soil from an oilfield in the United States (7). Based on 16S rRNA gene sequence analyses, the most closely related bacterium was *Acinetobacter junii* with 94% sequence similarity, which has rarely caused disease, doing so mainly in nosocomial bacteremia (35). "Dehydroabietic acid degrading bacterium DhA 73" was isolated in 1999 from a laboratory-scale bioreactor treating bleached mill effluent (64). "Unidentified Hailaer soda lake bacterium F1" was first detected in 2001 in a lake in China. This bacterium is closely related to the genus *Alkalibacterium*, which comprises alkaliphilic bacteria and is mainly present in water (42). "Uncultured bacterium HuCa4" was detected for the first time in 2002 in human intestinal flora (47).

Also surprising was the detection of seven 16S rRNA gene sequences exhibiting less than 97% sequence similarity to bacterial sequences deposited in GenBank, suggesting they were potential new species. All were anaerobes included in the genera *Anaerococcus* (3 isolates), *Peptoniphilus* (1 isolate), *Clostridium* (2 isolates), and *Prevotella* (1 isolate). These data confirm that anaerobes are probably an underestimated cause of bone and joint infections, as previously suggested (21, 34, 53).

It is interesting that all of the unusual microorganisms were only observed among the patients with mixed infections. We could hypothesize that these unusual microorganisms are dif-

ficult to isolate in culture and that they have low pathogenic potential. They could in some circumstances, such as polymicrobial infection, gain in pathogenicity and should be then considered in the antibiotic strategy. Indeed, among the 4 patients with a mixed infection for whom data about treatment and follow-up were available, 3 have benefited from an adapted antibiotic therapy against the identified microorganisms and were cured, whereas the lone patient who has not benefited from an adapted treatment had an amputation.

Overall, the 16S rRNA gene PCR assay followed by sequencing offers several advantages when used to complement culture results, but its use should be restricted. A strategy for the use of 16S rRNA gene PCR for the diagnosis of bone or joint infections is proposed in Fig. 5. We do not recommend performing 16S rRNA gene PCR in the following circumstances: (i) prior to prosthesis implantation; (ii) in cases where a diagnosis other than infection is established; or (iii) for patients with a positive culture of a single, highly pathogenic bacterium if they are not also at risk for a polymicrobial infection. We propose the use of the 16S rRNA gene PCR assay for culture-negative cases when infection is suspected on the basis of clinical signs and symptoms (33, 52, 66) or inflammatory syndrome is present, as highlighted by blood test results and purulent samples. In the case of suspected polymicrobial infection as detailed below, 16S rRNA gene PCR assay followed by cloning procedures should also be performed. These tools are not expensive and could be realized routinely in all laboratories with basic molecular biology competencies. The cloning step could be easily performed using commercialized kits. Only sequencing of all the clones is time-consuming. The presence of a polymicrobial infection should be suspected if a mixed sequence is observed on the DNA electropherogram, if $\geq$2 bacteria species were isolated using culture, or for patients with a suggestive clinical context such as an open lesion. These mixed infections were mainly observed in patients with hip infections and decubitus ulcers or in patients with an infected open fracture.

The use of molecular biology based on a broad-range PCR followed by sequencing and, when necessary, cloning procedures, provides a assay complementary to culture to identify microorganisms from bone or joint samples. This tool should be used in patients highly suspected of infection for whom culture was negative and in cases of suspected polymicrobial infection. Cloning procedures should allow the detection of previously unknown pathogens and potentially novel bacterial species.

ACKNOWLEDGMENTS

We are indebted to Veronique Brice and Stephanie Basone for technical assistance and Melanie Ihrig for reviewing the manuscript.

None of the authors have potential conflicts of interest with this research.

