Evaluation of 16S rDNA PCR Sensitivity and Specificity for Diagnosis of Prosthetic-Joint Infection: a prospective multicenter cross-sectional study

Pascale Bémer, 1# Chloé Plouzeau, 2 Didier Tande, 3 Julie Léger, 4 Bruno Giraudieu, 4 Anne Sophie Valentin, 5 Anne Jolivet-Gougeon, 6 Pascal Vincent, 6 Stéphane Corvec, 1 Sophie Gibaud, 1
1 Marie Emmanuelle Juvin, 1 Genevieve Héry-Arnaud, 3 Carole Lemarié, 7 Marie Kempf, 7
6 Laurent Bret, 8 Roland Quentin, 5 Carine Coffre, 4 Gonzague de Pinieux, 9 Louis Bernard, 10
7 Christophe Burucoa 2 and the CRIOGO (Centre de Référence des Infections Ostéo-articulaires du Grand Ouest) Study Team

1 CHU Nantes, Laboratoire de Bactériologie, Nantes, France; 2 CHU Poitiers, Laboratoire de Bactériologie, Poitiers, France; 3 CHU Brest, Laboratoire de Bactériologie, Brest, France; 4 Inserm, CIC 1415, Tours, France; 5 CHU Tours Laboratoire de Bactériologie, Tours, France; 6 CHU Rennes, Laboratoire de Bactériologie, Rennes, France; 7 CHU Angers University Hospital, d’Anatomo-Pathologie, Tours, France; 10 CHU Tours, Service des Maladies Infectieuses, Tours, France.

Keywords: Prosthetic-Joint-Infection, multicenter study, 16S rDNA PCR, sensitivity, specificity

Running title: 16S rDNA PCR for diagnosis of PJI on a routine basis

Corresponding author: Pascale Bémer, CHU Nantes, Laboratoire de Bactériologie, 1 Place Alexis Ricordeau, 44095 Nantes, France. Phone number: 33 2 40 08 39 85 – Fax number: 33 2 40 08 38 29 - Email: pascale.bemer@chu-nantes.fr
Abstract

Background There is no gold standard for the diagnosis of prosthetic-joint-infection (PJI). The contribution of 16S rDNA PCR sequencing on a routine basis remains to be defined. We performed a prospective multicenter study to assess the contribution of 16S rDNA in PJI diagnosis.

Methods Over a 2-year period, all patients suspected of PJI and few non-infected patients with primary arthroplasty (control group) were included. Five per-operative samples were collected per patient for cultures and 16S rDNA PCR-sequencing, and one for histology. Three multicenter quality controls were performed with both DNA extracts and crushed samples. The diagnosis of PJI was based on clinical, bacteriological and histological criteria according to IDSA guidelines. A molecular diagnosis was modelled on a bacteriological criterion (≥1 positive sample for strict pathogens; ≥2 for commensal skin flora). Molecular data were analyzed according to the diagnosis of PJI.

Findings From December 2010 to March 2012, 264 suspected cases of PJI and 35 control patients were included. PJI was confirmed in 215/264 suspicions, 192 with a bacteriological criterion (89%). The PJI was monomicrobial (n=163, 85%) (Staphylococci n=108, Streptococci n=22, Gram-negative bacilli n=16, anaerobes n=13, others n=4) or polymicrobial (n=29, 15%). The molecular diagnosis was positive in 151/215 confirmed cases of PJI (143 with bacteriological PJI documentation and 8 treated patients without bacteriological documentation), and in 2/49 patients without confirmed PJI (sensitivity 73.3%, specificity 95.5%).

