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Fluorescence In Situ Hybridization as Diagnostic Tool for Implant-associated Infections: A Pilot Study on Added Value

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Background: Implant-associated infections are a devastating complication in surgery. Especially in infections with biofilm-forming microorganisms, the identification of the causing microorganism remains a challenge. However, the classification as biofilm is not possible with conventional polymerase chain reaction or culture-based diagnostics. The aim of this study was to evaluate the additional value of fluorescence in situ hybridization (FISH) and nucleic acid amplification technique (FISHseq) to discuss a diagnostic benefit of the culture-independent methods and to map spatial organization of pathogens and microbial biofilms in wounds.

Methods: In total, 118 tissue samples from 60 patients with clinically suspected implant-associated infections (n = 32 joint replacements, n = 24 open reduction and internal fixation, n = 4 projectiles) were analyzed using classic microbiological culture and culture-independent FISH in combination with polymerase chain reaction and sequencing (FISHseq).

Results: In 56 of 60 wounds, FISHseq achieved an added value. FISHseq confirmed the result of cultural microbiological examinations in 41 of the 60 wounds. In 12 wounds, one or more additional pathogens were detected by FISHseq. FISHseq could show that the bacteria initially detected by culture corresponded to a contamination in three wounds and could exclude that the identified commensal pathogens were a contamination in four other wounds. In five wounds, a nonplanktonic bacterial life form was detected.

Conclusions: The study revealed that FISHseq gives additional diagnostic information, including therapy-relevant findings that were missed by culture. In addition, nonplanktonic bacterial life forms could also be detected with FISHseq, albeit less frequently than previously indicated. *(Plast Reconstr Surg Glob Open 2023; 11:e4994; doi: 10.1097/GOX.0000000000004994; Published online 22 May 2023.)*

INTRODUCTION

Implant-associated surgical site infections are a major challenge and show a frequency of up to more than 20%.[1–3] The increasing age of patients, the associated risk-increasing comorbidities, and the considerable increase in technically demanding operations[4] observed in recent decades contribute to a steadily rising number of septic soft tissue and bone infections, and make them one of the most relevant complications in modern surgery. This situation becomes further complicated, not only by the increasing multiresistance of the pathogens causing the infection, but also the ability of the pathogens to form a biofilm, especially around any implant surface.[5,6] A Biofilm is a physiological state of existence of bacteria in which special forms of communication, a greatly reduced metabolism, and the formation of a protective mucopolysaccharide layer embedding entire bacterial colonies lead to a pronounced resistance to the attacks of the human host immune system and to antibiotic therapies.[7–12] In implant-associated infections, biofilm formation very often forces implant removal.

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Therapeutic options are already hampered by the fact that conventional microbiological diagnostic techniques such as culture or PCR-based methods do not detect whether biofilms are present.[13,14] Culture technique only allows the detection of metabolically active and multiplying microorganisms. PCR-based techniques can detect minute components of bacterial DNA, but cannot distinguish between the DNA of vital and dead microorganisms.[1,15] In contrast, with modern molecular microscopic methods such as fluorescence in situ hybridization (FISH), it is possible to detect the growth form of the bacteria (planktonic, microcolonies or biofilms), to localize the biofilm in the tissue sample and even to identify the leading bacterial pathogen in the case of mixed infections.[16–20] Indeed, the FISH technique has been able to detect microorganisms that could not be identified by the conventional culture technique.[21–24]

However, despite the great importance of biofilm for implant-associated infections, there are no studies with precise information on the frequency of nonplanktonic growth forms. Similarly, no study has investigated whether molecular biology testing methods, including FISH techniques in combination with molecular nucleic acid amplification techniques (FISHseq), can provide additional information in the diagnosis of implant-associated infections. Therefore, in this study, we aimed to investigate the frequency of planktonic and nonplanktonic microbial burden in implant-associated infections, as well as a possible added value of molecular diagnostic techniques. For this purpose, the bacterial load of the infected wounds was investigated using both FISHseq and conventional microbiological methods.