REFERENCES

1. **Amann, R., J. Snaidr, M. Wagner, W. Ludwig, and K. H. Schleifer.** 1996. In situ visualization of high genetic diversity in a natural microbial community. J. Bacteriol. **178:**3496–3500.
2. **Balsam, L. B., G. M. Sheperd, and K. L. Ruoff.** 1997. *Streptococcus anginosus* spondylodiskitis. Clin. Infect. Dis. **24:**93–94.
3. **Bartz, H., C. Nonnenmacher, C. Bollmann, M. Kuhl, S. Zimmermann, K. Heeg, and R. Mutters.** 2005. *Micromonas* (*Peptostreptococcus*) *micros*: unusual case of prosthetic infection associated with dental procedures. Int. J. Med. Microbiol. **294:**465–470.
4. **Belzunegui, J., J. R. De Rios, J. J. Intxausti, and J. A. Iribarren.** 2000. Septic arthritis caused by *Stenotrophomonas maltophilia* in a patient with acquired immunodeficiency syndrome. Clin. Exp. Rheumatol. **18:**265.
5. **Benslimani, A., F. Fenollar, H. Lepidi, and D. Raoult.** 2005. Bacterial zoonoses and infective endocarditis, Algeria. Emerg. Infect. Dis. **11:**216–224.
6. **Bizet, J., and C. Bizet.** 1997. Strains of *Alcaligenes faecalis* from clinical material. J. Infect. **35:**167–169.
7. **Bogan, B. W., W. R. Sullivan, K. J. Kayser, K. D. Derr, H. C. Aldrich, and J. R. Paterek.** 2003. *Alkanindiges illinoisensis* gen. nov., sp. nov., an obligately hydrocarbonoclastic, aerobic squalane-degrading bacterium isolated from oilfield soils. Int. J. Syst. Evol. Microbiol. **53:**1389–1395.
8. **Borneman, J., P. W. Skroch, K. M. O'Sullivan, J. A. Palus, N. G. Rumjanek, J. L. Jansen, and J. Nienhhuis.** 1996. Molecular microbial diversity of an agricultural soil in Wisconsin. Appl. Environ. Microbiol. **62:**1935–1943.
9. **Branger, S., J. P. Casalta, G. Habib, F. Collard, and D. Raoult.** 2003. *Streptococcus pneumoniae* endocarditis: persistence of DNA on heart valve material 7 years after infectious episode. J. Clin. Microbiol. **41:**4435–4437.
10. **Bru, J. P., S. Bland, and A. Sédallian.** 2000. Aspects épidémiologiques et microbiologiques de 33 ostéites et ostéoarthrites anaérobies. Med. Mal. Infect. **30**(Suppl. 2)**:**102–108.
11. **Chhour, K. L., M. A. Nadkarni, R. Byun, F. E. Martin, N. A. Jacques, and N. Hunter.** 2005. Molecular analysis of microbial diversity in advanced caries. J. Clin. Microbiol. **43:**843–849.
12. **Denton, M., and K. G. Kerr.** 1998. Microbiological and clinical aspects of infection associated with *Stenotrophomonas maltophilia*. Clin. Microbiol. Rev. **11:**57–80.
13. **Drancourt, M., P. Berger, and D. Raoult.** 2004. Systematic 16S rRNA gene sequencing of atypical clinical isolates indentified 27 new bacterial species associated with humans. J. Clin. Microbiol. **42:**2197–2202.
14. **Drancourt, M., C. Bollet, A. Carlioz, R. Martelin, J. P. Gayral, and D. Raoult.** 2000. 16S ribosomal DNA sequence analysis of a large collection of environmental and clinical unidentifiable bacterial isolates. J. Clin. Microbiol. **38:**3623–3630.
15. **Fenollar, F., V. Gauduchon, J. P. Casalta, H. Lepidi, F. Vandenesch, and D. Raoult.** 2004. *Mycoplasma* endocarditis: two case reports and a review. Clin. Infect. Dis. **38:**e21–e24.