Interpretation 16S rDNA PCR showed a lack of sensitivity in the diagnosis of PJI on a multicenter routine basis.
Introduction. Prosthetic Joint Infection (PJI) is one of the most serious complications of orthopaedic surgery, increasing the risk of morbidity and mortality of this very frequent operation. The infection rate is estimated at about 1% after hip and shoulder replacement, and at about 2% for knee prosthesis (1). Despite the lack of a gold standard definition of PJI, bacterial documentation remains the cornerstone of the diagnosis. Bacterial adherence to biomaterials and tissues adjacent to prostheses is essential in the development of PJI (1). Bacteriological diagnosis requires the extraction of bacteria from a periprosthetic tissue biofilm. Culture of prosthetic sonicate-fluid was more sensitive than traditional tissue culture when antibiotics were stopped within 14 days before surgery (75% vs. 45%, p<0.001) (2). Nevertheless, the conventional bacteriological method used in comparison to sonication was simple homogenization of tissue specimens before culture (2). Recently, bacterial extraction using bead mill processing of specimens improved bacteriological diagnosis of PJI (3).

Histological examination of periprosthetic tissue is recommended if there is any suspicion of PJI (4,5). The gold standard is based on the count of polymorphonuclear neutrophils per high-power field (PMN/HPF). The cut-off point (number of neutrophils per field) to affirm infection differed among authors, but nevertheless, the Mirras’ criterion of ≥ 5 PMN/HPF (adapted by Feldman) remains the most commonly used (6-8).

Broad-range 16S rDNA gene PCR has already been evaluated for diagnosis of PJI. 16S rDNA PCR from periprosthetic tissues or periprosthetic sonicate-fluid showed a wide range of sensitivity and specificity values, from 50 to 92% and 65 to 94% respectively (9-15). Compared with conventional culture, the sensitivity of 16S rDNA PCR was higher, lower or equivalent,
sometimes to the detriment of specificity (9-15). More recent studies on 16S rDNA PCR of periprosthetic sonicate fluids have shown contradictory results too. In one study, 16S rDNA PCR and culture of sonicate fluid were shown to have equivalent performance for PJI diagnosis (16). In another study, 16S rDNA PCR of periprosthetic tissues or sonicate fluid did not diagnose more PJI than a culture of adequate periprosthetic tissues (17). Finally, a recent study found greater sensitivity with a multiplex PCR panel, including anaerobic bacteria applied to implant-derived sonicate fluid (18). These contradictory results may be due to different pre-treatment procedures applied to samples before DNA extraction, to the different number of samples per patient, and finally to the largest panel of bacteria, especially anaerobes (such as Propionibacterium acnes which is often missed by 16S rDNA PCR). The wide range of PCR performance through different monocenter studies underlines the interest of multicenter protocols for evaluating 16S rDNA PCR.

The Infectious Diseases Society of America (IDSA) provided recent guidelines, assessing definitive evidence of PJI when per-operative surgical features of infection were observed with multiple tissue specimens found to be positive in culture (4). At the end of the IDSA guidelines, gaps in information were identified, such as the role of PCR and bead mill processing in the diagnosis of PJI on a routine basis (4).

The main objective of our study was to assess the contribution of 16S rDNA PCR to PJI diagnosis. Our network organization of pluridisciplinary diagnosis and treatment of bone and joint infections in seven referent centers was used to achieve the first prospective multicenter study relating to molecular diagnosis of PJI.
Patients and methods.

Study design

This study is designed as a multicenter, prospective, observational, cross-sectional study of adult patients suspect of PJI. The study protocol was approved by the institutional review board or ethics committee at every site. Informed consent was obtained from each patient before inclusion.

Study population

Consecutive patients with clinical signs suggesting acute or chronic PJI were included in 7 French University hospitals from December 2010 through March 2012. During the same study period, 5 patients per center undergoing primary total arthroplasty with no previous history of joint surgery were included, and their tissue samples were processed as negative controls for the PCR and culture procedures. Six tissue samples were collected during surgery, five samples for the culture and PCR, and one periprosthetic membrane sample for histological analysis. Case report forms were created for collecting the following data for each patient: patient characteristics, arthroplasty localization, presentation of infection, and antibiotic treatment over the 15 days before surgery.

Definition of a PJI

Acute PJI was suspected in patients with pain, disunion, necrosis or inflammation of the scar in the months following prosthesis implantation. Chronic infection was suspected in the presence of chronic pain without systemic symptoms, as well as a loosened prosthesis (4,5). According to the IDSA proposals, PJI was diagnosed when at least one of the following criteria was positive:
1/ Clinical criterion with the presence of a sinus tract communicating with the prosthesis, and/or purulence around the prosthesis.

