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Improved diagnosis specificity in bone and joint infections using molecular techniques[☆]

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Summary *Objectives:* The microbiological diagnosis of osteoarticular infections currently relies on microbiological cultures, to specifically target treatment. However, these conventional methods sometimes lack of sensitivity and of specificity to establish definitive diagnosis. This study was conducted to determine whether molecular method could improve bacterial bone and joint infection diagnosis.

Methods: We evaluated the performance of nucleic acid extraction with the semi-automated NucliSens miniMAG instrument coupled to 16S rDNA sequencing on 76 samples collected from 51 patients with suspected infections: prosthetic-joint infection (15), spondylodiscitis (7) acute septic arthritis (11) and 18 controls. No pre-treatment of the sample was done before nucleic acid extraction. Classification in infected group required an accumulation of arguments.

Results: Our molecular method identified a broad spectrum of pathogenic bacteria including *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Enterococcus faecalis*, *Salmonella enterica*, *Escherichia coli*, *Pseudomonas aeruginosa*, and fastidious bacteria like *Neisseria gonorrhoeae* and *Fusobacterium nucleatum*. The overall PCR sensitivity was 73.3%: 53.8% for prosthetic-joint infections and 88.2% for infections without prostheses. The overall PCR specificity was 95.2%, whereas culture specificity was only 85.7%.

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Conclusions: The instrument was simple to use and provided nucleic acids free of PCR inhibitors and free of contamination by foreign bacterial DNA. Our study highlights the need for still improved molecular diagnostic sensitivity.

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Introduction

Osteoarticular infections are painful and disabling diseases that require antimicrobial treatment adapted to the micro-organisms implicated.^{1–3} Microbiological cultures are currently regarded as the reference for identification of pathogenic bacteria. However, the sensitivity of these cultures is very variable and depends both on the context in which clinical samples are taken, and on the pathogen involved. The rate of detection varies according to infection type: 50–70% for infectious spondylodiscitis,⁴ 65–95% for prosthetic-joint infections,² 50% for gonococcal arthritis and 90% for non-gonococcal arthritis.³ Patients undergoing antibiotic treatment during the time when samples are taken, and bacteria enclosed within biofilms on an orthopaedic implant^{2,5} are two situations that frequently explain false-negative cultures. Furthermore, the development of delayed chronic infections caused by low-virulence bacteria of the skin flora⁶ is often difficult to establish because of the risk of culture contamination during sample manipulations. This problem will probably be exacerbated by the growing number of prosthetic implants due to increased lifespan in industrialized countries. If an infection is unrecognized and is not treated with appropriate antibiotics, patients may be exposed to the risk of recurrent infection when prostheses are changed. Also, acute septic arthritis is a serious disease that, if not quickly and appropriately treated, exposes the patient to functional sequels with destruction of the joint surfaces.³ Fastidious organisms like *Neisseria gonorrhoeae* in young adults and *Kingella kingae* in children make diagnosis difficult with a synovial culture sensitivity lower than 50%.^{3,7} The continual increase in gonorrhoea observed in France since 1998⁸ could result in a resurgence in disseminated gonococcal infection and arthritis in the next few years. Lastly, infectious spondylodiscitis is the most frequent haematogenous osteomyelitis, and especially affects the increasing numbers of elderly patients with the risk of soft-tissue extension, para-vertebral abscesses and cord compression.¹

Molecular diagnostic methods are of interest because of the potential for rapid detection of the bacterial genome in a sample without prior knowledge of whether or not the bacteria can be cultured *in vitro*. Several authors already showed an improvement in etiologic diagnosis of bacterial infections by the use of a universal PCR strategy that targeted 16S rDNA in clinical samples.^{4,9–11} Molecular diagnostic methods applied to joint infections established the high incidence of *Kingella kingae* infections in children.⁷ However, the literature contained only a few studies that addressed the usefulness of PCR in prosthetic-joint infections.^{12–14} Only some of them¹¹ had information on the DNA sequences amplified whereas there is a major risk of contamination by exogenous DNA in the nucleic acid extraction step or in the PCR reactions.¹⁵

Several new commercial systems recently have become available to facilitate standardization of extraction protocols, optimal elimination of amplification inhibitors, and efficient nucleic acid recovery. We evaluated the usefulness of molecular diagnosis without pre-treatment of disc-vertebral samples, osseous biopsies or articular fluid punctures with a new semi-automated Nuclisens miniMAG system (bioMérieux, Marcy-l'Etoile, France). The originality of our study is to compare the diagnostic performances of microbiological cultures and our molecular method by considering for the definition of cases an accumulation of clinical, radiological and biological arguments usually used to establish the diagnosis of bone and joint infections.