Takeaways

Question: Implant-associated infections are a devastating complication. Especially in biofilms, the identification of causing microorganisms is a challenge. This study was created to evaluate the additional value of fluorescence in situ hybridization (FISH) as a diagnostic benefit compared to standard culture-independent methods.

Findings: Tissue samples were collected intraoperatively from patients with clinically suspected implant-associated infections and analyzed using microbiological culture and culture-independent FISH. In 56 of 60 wounds, FISH achieved an added value. In 41 of the 60 wounds, FISH confirmed the result of the standard microbiological tests.

Meaning: FISH gives additional diagnostic information and detects non-planktonic bacterial life forms, that were otherwise missed by routine culture methods.

METHODS

Study Design and Patient Population

The cohort study was conducted in a centre for septic and reconstructive surgery. We prospectively included 60 consecutively hospitalised patients (age ≥18 years) with suspected early- or late-onset implant- or foreign body-associated infection, similar to the classification of Li et al.[25] Inclusion conditions were clinical high-grade suspicion of infection according to the diagnostic criteria of McNally et al[26] and Zimmerli and Sendi.[27] Patients were excluded if they had not signed a written informed consent, if they were younger than 18 years, or if they had an additional septic focus at a different location. The study was approved by the local ethics committee (EA1/362/13) of the Charité University Medical Centre Berlin, Germany.

Sample Collection

Representative tissue samples were taken intraoperatively from patients with early- or late-onset periprosthetic, implant- or foreign body-associated infection. Tissue samples included, for example, the peri-implant membrane, the tissue at the implant-bone interface, or the cancellous bone in intramedullary implants such as intramedullary nails or endoprostheses. Cortical samples were not taken as they cause artifacts in the FISH images. The tissue samples were sent for routine microbiological diagnosis in transport medium or native form within less than 2 hours. A matched tissue sample was cooled down to 4°C and transported to a specialized laboratory for FISH diagnosis.

Microbiological Analysis

Standard Culture Methods

The processing of cultural diagnostics was carried out under the standard conditions used in microbiology (Schaedler agar, Columbia boiled blood agar, Columbia sheep blood agar, MacConkey agar, Candida selective agar) and various liquid enrichment media (brain-heart dextrose broth, thioglycolate broth) with incubation times of 2–14 days. A semiquantitative algorithm was used to assess growth on the solid media. Simple manual procedures as well as the BioMerieux Vitek 2 system, which was also used for antibiotic susceptibility testing (determination of Minimum Inhibitory Concentration; MIC), were available to identify the cultured microorganisms. Alternatively, the inhibition zone diameter was determined in the agar diffusion test, originally according to Kirby-Bauer[28], which was performed according to the standards of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

Molecular Analysis Using FISHseq

FISHseq describes the combination of molecular imaging of microorganisms using FISH with 16S rRNA-gene PCR and subsequent sequencing, both from consecutive methacrylate embedded tissue sections.

Sample Embedding and FISH

Tissue samples were fixed in FISHopt® fixation solution (MoKi Analytics, Berlin, Germany), embedded using cold polymerizing resin (Technovit 8100; Kulzer, Wehrheim, Germany), and sectioned as previously described.[22] FISH analysis was performed as follows[29]: Briefly, sections were hybridized with pan-bacterial probe $EUB338_{Cy3}$[30] to visualize the entire active, ribosome-containing bacterial population. A nonsense probe $NON\ EUB338_{Cy5}$[31] was used to exclude unspecific probe binding. For visualization of nucleic acids in host cell nuclei and bacteria, DAPI (4′,6-Diamidine-2′-phenylindole dihydrochloride) was included. In cases where a positive $EUB338_{Cy3}$ signal

was detected, specific probes corresponding to culture or sequencing results were used to confirm microbial findings by FISH. An epifluorescence microscope (Axioplan 2 and AxioImager Z2; Carl Zeiss, Jena, Germany) equipped with narrow band filter sets (AHF Analysentechnik, Tuebingen, Germany) was used for microscopy. Digital images were generated using the ZEN and the AxioVision software from Zeiss, Jena, Germany. Detected microorganisms were empirically classified as planktonic (single bacteria), microcolonies (clusters of up to 30 microorganisms) or biofilms (communities of more than three layers of adjacent bacteria over a length of more than 20 µm).