2/ Histology positive for infection (as specified above).

3/ Bacteriological criterion of infection (as specified below).

**Microbiological methods**

Cultures of periprosthetic tissues were performed in each center following a standardized protocol. For each patient, 5 per-operative specimens were collected in sterile vials with different surgical instruments. After the addition of 10 mL sterile water and 10 sterile stainless steel beads (4 mm diameter), the vials were shaken on a Retsch MM401 bead mill for 2 min 30 sec, at 30 Hertz per minute. Two aliquots of each of the 5 bead milled suspensions were collected for molecular assays. 2 mL aliquots were inoculated into a blood culture bottle and a Schaedler anaerobic liquid broth, both incubated at 37°C. Three further 50 µL volumes each were spread onto a blood agar plate and a Polyvitex chocolate agar plate, both incubated at 37°C in 5% CO₂ for 7 days, and a blood agar plate incubated at 37°C in an anaerobic atmosphere for 5 days. Schaedler anaerobic liquid broth was subcultured on blood agar plates in an anaerobic atmosphere for 72 hours if cloudy, or systematically at the 14th day. Isolated bacteria were identified according to standard laboratory procedures. Antibiotic susceptibility testing was determined as recommended (19). The bacteriological criterion was considered positive when at least one culture yielded a strict pathogen (*Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacteriaceae*, anaerobes), or two cultures if the pathogen was a skin commensal (such as coagulase-negative staphylococci (CoNS) or *Propionibacterium acnes*) (4).
Histological analysis

The periprosthetic membrane samples were fixed in buffered formalin, and paraffin block
sections were stained with haematoxylin and eosin. Following Feldman’s criteria (adapted from
Mirra’s criteria), the histology was considered positive for infection when at least 5 neutrophils
per high-power field (x400) were found after examination of at least five separate microscopic
fields (6,7). The specimens were examined by pathologists blinded to the presence of infection
and to the results of the cultures.

Molecular methods

PCRs of periprosthetic tissues were performed in a highly standardized manner from the 5
patient samples. All the PCRs were performed in parallel with cultures from the same bead
milled suspension.

i/ 16S rDNA PCR method. A 200-µL aliquot of each bead milled suspension was treated with
proteinase K (concentration of 2 g/L) for 3 hours at 65°C. Then, DNA extraction was performed
using Qiagen manual extraction (4 laboratories) or automated extraction (3 laboratories using
MagnaPur (Roche), EasyMag (BioMérieux), iPrep (Invitrogen)). Real-time PCR was performed
with SybrGreen to target the 5' part of the 16S rDNA gene (forward primer 27F, 5' - AGA GTT
TGA TCM TGG CTC AG 3', and reverse primer 685R3, 5' - TCT RCG CAT TYC ACC GCT AC
3'; 658-bp amplification product; NR024570 GenBank accession number). The corresponding
amplicons were sequenced in both strands, assembled, and the consensus sequences were
compared with those in the BIBI (Bioinformatics Bacteria Identification: http://umr5558-sud-
str1.univ-lyon1.fr/lebibiai/lebibi.cgi) and BLAST databases. The concordance rate between 16S
rDNA PCR and bacteriological results was based on the genus (≥ 96% similarity) and species
level (≥ 98% similarity). A negative control and a positive control with *Roseomonas* sp DNA were run in parallel with each series of 5 samples per patient. A fragment of human beta-globin gene was amplified for each negative sample to control DNA extraction and the absence of PCR inhibitors. All specimens where inhibition was observed were diluted 1/10 and re-tested. Patients with inhibitors in at least two specimens were excluded from analysis. The criterion for molecular diagnosis was modelled on the bacteriological criterion (≥1 positive sample for strict pathogens; ≥2 for commensal skin flora). Molecular data were analyzed according to the diagnosis of PJI.