Patients and methods

Patients

Seventy-six clinical joint or bone specimens (from 51 adults admitted to our hospital) were given to the Microbiology Department for routine microbiological culture between January 2004 and June 2005; residual material was stored at -80°C for further molecular analyses. The assay was not performed in a comparative study of sequential specimens but specimens were selected when clinical and other laboratory variables suggested a high likelihood of infection. None of the patients had received antibiotics during the 15 days preceding sampling. Fifteen patients (8 men and 7 women, average age 57 years) presented with suspicion of infection on orthopaedic implant¹⁶ (pain or functional disability, fever, radiological evidence of joint loosening, inflammation). Infections were classified as early (those that developed less than 2 months after surgery), delayed (2–24 months after surgery), or late acute (more than 24 months after surgery), as previously described.^{2,5} Seven patients (5 men and 2 women, average age 59 years) presented with signs of infectious spondylodiscitis¹⁷ (localized vertebral pain, fever, and changes consistent with infection on Magnetic Resonance Imaging). Eleven patients (9 men and 2 women, average age 53 years) presented with signs of acute septic arthritis without orthopaedic implant³ (effusion, fever, leukocyte count in synovial fluid $>2000/\text{mm}^3$ and/or polymorphonuclear cells $>90\%$). The controls were 19 specimens from 18 patients (5 with an orthopaedic prosthesis and 13 without) who did not have any signs of infection, had no previous history of infection, and who had not received local corticosteroid injection.

Each patient was classified by his physician in "infected" or "not infected" group on the basis of whole of arguments including clinical presentation, inflammatory assessment (ESR, CRP), the results of imagery, microbiological cultures, histology and the evolution of the whole of these parameters under antibiotic treatment adapted to the microbiological results. The patients considered as "not infected" did not receive antibiotic treatment and had a

clinical, biological and radiological follow-up during at least one year.

Clinical specimens

A total of 43 synovial fluids, including 4 joint lavage specimens, were recovered either during surgery, by puncture aspiration at the bedside, or by guided ultrasonography, all under sterile conditions (mask, sterile gloves, cautious skin preparation and towelling). The majority of the synovial fluids were transported to the Bacteriology department in sterile tubes without additional substrate in less than 4 h. Ten CT-guided discovertebral biopsies and 23 tissue samples from synovial membrane, or from bone in contact with the orthopaedic implant, were recovered for microbiological analysis under the same conditions of asepsis and carriage. In operating theatre, routine antibiotic prophylaxis was done after sampling.

Microbiological methods

For non-coagulated liquid samples, erythrocyte and leukocyte counts were done, and the polymorphonuclear percentage was determined for any count $>1000/\text{mm}^3$ (Shandon 3 Cytospin[®] cytocentrifuge preparation, Thermo Electron Corp., Saint-Herblain, France). Solid specimens were crushed with a sterile mortar and pestle, and then fine smears were made.

All samples were examined after Gram and auramine staining and cultured at 37 °C on Columbia sheep blood agar (aerobically and anaerobically for 5 days), chocolate agar supplemented with IsoVitaleX (with 5% CO₂ for 5 days) and brain heart infusion broth (anaerobically for 15 days with subcultures done after 10 days on the same agar media, even if aspect was not cloudy). All media were obtained from bioMérieux, Marcy l'Etoile, France. Bacteria from isolated colonies were identified using standard procedures,¹⁸ API strips (bioMérieux) or molecular methods as previously described.^{19,20} Mycobacterial culture was carried out on Lowenstein-Jensen and Coletsos media incubated for 3 months.