DNA Extraction, 16S rRNA-gene PCR Amplification, and Sequencing

DNA was extracted from consecutive sections of the embedded samples and PCR was performed using the pan-bacterial primer set TPU1 and RTU3[32] for the amplification of the 16S rRNA gene as described.[22] Subsequent sequencing of amplicons was performed using a commercial sequencing service (LGC Genomics, Berlin, Germany) and analyzed using the SmartGene commercial analysis pipeline (SmartGene, Lausanne, Switzerland) as previously described.[33]

Statistics

The data obtained were analyzed and compiled in tables and graphs (Microsoft Excel 2010, Microsoft Corp, Redmond, Wash.).

RESULTS

Demographics

Tissue samples were collected from 60 patients (n = 45, 75% men; n = 15, 25%, woman; median age 69 years) suspected of early- or late-onset infection of an arthroplasty (n = 32, 55.3%), fracture-fixating internal osteosynthesis (n = 24, 40%), or septic wound situation with a foreign body (metal ballistic projectile, n = 4, 6.7%) (Table 1). The time between primary implantation and the start of clinical treatment varied greatly (8 days to 12 years). Suspicion of infection was based on the presence of clinical signs in 47 patients (78.3%) and of radiological signs (eg, loosening, pseudarthrosis, callus deformity) in 34 patients (56.7%). Thirteen (21.7%) patients had positive findings on scintigraphy. Joint puncture was performed in 11 patients (17.3%), three of whom were positive, and the evaluation showed an increased cell count.

Table 1. Demographics

Age (n = 60)*		**69 years (41/78; 21–93)**
Gender	Female	15 (25.0%)
	Male	45 (75.0%)
Implants (n = 60)	TKA	11 (18.3%)
	THR	17 (28.3%)
	Hemiarthroplasty	4 (6.7%)
	Intramedullary nail	9 (15.0%)
	Plate osteosynthesis	15 (25.0%)
	Foreign body (3 projectiles, 1 shrapnel)	4 (6.7%)
Signs of infection (n = 60)	Clincal findings (pain, wound redness, swelling, secretion, abscess, wound healing disorder)	47 (78.3%)
	Labaratory signs (elevated WBC, CRP)	39 (65.0%)
	WBC in early infection*	8.7 (6.0/10.8; 3.4–27.7)
	WBC in late infection*	6.9 (5.6/9.2; 3.4–17.7)
	CRP in early infection*	45.6 (11.8/133.9; 0.4–390)
	CRP in late infection*	14.2 (3.3/ 56.9; 0.4–387)
	Radiological signs	34 (56.7%)
	Nuclearmedical signs	13 (21.7%)
	Culture positive [≥2 positive probes]	46 (76.7%) [29 (48.3%)]
Acuity of infection** (n = 53)	Early infection	16 (30.2%)
	Late infection	38 (69.8%)

*Data are presented as median, quartiles and range, otherwise as absolute frequency (in brackets: relative frequency).
TKA, total knee arthroplasty; THA, total hip arthroplasty; WBC, white blood cell count; CRP, c-reactive protein.