**ii/ Multicenter external quality control (MEQC)**

A MEQC was set up to validate the 16S rDNA PCR results obtained with the diverse molecular laboratory equipment. Three sets, including 4 bacterial DNA extracts and 4 bead milled osteo-articular tissues, were sent to each laboratory in November 2010, June 2011 and March 2012.

**Statistical analysis**

Categorical data were expressed as numbers and percentages, and continuous variables were reported as median with an interquartile range. Molecular data were analyzed using numbers and percentages according to the number of confirmed PJIs, or unconfirmed PJIs.

Sensitivity and specificity of the 16S rDNA PCR were estimated with a 95% exact confidence interval.

**Results**

**Patient characteristics**

Three-hundred and five patients were included, 6 were excluded, yielding 299 patients for analysis (Table 1). There were 264 suspected cases of PJI and 35 controls. Of the suspected cases
of PJI, 127 (48%) occurred in male patients, and the median age at the time of diagnosis was 73 years old. The suspected cases of PJI included 165 (63%) hip arthroplasty infections, and 88 (33%) knee arthroplasty infections. The patients presented with symptoms of acute infection in 19%, and chronic infection in 81% of cases. Seventy-six (29%, 19 in acute PJI, and 57 in chronic PJI) patients received antibiotics for two weeks before surgery.

**Diagnosis of infection**

After analysis of clinical, bacteriological and histological criteria, a definitive diagnosis of infection was confirmed in 215 out of 264 suspected cases of PJI (Fig. 1).

Of the 215 patients with confirmed PJI, 192 (89%) had a positive bacteriological criterion, monomicrobial in 163 (85%) cases (35 acute versus 128 chronic infections), and polymicrobial in 29 (15%) cases (10 acute versus 19 chronic infections) (Table 2). Of the monomicrobial infections, staphylococci were isolated in 108 (66%) cases, streptococci and enterococci in 22 (13.5%), Gram-negative bacilli in 16 (10%) cases, anaerobes in 13 (8%) cases, and other bacteria in 4 cases (2.5%) (Table 2). The 29 polymicrobial infections were caused by 2 bacterial species in 22 cases and 3 or 4 in the remaining 7 cases (Table 3).

Cultures remained sterile for 23 confirmed cases of PJI, including 16 (70%) patients treated with antibiotics at the time of surgery (Fig. 2).

Forty-nine patients showed no clinical, bacteriological or histological criteria for infection. The diagnosis of PJI could not be confirmed postoperatively for those patients, who were considered as aseptic failures.

**Analysis of 16S rDNA PCR results**
Of the 192 confirmed PJIs with bacteriological documentation, the molecular diagnosis was positive in 143 PJIs (121 monomicrobial and 22 polymicrobial infections), negative in 40 PJIs, and non-interpretable in 9 PJIs owing to presence of PCR inhibition (Fig. 2). Of the 40 bacteriologically documented PJIs with a negative molecular diagnosis, 33 were monomicrobial infections with \( S. \) epidermidis \((n=10), S. \) lugdunensis \((n=4), P. \) acnes \((n=7), S. \) aureus \((n=6), E. \) cloacae \((n=1), K. \) oxytoca \((n=1), P. \) mirabilis \((n=1), P. \) aeruginosa \((n=1), E. \) faecalis \((n=1), C. \) amycolatum \((n=1); among them, 8 patients had a single specimen positive in the PCR to the same bacterium as in culture \((S. \) epidermidis \(n=4), S. \) lugdunensis \(n=2), P. \) acnes \(n=2); the remaining 7 bacteriologically documented PJIs with a negative molecular diagnosis were polymicrobial infections. Regarding polymicrobial infections with a positive molecular diagnosis, sequencing of 16S rDNA PCR products found one bacterium in 19 cases, 2 bacteria in 1 case, and uninterpretable results due to unreadable sequences in 2 cases (Table 3).

Of the 23 confirmed PJIs which remained negative in culture, the molecular diagnosis was positive in 8 out of 16 patients treated with antibiotics at the time of surgery, and negative in 7 patients who did not receive antibiotics (Fig. 2).