Molecular methods

All DNA manipulations pre- and post-PCR were done in separate rooms with separate pipetting devices to prevent contamination of samples with foreign DNA. All equipment used in pre-PCR steps was DNA-free and UV-irradiated. DNA extraction based on Boom's method²¹ was done using NucliSens magnetic extraction reagents. The sample (200 µl) was added to the lysis buffer and incubated for 10 min at room temperature. Then 50 µl of magnetic silica was mixed with the lysis buffer-sample mixture for 10 min. The lysis buffer/silica/sample mixture was pelleted, and the supernatant was aspirated. The pellet was resuspended in 400 µl of wash buffer 1 and then transferred to a 1.5-ml centrifuge tube. Several wash steps were done using the miniMAG semi-automated instrument. After the last wash buffer was aspirated, 25 µl of elution buffer was added and incubated for 5 min at 60 °C. Tubes were moved against a magnetic rack while eluted DNA was pipetted. No

pre-treatment of the sample (e.g. with lysostaphin, lysozyme, or proteinase K) was done to improve bacterial lysis.

Each DNA amplification was done with 5 µl of nucleic acid extract in a final 50 µl reaction mixture containing 1.5 mM MgCl₂, 200 µM dNTPs (Eurobio, Les Ulis, France), 0.2 µM of each primer and 1.5 units of *Taq* DNA polymerase (Roche Applied Science, Meylan, France). Broad-range 16S rRNA gene amplification was done with three sets of primers previously described: 536f (5'-CAG CAG CCG CGG TAA TAC-3') and 1050r (5'-CAC GAG CTG ACG ACA-3')²²; 16S-cons-F (5'-YGG CGR ACG GGT GAG TAA-3'), 16S-GP-R (5'-CCG ATC ACC CTC TCA GGT CG-3') and 16S-GN-R (5'-AGT TAG CCG GTG CTT CTT CT-3')⁴; 16S-241bp-F (5'-GGA GGA AGG TGG GGA TGA CG-3') and 16S-241bp-R (5'-ATG GTG TGA CGG GCG GTG TG-3').^{23,24} The corresponding amplicons were sequenced in both directions on an ABI PRISM 3100 analyser (Applied Biosystems, Courtaboeuf, France). Sequence similarities were searched in GenBank using the BLASTN program through the website of the National Center of Biotechnology Information. An internal control for extraction and PCR inhibition was the amplification of the beta-actin gene with the primers 5'-CCA GAG CAA GAG AGG CAT CC-3' and 5'-GCT GGG GTG TTG AAG GTC TC-3'.²⁵

Results

Prosthetic-joint infection

Among the 15 patients with suspected prosthetic-joint infection, 14 patients had positive cultures from 31 specimens (Table 1). A single pathogen was isolated from each of 13 patients: *Staphylococcus aureus* ($n = 7$), *Staphylococcus epidermidis* ($n = 2$), *Streptococcus oralis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Moraxella osloensis* ($n = 1$ each). For patient 2, two bacteria were found: *S. aureus* was found on all media and *S. epidermidis* was found in brain heart infusion broth only. The physician concluded that the presence of *S. epidermidis* was an unreliable result; this conclusion had no effect on patient outcome. For patient 3, no pathogen was identified in the liquid sample included in the study. However, *S. aureus* was found in a bone biopsy carried out later from bone in contact with the prosthesis. For solid samples, the number of positive cultures out of the total number tested was 18/19 (95%), and for synovial fluid samples it was 9/12 (75%). For patients 14 and 15, physicians did not diagnose infection (despite positive culture results) because of the low number of positive culture media (only one for each sample) and because of negative plain radiograph and leukocyte labelled scintigraphy findings. These patients were clinically well after 1 year of follow-up without antibiotic treatment. Finally, only 13 patients (nos. 1–13) were considered infected on the basis of these results.

We used 16S rDNA sequences to identify the bacteria in 10 PCR-positive samples of 36 tested. They were from 8 patients with positive cultures: *S. aureus* was found in 5 cases, *E. coli*, *P. aeruginosa* and *M. osloensis* in one case each. In all cases, there was full agreement between the molecular bacterial identifications and those obtained with conventional methods. For solid samples, we obtained a positive PCR on 8 of the 19 samples included in the study

Table 1 Clinical and laboratory results of patients with suspected Early (E), Delayed (D) or Late Acute (LA) prosthetic-joint infection, or control group (C)