16. **Giovannoni, S. J., T. B. Britschgi, C. L. Moyer, and K. G. Field.** 1990. Genetic diversity in Sargasso Sea bacterioplankton. Nature **345:**20.
17. **Goldenberger, D., A. Kunzli, P. Vogt, R. Zbinden, and M. Altwegg.** 1997. Molecular diagnosis of bacterial endocarditis by broad-range PCR amplification and direct sequencing. J. Clin. Microbiol. **35:**2733–2739.
18. **Gray, J. P., and R. P. Herwig.** 1996. Phylogenic analysis of the bacterial communities in marine sediment. Appl. Environ. Microbiol. **62:**4049–4059.
19. **Greub, G., H. Lepidi, C. Rovery, J. P. Casalta, and D. Raoult.** 2005. Diagnosis of infectious endocarditis in patients undergoing valve surgery. Am. J. Med. **118:**320–328.
20. **Harris, K. A., K. J. Fidler, J. C. Hartley, J. Vogt, N. J. Klein, F. Monsell, and V. M. Novelli.** 2002. Unique case of *Helicobacter* sp. osteomyelitis in an immunocompetent child diagnosed by broad-range 16S PCR. J. Clin. Microbiol. **40:**3100–3103.
21. **Harris, K. A., and J. C. Hartley.** 2003. Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service. J. Med. Microbiol. **52:**685–691.
22. **Hebb, J. K., C. R. Cohen, S. G. Astete, E. A. Bukusi, and P. A. Totten.** 2004. Detection of novel organisms associated with salpingitis, by use of 16S rDNA polymerase chain reaction. J. Infect. Dis. **190:**2109–2120.
23. **Hugenholtz, P., and N. R. Pace.** 1996. Identifying microbial diversity in the natural environment: a molecular phylogenetic approach. Trends Biotechnol. **14:**190–197.
24. **Hunfeld, K. P., M. Rittmeister, T. A. Wichelhaus, V. Brade, and R. Enzensberger.** 1998. Two cases of chronic arthritis of the forearm due to *Mycobacterium tuberculosis*. Eur. J. Clin. Microbiol. Infect. Dis. **17:**344–348.
25. **Hutter, G., U. Schlagenhauf, G. Valenza, M. Horn, S. Burgemeister, H. Claus, and U. Vogel.** 2003. Molecular analysis of bacteria in periodontitis: evaluation of clone libraries, novel phylotypes and putative pathogens. Microbiology **149:**67–75.
26. **Hykin, P., K. Tobal, G. McIntyre, M. Matheson, H. Towler, and S. Lightman.** 1994. The diagnosis of delayed post-operative endophtalmitis by polymerase chain reaction of bacterial DNA in vitreous samples. J. Med. Microbiol. **40:**408–415.
27. **Ince, A., J. Rupp, L. Frommelt, A. Katzer, L. Gille, and J. F. Löhr.** 2004. Is "aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low grade infection? Clin. Infect. Dis. **39:**1599–1603.
28. **Kroes, I., P. W. Lepp, and D. A. Relman.** 2005. Bacterial diversity within the human subgingival crevice. Proc. Natl. Acad. Sci. USA **96:**14547–14552.
29. **Laiho, K., and P. Kotilainen.** 2001. Septic arthritis due to *Prevotella bivia* after intra-articular hip joint infection. Joint Bone Infect. **68:**443–444.
30. **La Scola, B., G. Michel, and D. Raoult.** 1997. Use of amplification and sequencing of the 16S rRNA gene to diagnose *Mycoplasma pneumoniae* osteomyelitis in a patient with hypogammaglobulinemia. Clin. Infect. Dis. **24:**1161–1163.