Of the 49 patients without a confirmed diagnosis of PJI, the molecular diagnosis was positive in 2 patients, negative in 42 PJIs, and non-interpretable in 5 PJIs owing to PCR-inhibitors (Fig. 2). Both patients with PCR positive to \( L. \) monocytogenes and \( S. \) aureus respectively had been treated with antibiotics several months before for a previous PJI due to these bacteria; they were not treated after the current surgery. In 3 out of 49 unconfirmed PJIs, 1 sample out of 5 was PCR-positive to \( Acinetobacter johnsonii, Corynebacterium lipoliflavum \) and \( P. \) acnes respectively.
The sensitivity and specificity of 16S rDNA PCR according to the diagnosis of PJI were 73.3% [66.7; 79.2] and 95.5% [84.5; 99.4] respectively.

As regards the external quality control, from 168 quality controls, 160/168 controls (one laboratory did not participate in the first QC series) could be analyzed, 80 DNA extracts and 80 crushed samples. The overall rate of correct answers was 93.8% (150/160), with the same proportion for bacterial DNA extracts and crushed samples. Our results showed that manual or automated extraction had similar performances for osteoarticular specimens, whatever the equipment used for 16S rDNA PCR.

**Control patient analysis**

All 35 control patients were found to be negative by culture. Among them, the molecular diagnosis was negative in 33 patients, and non-interpretable in 2 patients owing to presence of PCR inhibition.

**Discussion**

Our study is the first prospective multicenter study to explore the performances of 16S rDNA PCR in a large number of PJIs. Clinical, bacteriological and histological criteria were chosen according to the latest IDSA guidelines on prosthetic-joint infections (4). Molecular biology results were validated through a multicenter external quality control, which will be submitted soon for publication. The correct results obtained uniformly showed that 16S rDNA PCR may be used with different laboratory equipment for molecular bone and joint infection diagnosis. DNA samples were extracted from the same bead milled suspensions used for the cultures. The PCR and cultures were performed from the five periprosthetic tissues collected for each patient.
included in the study. The criterion for molecular diagnosis was adapted from the bacteriological one, depending on whether the bacteria belonged to skin flora or were strict pathogens (4,20).

One of the main data from our study is the excellent specificity of our 16S rDNA PCR results. PCR positive to environmental or skin bacteria, controlled negative when performed from the second DNA extract, allowed us to eliminate rare contamination cases. Samples which tested positive for *S. aureus* and *L. monocytogenes* using PCR were from two patients who were treated for a PJI caused by these bacteria several months ago. The persistence of DNA from nonviable bacteria several months after clinical cure has already been reported in infective endocarditis, and is shown here for the first time in PJI (21). The excellent specificity of broad-range PCR, when standard recommendations are followed to prevent contamination, was already reported in many other studies (11, 13, 14, 15).

A lack of sensitivity of broad-range PCR was observed in our multicenter study on a large number of patients with PJI. Indeed, 16S rDNA PCR was not contributory in the diagnosis of 64 confirmed cases of PJI (30%), including 40 patients with positive cultures. A number of them (n=8) had a single 16S rDNA PCR sample positive to *S. epidermidis, S. lugdunensis* or *P. acnes,* which can be explained by the low bacterial inoculum in chronic infections and thus, the small amount of bacterial DNA in the extract. One question remaining unresolved is that we do not know if the bacterium identified by 16S rDNA PCR in a single sample can be made responsible for the infection. The lack of sensitivity of broad-range PCR was already shown in two main previous studies, including 13 and 18 PJI respectively (11, 17) despite the use of a pre-treatment with lytic enzymes (proteinase K, lysosome, lysostaphin and mutanolysin) to ensure optimum lysis of bacteria (17).
Conversely, the PCR became positive after DNA dilution in five patients with positive cultures, which underlined the risk of PCR inhibition caused by excessively high DNA concentration and the need to test diluted and non-diluted DNA when performing PCR. Finally, from 16 patients who were receiving antibiotics at the time of surgery, with negative cultures, 8 were also negative in PCR, highlighting the lack of sensitivity of the 16S rDNA PCR system. Concerning polymicrobial infections, one potential benefit of 16S rDNA PCR should be the identification of all bacteria involved, which is time-consuming using traditional cultures. Nevertheless, cloning of PCR products is often needed to analyze mixed sequence results, which is impossible on a routine basis. Unfortunately in our study, almost one quarter of the polymicrobial PJIs were negative in PCR, and 66% were positive to only one species.