Patient	Sex ^a	Age (years)	Condition suspected	No. of samples	Laboratory results ^b					
					Positive cultures		Conventional identification	Positive PCR		Molecular identification
					Solid samples	Synovial fluid		Solid samples	Synovial fluid	
<i>Diagnoses retained by physicians</i>										
1	M	27	LA	4	4	—	<i>S. aureus</i>	2	—	<i>S. aureus</i>
2	F	54	D	1	1	—	<i>S. aureus</i>	1	—	<i>S. aureus</i>
3	F	55	LA	1	—	0	<i>S. epidermidis</i> ^c	—	0	—
4	F	83	LA	1	—	1	<i>S. aureus</i>	—	1	<i>S. aureus</i>
5	M	41	D	3	3	—	<i>S. aureus</i>	1	—	<i>S. aureus</i>
6	M	75	E	4	1	2	<i>S. aureus</i>	1	1	<i>S. aureus</i>
7	F	50	E	2	1	1	<i>E. coli</i>	1	0	<i>E. coli</i>
8	M	77	E	4	3	1	<i>P. aeruginosa</i>	1	0	<i>P. aeruginosa</i>
9	M	82	E	2	1	0	<i>S. aureus</i>	0	0	—
10	M	38	LA	2	—	1	<i>S. aureus</i>	—	0	—
11	M	44	E	1	—	1	<i>S. aureus</i>	—	0	—
12	f	49	E	2	2	—	<i>S. epidermidis</i>	0	—	—
13	F	78	LA	1	—	1	<i>S. oralis</i>	—	0	—
<i>Diagnoses discarded by physicians</i>										
14	F	58	D	2	1	1	<i>S. epidermidis</i>	0	0	—
15	M	46	LA	1	1	—	<i>M. osloensis</i>	1	—	<i>M. osloensis</i>
<i>Control group</i>										
16	F	57	C	1	—	0	—	—	0	—
17	F	66	C	1	—	0	—	—	0	—
18	F	45	C	1	—	0	—	—	0	—
19	F	41	C	1	—	0	—	—	0	—
20	F	43	C	1	—	0	—	—	0	—
Total				36	18	9		8	2	
					27			10		

^a M, male, F, female.

^b "0" represents negative results, "—" , not done.

^c Culture showed the presence of *S. aureus* and *S. epidermidis* (see text). However, PCR detected only *S. aureus* but no *S. epidermidis*.

^d Biopsies at the edges of the prosthesis (not included in the study) and blood cultures showed *S. aureus* infection.

(42%). Puncture fluid samples showed 2 specimens that were PCR positive out of 12 tested (17%). Of 4 culture negative samples from infected patients (1 biopsy and 3 synovial fluids), none was PCR positive. Finally, sequencing allowed etiologic diagnosis for 7 of the 13 infected patients (54%). We did not observe any significant difference in results between various types of prosthetic-joint infection (early, delayed and late acute). PCR inhibitors were not present since the control reaction with the beta-actin gene was positive in all cases (data not shown).

The cultures and 16S rDNA PCR were negative for all control patients (nos. 16–20).

Spondylodiscitis and acute septic arthritis

Among the 18 patients with suspected infection without orthopaedic implant (spondylodiscitis for patients 21–27; acute septic arthritis for patients 28–38), all 26 samples

tested were positive in culture except for one knee synovial fluid for patient 32 (Table 2). A broad range of pathogenic bacteria was found, with only a single pathogen isolated from each of 17 patients. For spondylodiscitis, 1 *S. epidermidis*, 1 *Enterococcus faecalis*, 2 *Streptococcus agalactiae*, 1 *E. coli*, 1 *Salmonella enterica* serotype Muenchen and 1 *Fusobacterium nucleatum* were found. For acute septic arthritis, 4 *S. aureus*, 1 *Streptococcus pyogenes*, 2 *S. agalactiae*, 3 *N. gonorrhoeae* and 1 *Streptococcus bovis* were found. For patient 36, one synovial fluid aspiration out of three yielded a culture of *Propionibacterium acnes* in brain heart infusion broth in 15 days. This was not the case with the 2 other specimens from the same patient, which both contained *Neisseria gonorrhoeae* (more than 10³ per ml). The acute clinical course of the disease also seemed incompatible with an infection by *P. acnes*. The patient was allergic to β -lactams and was treated by ciprofloxacin (400 mg i.v. every 8 h) although this antibiotic was inactive *in vitro* on the *P. acnes* isolate (data not shown). He was clinically

