Accurate diagnosis of orthopedic device-associated infections can be challenging. Culture of tissue biopsy specimens is often considered the gold standard; however, there is currently no consensus on the ideal incubation time for specimens. The aim of our study was to assess the yield of a 14-day incubation protocol for tissue biopsy specimens from revision surgery (joint replacements and internal fixation devices) in a general orthopedic and trauma surgery setting. Medical records were reviewed retrospectively in order to identify cases of infection according to predefined diagnostic criteria. From August 2009 to March 2012, 499 tissue biopsy specimens were sampled from 117 cases. In 70 cases (59.8%), at least one sample showed microbiological growth. Among them, 58 cases (82.9%) were considered infections and 12 cases (17.1%) were classified as contaminations. The median time to positivity in the cases of infection was 1 day (range, 1 to 10 days), compared to 6 days (range, 1 to 11 days) in the cases of contamination ($P < 0.001$). Fifty-six (96.6%) of the infection cases were diagnosed within 7 days of incubation. In conclusion, the results of our study show that the incubation of tissue biopsy specimens beyond 7 days is not productive in a general orthopedic and trauma surgery setting. Prolonged 14-day incubation might be of interest in particular situations, however, in which the prevalence of slow-growing microorganisms and anaerobes is higher.

Surgical implants play a major role in orthopedic trauma surgery and in the management of degenerative and inflammatory joint diseases. However, the rising number of indwelling devices is associated with increases in related complications. Along with device loosening or malfunctions and foreign-material reactions, infection remains one of the most serious problems encountered with surgical implants. Although orthopedic device-associated infections (ODAI) are uncommon, occurring in only 1 to 2% of patients with hip and knee replacements and up to 6% of patients after internal fixation of fractured bones, their management is difficult (1). Management can require multiple revision surgeries and prolonged antibiotic treatment, may result in permanent disabilities, and is associated with high costs (2, 3).

Despite the promising results reported with newer techniques, such as sonic cultures and molecular testing, the diagnosis of ODAI remains a medical challenge, as routinely used methods lack sensitivity and specificity (4–7). Synovial fluid sample culture, tissue biopsy specimen culture, and histopathological examination show high sensitivities and are frequently considered the gold standard. A reliable microbiological diagnosis is crucial for determining appropriate treatment (8).

There is currently no consensus regarding the appropriate incubation time for ODAI tissue biopsy specimens. The duration of incubation is not specified in most studies, but a 5-day period has often been reported (9–11). Recently, some authors have proposed prolonging the incubation period to 7 or 14 days in order to reveal microorganisms with low virulence, such as Propionibacterium acnes, Peptostreptococcus spp., and Corynebacterium spp. (12–15). Low-virulence, foreign-material-adherent bacteria are typically in a dormant starved state with a slow replicating rate (16). This particular behavior may require a longer culture incubation time (16–19). However, prolonging the incubation time is costly and labor-intensive and could increase the likelihood of detecting organisms that are not clinically relevant. Thus, the aim of our study was to determine if an incubation time of 14 days for tissue biopsy specimens is useful in the diagnosis of ODAI.

**MATERIALS AND METHODS**

**Study design.** Microbiological samples of tissue biopsy specimens that were taken from orthopedic device revision surgery (joint replacement and internal fixation devices) between August 2009 and March 2012 and were incubated for 14 days were analyzed. At our institution, 14-day incubation is standard for implant-associated samples and is performed on request for other bone and joint infections. In this study, case identification was prospective and continuous, while the study was retrospective. When there were several interventions for the same joint, only the first revision surgery was considered. The time until microbial growth was recorded. In cases of polymicrobial growth, infection was diagnosed if at least one microorganism fulfilled the diagnostic criteria (see below). The day of growth of the most slowly growing microorganism was used to avoid overlooking late-growing bacteria.

The study was performed in a hospital acting as a primary care and referral center for a population of about 280,000 inhabitants. Elective orthopedic surgery and trauma surgery each account for about one-half of the activity of the Department of Orthopedic Surgery and Traumatology at this hospital. Medical records were reviewed in order to determine if infection was present. Infection was diagnosed according to predefined diagnostic criteria.
diagnostic criteria (see below). Cases were reviewed by an infectious disease specialist and an orthopedic surgeon. Patient files were scanned for indications of clinical signs of infection (fever, erythema, edema, local hyperthermia, wound discharge, and/or the presence of a sinus tract). A temperature above 38.5°C was considered fever, and fracture nonunion was taken as a potential sign of infection. Preoperative antimicrobial treatment was defined as the administration of any type of antibiotic for more than 24 h during the 14 days preceding surgery. The histopathological findings were divided into 3 categories depending on the average number of polymorphonuclear cells (PMN) per high-power field (HPF) (×400 magnification) on microscopic analysis, as a mean value of at least 10 fields examined, i.e., <1 PMN/HPF, 1 to 5 PMN/HPF, or >5 PMN/HPF.