Our study is the first multicenter study which proposes a multicenter homogenization of culture techniques. We have chosen a uniform number of samples with many of them collected at the bone-prosthesis interface. Using a bead mill provides better bacterial extraction from the tissue matrix. The beadmilled suspensions can be used to seed solid and culture media, and to freeze part of the sample for molecular tests. All this enabled us to document the infection in 89% versus 61% or 70% in other reported studies, and in 96% of patients who were not receiving antibiotics at the time of surgery (2,18).

Concerning the distribution of bacteria in PJI, our study confirms the predominance of staphylococci, accounting for 56% of infections, and the same representation of Gram-negative bacilli (8%) and polymicrobial infections (15%) over the decades (1,22-25). There was an almost equal distribution between *S. aureus* and CoNS. Of the CoNS, *S. lugdunensis* is the second most frequently isolated species after *S. epidermidis*, confirming its strong virulence as previously
Concerning streptococcal infections, our study confirms the importance of *Streptococcus agalactiae*, and shows the existence of true PJI caused by the *Streptococcus mitis* and *milleri* groups (27). *P. acnes* infections represented 7% of monomicrobial anaerobic PJIs, highlighting its role in the pathogenicity of implant-associated infections (28).

Considering the performance of the 16S rDNA PCR according to the different bacteria, sensitivity was excellent for *S. aureus* and streptococci, poorer for CONS and bad for *P. acnes*, as only 11% of *P. acnes* and 72% of CoNS infections were detected by PCR versus 92% and 100% of *S. aureus* and streptococci respectively.

In conclusion, our prospective study demonstrated the reliability of 16S rDNA PCR on a multicenter routine basis through the use of a multicenter quality control. Nevertheless, its lack of sensitivity even in treated patients, did not allow us to recommend the systematic use of 16S rDNA PCR for optimal detection of microorganisms causing mono- or polymicrobial PJI.

Finally, the use of other techniques in addition to cultures, such as multiplex PCR or pathogen-specific PCR assays, should be considered for infections remaining negative in culture (18,29).

**Contributors**

PB, CP, DT, BG, GDP, LB, and CB conceived and designed the study. PB, CP, DT, ASV, AG, PV, SC, SG, MEJ, GHA, CL, MK, LB, RQ and CB were site investigators. JL, BG, and CC were study statisticians. PB, CP, DT, ASV, AG, PV, SC, GHA, CL, MK, LB, and CB wrote the paper. PB was the principal investigator.

**Conflicts of interest**
All authors declare that they have no conflicts of interest.

Acknowledgements

This study was supported by a grant from the French Ministry of Health [Programme Hospitalier de Recherche Clinique Interrégionale API/N/041], and a grant from the CRIOGO (Centre de Référence des Infections Ostéo-articulaires du Grand Ouest).

We gratefully acknowledge Karine Fèvre and Line Happi for their help with the study and technical assistance. We are indebted to Jane Cottin† for her enthusiasm until the end. We are also very grateful to all the members of the CRIOGO for their continuing support.