Table 2 Clinical and laboratory results of patients with suspected spondylodiscitis (S), acute septic arthritis (ASA), or control group (C)

Patient	Sex ^a	Age (years)	Condition suspected	No. of samples	Laboratory results ^b					
					Positive cultures		Conventional identification	Positive PCR		Molecular identification
				Solid samples	Synovial fluid			Solid samples	Synovial fluid	
<i>Diagnoses retained by physicians</i>										
21	M	73	S	2	2	—	<i>S. epidermidis</i>	0	—	—
22	M	79	S	1	1	—	<i>E. faecalis</i>	1	—	<i>E. faecalis</i>
23	M	76	S	1	1	—	<i>S. agalactiae</i>	1	—	<i>S. agalactiae</i>
24	F	46	S	1	1	—	<i>S. agalactiae</i>	1	—	<i>S. agalactiae</i>
25	M	63	S	1	1	—	<i>E. coli</i>	1	—	<i>E. coli</i>
26	F	26	S	2	2	—	<i>S. enterica</i>	1	—	<i>S. enterica</i>
27	M	51	S	2	2	—	<i>F. nucleatum</i>	2	—	<i>F. nucleatum</i>
28	M	54	ASA	1	1	—	<i>S. aureus</i>	1	—	<i>S. aureus</i>
29	F	67	ASA	1	—	1	<i>S. aureus</i>	—	1	<i>S. aureus</i>
30	M	29	ASA	1	1	—	<i>S. aureus</i>	1	—	<i>S. aureus</i>
31	M	83	ASA	1	—	1	<i>S. pyogenes</i>	—	1	<i>S. pyogenes</i>
32	M	46	ASA	2	—	1	<i>S. agalactiae</i>	—	2	<i>S. agalactiae</i> ^c
33	M	48	ASA	2	2	—	<i>S. agalactiae</i>	1	—	<i>S. agalactiae</i>
34	M	39	ASA	2	—	2	<i>N. gonorrhoeae</i>	—	2	<i>N. gonorrhoeae</i>
35	M	49	ASA	1	—	1	<i>N. gonorrhoeae</i>	—	1	<i>N. gonorrhoeae</i>
36	M	45	ASA	3	—	3	<i>N. gonorrhoeae</i> <i>P. acnes</i> ^d	—	1	<i>N. gonorrhoeae</i>
37	F	89	ASA	1	—	1	<i>S. bovis</i>	—	0	—
<i>Diagnoses discarded by physicians</i>										
38	M	34	ASA	1	—	1	<i>S. aureus</i>	—	0	—
<i>Control group</i>										
39	F	41	C	1	—	0	—	—	0	—
40	M	40	C	1	—	0	—	—	0	—
41	M	43	C	1	—	0	—	—	0	—
42	M	48	C	1	—	0	—	—	0	—
43	F	58	C	2	—	0	—	—	0	—
44	F	58	C	1	—	0	—	—	0	—
45	M	82	C	1	—	0	—	—	0	—
46	F	45	C	1	—	0	—	—	0	—
47	M	53	C	1	—	0	—	—	0	—
48	F	56	C	1	—	0	—	—	0	—
49	F	68	C	1	—	0	—	—	0	—
50	F	34	C	1	—	0	—	—	0	—
51	F	84	C	1	—	0	—	—	0	—
Total				40	14	11		10	8	
					25			18		

^a M, male, F, female.

^b "0" represents negative results, "—" , not done.

^c PCR showed bacteria in two different joints (ankle and knee) whereas culture showed bacteria only in one liquid puncture (ankle).

^d One synovial fluid culture was positive in 15 days with *Propionibacterium acnes*. The PCR on this sample was negative.

well after 1 year of follow-up, which argues that *P. acnes* was a contamination. For patient 38, physicians concluded that there was no infection because of the low number of positive cultures (only one brain heart infusion broth), the long culture time (10 days) and because the symptoms disappeared without antibiotic treatment. Finally, only 17 patients (nos. 21–37) were considered infected on the basis of these results.