31. **Leder, K. S., and T. F. Barlam.** 2000. A case of paraspinal abscess and diskitis due to *Peptostreptococcus micros*. Clin. Infect. Dis. **30:**622–623.
32. **Leitner, T., E. Halapi, G. Scarletti, P. Rossi, J. Albert, E. M. Fenyö, and M. Uhlen.** 1993. Analysis of heterogeneous viral populations by direct DNA sequencing. BioTechniques **15:**120–127.
33. **Lew, D. P., and F. A. Waldvogel.** 2004. Osteomyelitis. Lancet **364:**369–379.
34. **Lewis, R. P., V. L. Sutter, and S. M. Finegold.** 1978. Bone infections involving anaerobic bacteria. Medicine (Baltimore) **57:**279–305.
35. **Linde, H. S., J. Hahn, H. Holler, U. Reischl, and N. Lehn.** 2002. Septicemia due to *Acinetobacter junii*. J. Clin. Microbiol. **40:**2696–2697.
36. **Mariani, B. D., and R. S. Tuan.** 1998. Advances in the diagnosis of infection in prosthetic joint implants. Mol. Med. Today **4:**207–213.
37. **Mariani, B. D., D. S. Martin, M. J. Levine, R. E. Booth, Jr., and R. S. Tuan.** 1996. Polymerase chain reaction detection of bacterial infection in total knee arthroplasty. Clin. Orthop. Relat. Res. **331:**11–22.
38. **Mateo Soria, L., J. Miquel Nolla Sole, A. Rozadilla Sacanell, J. Valverde Garcia, and D. Roig Escofet.** 1992. Infectious arthritis in patients with rheumatoid arthritis. Ann. Rheum Dis. **51:**402–403.
39. **Metzker, M. L., M. A. Ansari-Lari, X. M. Liu, D. J. Holder, and R. A. Gibbs.** 1998. Quantification of mixed-based populations of HIV-1 variants by automated DNA sequencing with BODIPY dye-labeled primers. BioTechniques **25:**446–447.
40. **Moumile, K., J. Merckx, C. Glorion, P. Berche, and A. Ferroni.** 2003. Osteoarticular infections caused by *Kingella kingae* in children: contribution of polymerase chain reaction to the microbiologic diagnosis. Pediatr. Infect. Dis. J. **22:**837–839.
41. **Ni, H., A. Knight, K. Cartwright, W. Palmer, and J. McFadden.** 1992. Polymerase chain reaction for diagnosis of meningococcal meningitis. Lancet **340:**1432–1434.
42. **Ntougias, S., and N. J. Russell.** 2001. *Alkalibacterium olivoapovliticus* gen. nov., sp. nov., a new obligately alkaliphatic bacterium isolated from edible-olive wash-waters. Int. J. Syst. Evol. Microbiol. **51:**1161–1170.
43. **Papasian, C. J., D. H. McGregor, G. R. Hodges, and J. Kennedy.** 1986. Peptostreptococcal vertebral osteomyelitis. J. Clin. Microbiol. **24:**633–635.
44. **Parker, L. T., H. Zakeri, Q. Deng, S. Spurgeon, P. Y. Kwok, and D. A. Nickerson.** 1996. AmpliTaq* DNA polymerase, FS Dye-Terminator sequencing: analysis of peak height patterns. BioTechniques **21:**694–792.
45. **Paster, B. J., S. K. Boches, J. L. Galvin, R. E. Ericson, C. N. Lau, V. A. Levanos, J. Sahasrabudh, and F. E. Dewhirst.** 2001. Bacterial diversity in human subgingival plaque. J. Bacteriol. **183:**3770–3783.
46. **Petrini, B., T. Welin-Berger, and C. E. Nord.** 1979. Anaerobic bacteria in late infections following orthopedic surgery. Med. Microbiol. Immunol. (Berlin) **167:**155–159.
47. **Pryde, S. E., S. H. Duncan, G. L. Hold, C. S. Stewart, and H. J. Flint.** 2002. The microbiology of butyrate formation in the human colon. FEMS Microbiol. Lett. **17:**133–139.
48. **Riesback, K., and L. Sanzen.** 1999. Destructive knee joint infection caused by *Peptostreptococcus micros*: importance of early microbiological diagnosis. J. Clin. Microbiol. **37:**2737–2739.
49. **Riesbeck, K.** 2003. Paronychia due to *Prevotella bivia* that resulted in amputation: fast and correct bacteriological diagnosis is crucial. J. Clin. Microbiol. **41:**4901–4903.
50. **Rovery, C., G. Greub, H. Lepidi, J. P. Casalta, G. Habib, F. Collart, and D. Raoult.** 2005. PCR detection of bacteria on cardiac valves of patients with treated bacterial endocarditis. J. Clin. Microbiol. **43:**163–167.
51. **Seifert, H., A. Strate, A. Schulze, and G. Pulverer.** 1993. Vascular catheter-related bloodstream infection due to *Acinetobacter johnsonii* (formerly *Acinetobacter calcoaceticus var. lwoffi*): report of 13 cases. Clin. Infect. Dis. **17:**632–636.
52. **Shirtliff, M. E., and J. T. Mader.** 2002. Acute septic arthritis. Clin. Microbiol. Rev. **15:**527–544.
53. **Song, Y., C. Liu, M. McTeague, A. Vu, J. Y. Liu, and S. M. Finegold.** 2003. Rapid identification of gram-positive anaerobic coccal species originally classified in the genus *Peptostreptococcus* by multiplex PCR assays using genus- and species-specific primers. Microbiology **149:**1719–1727.
54. **Sonnenwirth, A. C.** 1970. Bacteremia with and without meningitis due to *Yersinia enterocolitica*, *Edwarsiella tarda*, *Comamonas terrigena*, and *Pseudomonas maltophilia*. Ann. N. Y. Acad. Sci. **174:**488–502.
55. **Stackebrandt, E., and B. M. Goebel.** 1994. Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int. J. Syst. Bacteriol. **44:**846–849.
56. **Stahelin, J., D. Goldenberger, H. E. Gnehm, and M. Altwegg.** 1998. Polymerase chain reaction diagnosis of *Kingella kingae* arthritis in a young child. Clin. Infect. Dis. **27:**1328–1329.