The CRIOGO Study Team members included the following: J. Cottin†, M.C. Rousselet, P. Bizot, P. Abgueguen (CHU Angers); I. Quentin-Roue, R. Gérard, E. Stindel, S. Ansart (CHU Brest); R. Boisson, A. Guilloux, L. Crémet, A. Moreau, S. Touchais, F. Gouin, D. Boutoille, N. Asseray (CHU Nantes); A. Guigon, J. Guinard, P. Michenet, F. Razanabola, C. Mille (CHU Orléans); A.S. Cognée, S. Milin, L.E. Gayet, F. Roblot, G. Le Moal (CHU Poitiers); J. Guinard, N. Stock, J.L. Polard, C. Arvieux (CHU Rennes); P. Rosset, G. Gras (CHU Tours).
References


Table 1. Characteristics of the 299 patients analysed

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls n=35 (%)</th>
<th>Suspected PJI n=264 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, median years [interquartile range]</td>
<td>70 [63-77]</td>
<td>73 [63-79]</td>
</tr>
<tr>
<td>Male</td>
<td>23 (66)</td>
<td>127 (48)</td>
</tr>
<tr>
<td>Location of Arthroplasty</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hip</td>
<td>23 (66)</td>
<td>165 (63)</td>
</tr>
<tr>
<td>Knee</td>
<td>12 (34)</td>
<td>88 (33)</td>
</tr>
<tr>
<td>Shoulder</td>
<td>0</td>
<td>10 (4)</td>
</tr>
<tr>
<td>Elbow</td>
<td>0</td>
<td>1 (&lt;1)</td>
</tr>
<tr>
<td>Presentation of infection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute</td>
<td>0</td>
<td>50 (19)</td>
</tr>
<tr>
<td>Chronic</td>
<td>0</td>
<td>214 (81)</td>
</tr>
<tr>
<td>Antimicrobial therapy over the 15 days before surgery&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>76 (29)</td>
</tr>
<tr>
<td>Betalactams</td>
<td>0</td>
<td>44 (58)</td>
</tr>
<tr>
<td>Fluroquinolones</td>
<td>0</td>
<td>16 (20)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>0</td>
<td>12 (16)</td>
</tr>
<tr>
<td>Other antibiotics&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>25 (33)</td>
</tr>
</tbody>
</table>

<sup>a</sup> Some patients received two antibiotics.

<sup>b</sup> cotrimoxazole n=11, rifampicin n=9, others n=5.
Table 2 - Results obtained by 16S rDNA PCR and culture for 192 microbiologically documented infections

<table>
<thead>
<tr>
<th>Microbiologically documented PJI</th>
<th>Available PCR results</th>
<th>Positive 16S rDNA PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>63 (33)</td>
<td>62 (34)</td>
</tr>
<tr>
<td>CoNS a</td>
<td>45 (23)</td>
<td>39 (21)</td>
</tr>
<tr>
<td>Polymicrobial infection</td>
<td>29 (15)</td>
<td>29 (16)</td>
</tr>
<tr>
<td>Streptococci b</td>
<td>19 (10)</td>
<td>19 (10)</td>
</tr>
<tr>
<td>Enterococcus faecalis</td>
<td>3 (2)</td>
<td>3 (2)</td>
</tr>
<tr>
<td>Gram-negative bacilli c</td>
<td>16 (8)</td>
<td>16 (9)</td>
</tr>
<tr>
<td>Anaerobes d</td>
<td>13 (7)</td>
<td>11 (6)</td>
</tr>
<tr>
<td>Other e</td>
<td>4 (2)</td>
<td>4 (2)</td>
</tr>
<tr>
<td>Total</td>
<td>192</td>
<td>183 f</td>
</tr>
</tbody>
</table>

a CoNS: S. epidermidis n=31, S. lugdunensis n=6, S. capitis n=4, S. simulans n=2, S. caprae n=1, S. haemolyticus n=1.
b Streptococci: S. agalactiae n=7, S. dysgalactiae n=3, Streptococcus mitis group n=4, S. milleri group n=3, S. pneumoniae n=1, S. salivarius n=1.
c Gram-negative bacilli: Escherichia coli n=5, Klebsiella sp. n=3, Enterobacter cloacae n=2, Proteus mirabilis n=2, P. aeruginosa n=4.
d Anaerobes: P. acnes n=10, P. avidum n=1, Peptoniphilus asacharolyticus n=1, Parvimonas micra n=1.
e Other: Listeria monocytogenes n=2, Corynebacterium amycolatum n=1, Bacillus cereus n=1.
f 9 patients with PCR inhibitors.
Table 3 – Comparison of results obtained by 16S rDNA PCR and culture for 29 polymicrobial infections