Definition of infection. Infection was diagnosed if one of the following criteria was fulfilled: (i) positive culture with ≥3 positive samples showing identical microorganisms (20) (microbiological criterion), (ii) positive culture with any number of positive samples and histopathological examination showing >5 PMN/HPF not explained by an acute fracture (21–23) (histopathological criterion), or (iii) positive culture with any number of positive samples and clinical signs of infection, i.e., erythema, edema, local hyperthermia, wound discharge, presence of a sinus tract, or fracture nonunion (8, 24) (clinical criterion). Patients who had not been treated postoperatively with antibiotics and who showed no signs of infection after 12 months of follow-up were not considered to be infected, independent of the diagnostic criteria. Cases with positive cultures that did not fulfill the criteria for infection were classified as contamination.

Culture methods. Tissue sampling was performed in the operating room according to usual surgical methods. The standard procedure was to obtain 3 to 6 samples, with priority given to tissue biopsy specimens if not limited by anatomical restrictions (as in the finger, hand, and foot) (20, 25–27). In order, tissues were sampled from the inflammatory membrane around the implant, the joint capsule, and any macroscopically suspect tissue (28, 29). Each biopsy specimen was stored in transportation medium (BBCPort-A-Cul; Becton, Dickinson, and Company, Sparks, MD) to ensure the survival of all bacteria, including anaerobic microorganisms.

Homogenization of the tissue biopsy specimens was carried out using a disposable closed tissue homogenization system (gentleMACS dissociator; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), with the addition of normal saline solution as necessary to obtain a heavy suspension. All manipulations were performed under sterile conditions and under laminar airflow. One hundred microliters of this suspension was inoculated on each of the following agar plates: (i) blood agar, i.e., Columbia-D agar base (bioMérieux, Marcy l’Etoile, France) with 5% sheep blood; (ii) chocolate agar, i.e., Columbia-D agar base (bioMérieux, Marcy l’Etoile, France), with 5% sheep blood (heated to lyse blood cells) supplemented with Vitox SR0090 growth factors (Oxoid-Thermo Fisher Scientific, Basingstoke, United Kingdom); or (iii) preseeded Brucella agar with 5% sheep blood, hemin, and vitamin K1 (bioMérieux, Marcy l’Etoile, France). After inoculation, the plates were sealed with Parafilm laboratory film (Bemis Company, Inc., Oshkosh, WI) to avoid desiccation. The first 2 media were incubated at 35°C in a 5% CO2 atmosphere for cultivation of aerobic and facultative organisms. The third plate was incubated at 35°C in an anaerobic atmosphere for the cultivation of anaerobic and facultative organisms. The remainder of the suspension was inoculated into thioglycolate broth medium CM0173 (Oxoid-Thermo Fisher Scientific, Basingstoke, United Kingdom) and incubated at 35°C for enrichment of aerobic, anaerobic, and facultative microorganisms. Quality control assessments showed the presence of adequate anaerobic conditions in the lower part of the broth. Each medium was inspected for signs of growth every day for a period of 14 days.

Statistical methods. Continuous variables are presented as medians and ranges and categorical variables as rates. Statistical significance was assessed using the chi-square test or Fisher’s exact test for categorical variables and the Mann-Whitney U test (Kruskal-Wallis test) for continuous variables. All tests were performed using SPSS version 21 (SPSS Inc., Chicago, IL). P values of <0.05 were considered statistically significant. For graphical representation, Microsoft Excel 2008 (Microsoft Corp., Redmond, WA) was used.

RESULTS

Study population. During the study period, 499 tissue biopsy specimens were collected from 117 cases of revision surgery, corresponding to a median number of 4.0 samples per case (range, 1 to 12 samples per case). In 70 cases (59.8%), a minimum of one sample was positive for microbiological growth, leaving 47 cases (40.2%) as sterile during the incubation period.

The study population consisted of 50 women (42.7%) and 67 men (57.3%), with a median age of 68.0 years (range, 14 to 94 years). The time between index surgery and revision surgery was <1 month in 31 cases (26.5%), 1 to 12 months in 32 cases (27.4%), and >12 months in 54 cases (46.2%). Orthopedic devices included 62 cases (53.0%) of joint prostheses and 55 cases (47.0%) of internal fixation devices. Localization of the devices varied from the hip in 51 cases (43.6%), the knee in 29 cases (24.8%), the lower extremity in 22 cases (18.8%), and the upper limb in 9 cases (7.7%) to the spine in 6 cases (5.1%). Articular devices were labeled according to the joint region involved. Histopathological analysis results were available for 85 cases (72.6%). Among the 70 cases with positive culture results, 58 cases (82.9%) were classified as infections and 12 cases (17.1%) as contaminations. The proportion of infections among the 117 cases of revision surgery was 49.6%, with 41.9% for joint replacement and 58.2% for internal fixation.