56a. **Summanen, P. H., B. Durmaz, M.-L. Väisänen, C. Liu, D. Molitoris, E. Eerola, I. M. Helander, and S. M. Finegold.** 2005. *Porphyromonas somerae* sp. nov., a pathogen isolated from humans and distinct from *Porphyromonas levii*. J. Clin. Microbiol. **43:**4455–4459.

57. **Thompson, J., D. Higgins, and T. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple-sequence alignment through sequence weighting, position-specific gap penalties, and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
58. **Titov, A. G., E. B. Vyshnevskaya, S. I. Mazurenko, S. Santavirta, and Y. T. Konttinen.** 2004. Use of polymerase chain reaction to diagnose tuberculous arthritis from joint tissues and synovial fluid. Arch. Pathol. Lab. Med. **128:** 205–209.
59. **Tronel, H., P. Plesiat, E. Ageron, and P. A. Grimont.** 2003. Bacteremia caused by a novel species of *Sphingobacterium*. Clin. Microbiol. Infect. **9:**1242–1244.
60. **Tunney, M., S. Patrick, M. Curran, G. Ramage, D. Hanna, J. Nixon, S. Gorman, R. Davis, and N. Anderson.** 1999. Detection of prosthetic hip protection at revision arthroplasty by immunofluorescence microscopy and PCR amplification of the bacterial 16S rRNA gene. J. Clin. Microbiol. **37:**3281–3290.
61. **Verettas, D., C. Kazakos, C. Tilkeridis, A. Dermon, H. Petrou, and V. Galanis.** 2003. Polymerase chain reaction for the detection of *Mycobacterium tuberculosis* in synovial fluid, tissue samples, bone marrow aspirate and peripheral blood. Acta Orthop. Belg. **69:**396–399.
62. **Ward, D. M., R. Weller, and M. M. Bateson.** 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in natural community. Nature **345:**63–65.
63. **Whiley, R. A., D. Beighton, T. G. Winstanley, H. Y. Frazer, and J. M. Hardie.** 1992. *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): association with different body sites and clinical infections. J. Clin. Microbiol. **30:**243–244.
64. **Yu, Z., and W. W. Mohn.** 1999. Isolation and characterization of thermophilic bacteria capable of degrading dehydroabietic acid. Can. J. Microbiol. **45:**513–519.
65. **Zannier, A., M. Drancourt, J. P. Franceschi, J. M. Aubaniac, and D. Raoult.** 1991. Intérêt d'une technique de lyse cellulaire par choc thermique dans l'isolement de bactéries responsables d'infections ostéo-articulaires. Pathol. Biol. **39:**543–546.
66. **Zimmerli, W., A. Trampuz, and P. E. Ochsner.** 2004. Prosthetic-joint infections. N. Engl. J. Med. **351:**1645–1654.