Specimen Collection and Bacterial Load

For microbiological diagnosis, 118 tissue samples were obtained from the 60 wounds for standard culture analysis and for FISHseq (median two samples per wound, halved for both methods). In all 60 wounds, 22 different pathogen species were detected (Table 2). Seven of 60 patients did not have microorganisms detected by culture or molecular techniques, and infection was also ruled out by FISHseq, although initial clinical suspicion of infection was evident. Sixteen of the 53 remaining patients (30.2%) had early-onset infection, and 37 patients (69.8%) had late-onset infection. In 46 of the 53 (86.8%) infected wounds, the responsible pathogens could be identified. In the other seven wounds, a bacterial pathogen was detected but could not be clearly identified by PCR and sequencing due to unfavorable host DNA to pathogen DNA ration, or DNA degradation. The most numerous and identifiable pathogens were *Staphylococcus aureus* (16 of 46 wounds, 34.8%), *Staphylococcus epidermidis* (nine of 46, 19.6%), *Enterobacter cloacae* (five of 46, 10.9%), *Cutibacterium acnes* (four of 46, 8.7%). Pathogens from the *Enterobacteriaceae* family (eg, *Escherichia coli*, *Klebsiella* spp., *Enterobacter* spp.) were detected in 14 of 46 wounds (30.4%). Wound infection was monobacterial in 34 (73.9%) and polymicrobial in 12 of these 46 wounds (26.1%, with two to four pathogens).

Added Value of FISHseq

Overall, in 56 of the 60 (93.3%) wounds, a diagnostic added value could be achieved by the additional examination using molecular biological methods. [**See table, Supplemental Digital Content 1,** which displays P: patient number, G/A: gender/age, THA: total hip replacement, TKA: total knee replacement, POS: plate osteosynthesis, IMN: intramedullary nail, HAP: hip arthroplasty.

Table 2. Spectrum and Frequency of Bacterial Microorganisms Detected by Standard Culture Technique and FISHseq

Pathogen Species	No. Tissue Specimen (118 Specimens from 60 Wounds*)		No. Infected Wounds (53 Infected Wounds of 60 Patients*)	
Staphylococcus aureus	27	22.9%	16	26.2%
Staphylococcus epidermidis	20	17.0%	9	14.8%
Enterobacter cloacae complex	13	11.0%	5	8.2%
Escherichia coli	8	6.8%	4	6.6%
Staphylococcus capitis	6	5.1%	3	4.9%
Cutibacterium acnes	6	5.1%	4	6.6%
Micrococcus sp	3	2.5%	1	1.6%
Enterococcus faecalis	3	"	2	3.3%
Klebsiella pneumoniae	3	"	2	"
Finegoldia magna	2	1.7%	2	"
Klebsiella oxytoca	2	"	2	"
Proteus mirabilis	2	"	1	1.6%
Pseudomonas aeruginosa	2	"	1	"
Enterobacteriaceae** (not further specified)	2	1.7%	1	"
Staphylococcus caprae	1	"	1	"
Acinetobacter baumannii	1	0.85%	1	"
Corynebacterium glucoronolyticum	1	"	1	"
Corynebacterium striatum	1	"	1	"
Enterococcus faecium	1	"	1	"
Methylobacterium sp	1	"	1	"
Paracoccus sp	1	"	1	"
Streptococcus agalactiae	1	"	1	"
Cutibacterium avidum	1	"		
Not identifiable***	21	17.8%		
Negative	10	8.5%		

*The percentage calculation refers to pathogens detected 139 times in 118 specimens (left column; right column: 61 identified pathogens in 53 wounds, in which the pathogen could not be identified in 7 wounds).
**According to the rules of the Bacteriological Code (ICBN), Enterobacteriaceae is a family of bacteria from the order Enterobacterales, officially established in 2016. They mostly reside in the digestive tract and partly belong to the normal intestinal flora. Examples are *Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae.*
***Pathogens could not be clearly identified by PCR and sequencing due to unfavorable host DNA/pathogen DNA ration, or DNA degradation.
An estimated 108 of 118 specimens were positive; 53 of 60 wounds were infected. The " symbol indicates that the number is the same as the number in the row above.