Microbiology. The majority of the isolated microorganisms were Gram-positive bacteria, mainly Staphylococcus aureus in 22 cases (31.4%) and coagulase-negative staphylococci in 18 cases (25.7%). Streptococcus spp. accounted for 2 cases (2.9%), Enterococcus spp. for 2 cases (2.9%), P. acnes for 3 cases (4.3%), Gram-negative bacteria for 6 cases (8.6%), and polymicrobial culture results for 17 cases (24.3%). The full spectrum of bacteria according to case classification is illustrated in Table 1. The two types of
orthopedic devices showed similar spectra of microorganisms except for *S. aureus*, which was significantly more frequent for prostheses than for internal fixation devices (15 cases versus 7 cases; \( P = 0.04 \)).

**Diagnostic criteria.** Of the 58 cases of infection, most were diagnosed by at least 2 diagnostic criteria, leaving only 9 cases diagnosed with a single criterion (the microbiological criterion in 3 cases and the clinical criterion in 6 cases). Discrepancy between the study’s definition of infection and the treating medical team’s diagnosis occurred in one case, which was treated as an infection based on one of 8 samples showing *P. acnes* on day 7. This case was classified as contamination according to our criteria.

**Time to culture positivity.** The median time to culture positivity for the 70 cases with positive results for tissue biopsy specimens was 1 day (range, 1 to 11 days). A total of 47 cases (67.1%) became positive within the first 2 days of incubation, 57 cases (81.4%) within 5 days, and 65 cases (92.9%) within 7 days.

The median time to positivity in cases classified as infections was 1 day (range, 1 to 10 days), compared to 6 days (range, 1 to 11 days) for cases considered contaminations (\( P < 0.001 \)). A total of 52 cases (89.7%) of infections were diagnosed within 5 days of incubation and 56 cases (96.6%) within 7 days (Fig. 1). No infection was diagnosed beyond 10 days. Twenty-five percent of contaminants grew after 7 days, representing the majority (60%) of late-growing microorganisms. Because the absolute numbers of cases of infections or contaminations are also relevant, graphical representation of the results is shown in a histogram in Fig. 2.

Only 2 cases of infection were detected after 7 days of incubation, at day 8 and day 10 (Table 2). The first case was a late postoperative infection, which showed a large amount of *Corynebac-
though without assessment of their benefits (33, 34). The aim of alternative diagnostic procedures, such as 16S rRNA PCR or sonication, 14-day incubations have sometimes been reported, although to culture positivity (40). This can be explained by the inclusion of trauma cases, for which early revision surgery was indicated on the basis of a high preoperative suspicion of infection, whereas the systematic sampling of loose prostheses used in other studies obviously had lower yields (39, 40).

Most reports focus on hip and knee prostheses (1, 13, 24, 37). We believe that including both prostheses and internal fixation devices in our study makes sense because similar pathogens have been described in the two settings (41–43) and biofilm formation is common to all types of foreign-body infections (44–46). We found that slow-growing microorganisms such as coagulase-negative staphylococci and P. acnes were equally represented in the two groups.

In conclusion, the results of our study show that extension of culture incubation times beyond 7 days has a low yield in a general orthopedic and trauma setting, where both acute and chronic infections are encountered.

We found that an incubation period of 7 days was sufficient to identify 56 of 58 cases (96.6%) of infection. The major difference between our data and those of Schaefer et al. (13) is the proportion of low-virulence microorganisms such as Propionibacterium spp., coryneform bacteria, and coagulase-negative staphylococci, which accounted for 80% of infections in their study population. These microorganisms are known to have slow growth rates. In our study, only 54% of the cases showed low-virulence microorganisms. In particular, P. acnes accounted for 4.3% of isolated bacteria, which corresponds to reports from other general orthopedic and trauma surgery departments (8, 35, 36).

The diagnosis of ODAI is a well-known challenge. In our study, we have tried to provide clear reproducible diagnostic criteria that are applicable to retrospective analysis, which has well-established limitations. For the microbiological criterion, our threshold of at least 3 positive samples with identical microorganisms could be viewed as stringent, in comparison with other studies in which 2 culture-positive specimens are considered sufficient for the diagnosis of infections (10, 37, 38). However, none of our contamination cases had more than one positive sample, meaning that our results would not have been different if we had adopted a lower threshold. The same is true for the histopathological criterion, as no contaminant showed an intermediate result of 1 to 5 PMN/HPF. Although the clinical diagnostic criterion is somewhat subjective, all of the cases classified as infections on this basis were quite evident; 10% of infections would have been missed without this strategy.

Overall, we identified a large proportion of infections (22, 39, 40). This can be explained by the inclusion of trauma cases, for which early revision surgery was indicated on the basis of a high preoperative suspicion of infection, whereas the systematic sampling of loose prostheses used in other studies obviously had lower yields (39, 40).

REFERENCES