Molecular methods allowed etiologic diagnosis for 15 of these 17 infected patients (88%). For solid samples, we obtained a positive PCR from 10 of the 14 samples (71%). Puncture fluid samples showed a positive PCR for 8 out of 10 specimens (73%). In all cases, there was full agreement between the bacterial identification obtained with molecular and conventional methods. For patient 32, molecular methods showed a false negative culture with

Streptococcus agalactiae detection in knee synovial liquid of a patient also presenting with a septic arthritis of the ankle. No antibiotic treatment was done between the two aspirations. No amplification was obtained for patient 38 whose culture was regarded as a false positive. PCR negative samples did not have amplification inhibitors, based on internal controls with the beta-actin gene.

The cultures and 16S rDNA PCR were negative for all control patients (nos. 39–51).

Performance

For patients with suspected prosthetic-joint infection, molecular diagnosis (16S rDNA PCR and sequencing) had a sensitivity of 53.8% when the physician's final decision was considered the gold standard (Table 3). This sensitivity was 75% if only solid specimens were considered (data not shown), and the specificity was 85.7%.

For patients without prostheses, the diagnosis sensitivity was 88.2%. As we had no false positive PCRs in our series, the specificity and positive predictive values were excellent. For molecular diagnosis, the overall sensitivity was 73.3% and specificity was 95.2%. For microbiological cultures, overall sensitivity was 96.7% and specificity was 85.7%.

Discussion

In this study of 30 cases of definite osteoarticular infections confirmed by conventional culture, we evaluated the performance of nucleic acid extraction by the new NucliSens miniMAG instrument coupled to broad range 16S rDNA PCR to identify the bacterial agent responsible. We chose this molecular target because analysis of this sequence allows identification of a broad spectrum of bacteria²⁶ with good sensitivity.^{4,9,10} In addition, PCR product sequencing may detect possible contamination by foreign DNA at the specimen extraction or amplification steps if all results in one run are identical.

We evaluated the semi-automated NucliSens miniMAG instrument because its technology seemed particularly adapted to bacterial detection in clinical samples. First, nucleic acid extraction was done using silica particles that bind nucleic acids in the presence of high concentrations of the chaotropic agent guanidinium thiocyanate (GuSCN).²¹ This methodology efficiently extracted and concentrated the nucleic acid target and was especially effective in the elimination of amplification inhibitors.²⁷ Two recent

studies confirmed this for blood²⁸ and urinary samples.²⁹ Moreover, the miniMAG instrument can process between 1 and 12 specimens per run, which allows for the inclusion of controls, and which is compatible with the multiple sampling usually done when prosthesis joint infection or infectious spondylodiscitis is suspected.

We could have chosen, like the majority of the studies published to date, to compare our molecular method with another method of diagnosis (culture, histology). However, there is no current gold standard and the diagnosis of osteoarticular infections generally rests on an accumulation of arguments. To determine, with less possible error, if each patient included were "infected" or "not infected", we adopted a methodology close to daily clinical reality and trusted in physician who was treating the patient.

In our study, molecular methods never yielded false positive results, leading to excellent specificity. There was complete agreement between identification to the species level obtained by conventional methods and 16S rDNA fragment sequencing. Thus, our molecular identification method predicted the conventional identification results in all cases. To achieve these results, we also took precautions to avoid any risk of sample contamination by foreign bacterial DNA. For example, *Taq* DNA polymerase is a recombinant form of an enzyme from the thermophilic eubacterium *Thermus aquaticus*, and it is expressed in *E. coli* cells. This enzyme is highly purified and is guaranteed free of endo- or non-specific exo-nucleases, but is generally not free from exogenic bacterial DNA. Thus, we checked each new batch of enzyme by doing 16S rDNA amplification on DNA-free water before use. This control may not be necessary anymore, as new *Taq* DNA polymerases certified DNA-free are currently available, although they weren't at the time of our study. Moreover, the control group selection of patients, who had no previous infection, rheumatoid arthritis or reactive arthritis avoided any detection of residual bacterial DNA as described in other studies.^{30–32}

We decided to analyse separately prosthetic-joint infections and infections without prosthesis. It is well established that the pathogenesis of these infections is different.^{1,2,5} In prosthetic-joint infections, bacteria are in a biofilm and are often in a slow-growing state that makes them more difficult to detect by culture, especially from synovial aspiration. Our results agree with this assumption since the diagnostic yield of solid sample cultures (95%) was higher than for liquid sample cultures (75%). Molecular methods had a diagnostic sensitivity of 53.8%, which may be disappointing at first sight. Indeed, similar methods