<table>
<thead>
<tr>
<th>Culture results</th>
<th>Number of infections</th>
<th>Available 16S rDNA PCR results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1 bacterium</td>
</tr>
<tr>
<td>2 bacteria</td>
<td>22(^a)</td>
<td>14</td>
</tr>
<tr>
<td>3 bacteria</td>
<td>3(^d)</td>
<td>2</td>
</tr>
<tr>
<td>4 bacteria</td>
<td>4(^d)</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\) One patient had 1 sample positive in PCR to *Staphylococcus aureus*, and another sample to *Streptococcus oralis*.

\(^b\) Results were uninterpretable because of unreadable sequences.

\(^c\) 22 polymicrobial infections involved 2 different bacteria: 2 different staphylococcal species (n=3); *staphylococci* with anaerobes (n=5), *staphylococci* with *enterobacteriaceae* (n=5), *staphylococci* with *P. aeruginosa* (n=1), *staphylococci* with *streptococci* or *enterococci* (n=4), *staphylococci* with *corynebacteriae* (n=2); *E. coli* with *P. aeruginosa* (n=1), *Finegoldia magna* with *Anaerococcus vaginalis* (n=1).

\(^d\) 7 polymicrobial infections were due to 3 or 4 bacteria involving Gram-positive cocci in association with Gram-negative bacilli and anaerobes.
Figure 1 – Flow-Chart of enrolled patients

305 patients enrolled

6 patients \(^a\) excluded from the study

299 patients analysed

35 patients \(^b\) as a control group undergoing total primary hip arthroplasty

264 patients with suspected PJI \(^c\)

49 unconfirmed PJI \(^d\)

215 confirmed PJI

192 confirmed PJI with positive microbiological culture

23 confirmed PJI with negative microbiological culture

163 with monomicrobial PJI

29 with polymicrobial PJI

[^a]: 6 patients excluded from the study
[^b]: 35 patients as a control group undergoing total primary hip arthroplasty
[^c]: 264 patients with suspected PJI
[^d]: 49 unconfirmed PJI
6 patients excluded for the following reasons: inclusion criteria not met (n=4), microbiological protocol not respected (n=1), patient included twice (n=1).

The 35 patients were negative in culture.

PJI: prosthetic-joint infection.

The 49 unconfirmed cases of PJI had no clinical, bacteriological, or histological criteria.
Figure 2 – Molecular results

264 patients with suspected PJI

49 unconfirmed PJI

Negative molecular diagnosis n=42

Positive molecular diagnosis n=2

PCR-inhibitors n=5

215 confirmed PJI

192 confirmed PJI with positive culture

163 with monomicrobial PJI

121 with a positive molecular diagnosis

33 with a negative molecular diagnosis

9 with PCR-inhibitors

29 with polymicrobial PJI

22 with a positive molecular diagnosis

7 with a negative molecular diagnosis

0 with PCR-inhibitors

23 confirmed PJI with negative culture

16 treated patients

8 with a positive molecular diagnosis

8 with a negative molecular diagnosis

7 untreated patients

7 with a negative molecular diagnosis

192 confirmed PJI with positive culture n=42

Positive molecular diagnosis n=2

PCR-inhibitors n=5

163 with monomicrobial PJI

121 with a positive molecular diagnosis

33 with a negative molecular diagnosis

9 with PCR-inhibitors

29 with polymicrobial PJI

22 with a positive molecular diagnosis

7 with a negative molecular diagnosis

0 with PCR-inhibitors
a PJI: prosthetic-joint infection.
b The 49 unconfirmed cases of PJI had no clinical, bacteriological, or histological criteria.
c Two patients with PCR positive to *Listeria monocytogenes* and *Staphylococcus aureus* respectively had been treated with antibiotics several months previously for PJI caused by these bacteria.
d The diagnosis of 215 cases of PJI was confirmed according to guidelines.