Table 3 Diagnostic yield of molecular methods and cultures when physician's final decision is considered the gold standard

	Test	% Sensitivity (n)	% Specificity (n)	PPV ^a (n)	NPV ^b (n)
Prosthetic-joint infections	PCR	53.8 (7/13)	85.7 (6/7)	0.875 (7/8)	0.5 (6/12)
	Culture	92.3 (12/13)	71.4 (5/7)	0.857 (12/14)	0.833 (5/6)
Osteoarticular infections without prosthesis	PCR	88.2 (15/17)	100 (14/14)	1 (15/15)	0.875 (14/16)
	Culture	100 (17/17)	92.9 (13/14)	0.944 (17/18)	1 (13/13)
All infections	PCR	73.3 (22/30)	95.2 (20/21)	0.957 (22/23)	0.714 (20/28)
	Culture	96.7 (29/30)	85.7 (18/21)	0.906 (29/32)	0.947 (18/19)

^a PPV, positive predictive value.

^b NPV, negative predictive value.

previously described in the literature reported higher rates ranging from 63% to 71%.^{12,14} In a study of 120 revision arthroplasties¹³ detection of bacterial DNA was reported in 72% of specimens, whereas the culture was positive in only 33% of the cases. However, our results are not easily comparable because our series showed a high positive culture rate with 12 positive cases from 13 definitely infected patients. This high rate may be because no patient was under antibiotic treatment and routine antibiotic prophylaxis was done after sampling. Furthermore, in any of these studies, PCR products were sequenced to confirm that the amplified DNA was representative of the organism found in culture, or to exclude false positives. Lastly, our results were obtained without any sample pre-treatment because the main goal of this study was to evaluate a new method of DNA extraction without multiplying the parameters to be analysed. Methods to dislodge adherent bacteria from biofilms such as sonication, mechanical disruption or enzyme treatment should improve our results.¹⁵

Acute septic arthritis and primary vertebral osteomyelitis are most frequently caused by haematogenous spread of the pathogenic micro-organism from a distant focus. Host immune responses and bacterial virulence factors may contribute to the release of bacterial cells in surrounding spaces, causing spread of the infection.^{1,3} Under these conditions, conventional microbiological cultures generally lead to etiological diagnosis. The causes of false negative cultures are the involvement of slow- or difficult-growing bacteria and antibiotic treatment during sampling. In this study, molecular methods correctly identified fastidious bacteria (3 *N. gonorrhoeae* and 1 *F. nucleatum*) and these etiological diagnoses were done in 48 h whereas conventional methods had required between 2 and 5 days to give the same result. This reduced diagnosis delay could lead to an improved prognosis for certain patients. In one case (patient 32), molecular methods produced a diagnosis that had been missed by conventional cultures. It seems that the microbiological cultures are sufficient in most cases to establish the diagnosis. These results are in agreement with the literature³² but it is possible that diagnosis improvement would be more significant if it included patients who had already received antibiotics. A broad prospective study is under development in our hospital to confirm this assumption.

Our overall results (Table 3) are comparable with those of a recent larger study.¹¹ For diagnosis of bone and joint infections (prosthetic or not are mixed), the authors reported that 16S rDNA sequencing had 92.5% sensitivity of and 95.7% specificity, and that culture had 86.7% sensitivity and 89% specificity. The use of an overnight pre-treatment by proteinase K to release bacteria before extraction could explain the difference in sensitivity. Moreover, our study had a high rate of positive cultures, because it was retrospective, which introduced a bias in sensitivity results. In addition, our results highlight that molecular methods are more specific than microbiological cultures even if several samples from one patient are analysed simultaneously.

In 1.5 h, we obtained with semi-automated NucliSens miniMAG instrument nucleic acids free of PCR inhibitors and free of contamination by foreign bacterial DNA. These results are compatible with use in emergency situations. Unfortunately, the attention of readers must be drawn to

different points: (i) molecular methods are not yet rather sensitive and not useful clinically when PCR results are negative; (ii) our method does not provide any false positive by PCR contamination and could be regarded as more specific than cultures—a PCR positive result would be a supplementary argument to establish the diagnosis of infection; (iii) high-quality nucleic acid extraction may extend the application of molecular methods to areas such as the detection of bacterial antibiotic resistance and virulence genes directly from clinical samples.

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