Table 3. Patients with Nonplanktonic Bacterial Growth

Patient (Age, Gender/ Comorbidity)	Type of Implant, Duration of Implant Placement	Acuity of Infection	Sample Type	Nonplanktonic Growth Form	Bacterial Species
71, female/allergic bronchial asthma	Hip endoprothesis, 4 y	Late infection	Near hip cup	Biofilm	Culture: *Staphylococcus epidermidis* FISHseq: "coccoid biofilms"
33, male/no comorbidities	Knee endoprosthesis,1 year	Late infection (symptoms since about 8 wk)	Tibial marrow space with remnants of bone cement	Biofilm	Culture and FISHseq: *Staphylococcus aureus* (MRSA), *Pseudomonas aeruginosa* (MDR)
28, male/no comorbidities	Clavicular plate, ORIF 9 d	Early infection	Soft tissue near osteosynthesis plate	Biofilm	Culture and FISHseq: *Staphylococcus aureus/ argenteus*
46, male/HIV+, benign bone cysts in affected lower leg with multiple conversion osteotomies	Tibial plate, ORIF 10 d	Early infection	Soft tissue near osteosynthesis plate	Microcolonies	Culture: *Klebsiella oxytoca, Staphylococcus aureus* FISHseq: *Staphylococcus aureus*
73, male/hypertension, hypothyroidism, history of laryngeal carcinoma, coxarthrosis	Knee endoprosthesis, 4 weeks	Early infection	Peri-implant capsular tissue	Microcolonies	Culture: negative FISHseq: Candida, unspecified bacterial microorganisms

Positive diagnostic findings: LAB: increased infection markers (WBC, CRP), RAD: sign of infection (plane radiographs, CT-scan), NUC: sign of infection (3-phase-bone-scintigraphy, granulocyte scintigraphy, CUL: positive culture, CUL≥2: positive culture in ≥ two or more samples (same microorganism). **http://links.lww.com/PRSGO/C551**.] Thus, FISHseq confirmed the result of the cultural microbiological examination in 41 of the 60 wounds (CON, 68.3%). In four patients, the negative culture result was confirmed (in Table 3, the term "NEG"

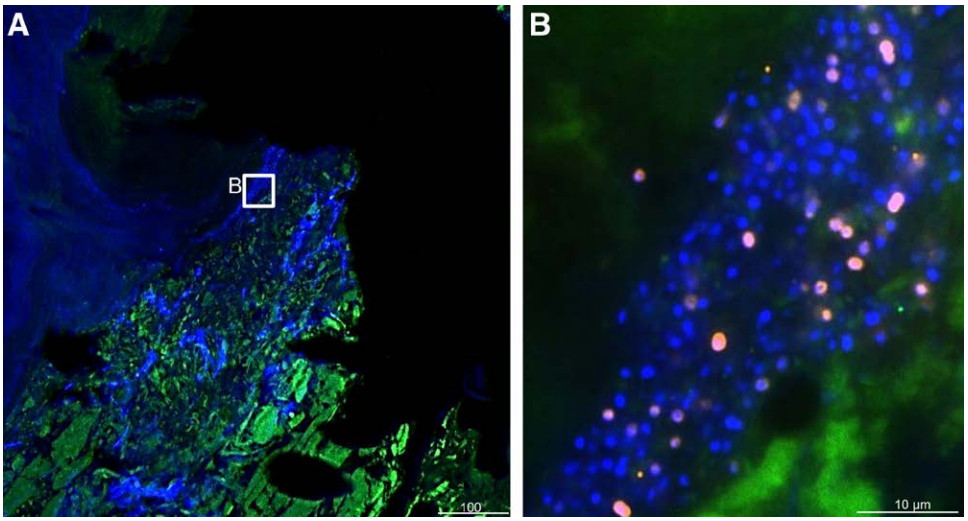

Fig. 1. FISH of wound tissue from a patient, where culture was positive for *Acinetobacter* sp., *Corynebacterium* sp., and *Enterococcus* sp. A, Overview shows host cell nuclei, stained with DAPI (blue); the tissue background appears in green. At higher magnification (B), FISH-positive bacteria are visible within a biofilm, which are detected by the *Enterococcus* genus-specific FISH probe EFAEC (orange).

refers to the patients numbered 1, 3, 7, 11), and in three patients, FISHseq ultimately identified the pathogen as noncausative wound contamination, thus ruling out bacterial wound infection (contamination in the sense of iatrogenic application error during sampling or processing) (NCC, patients 2, 13, 17). In another four wounds, FISHseq confirmed the culture result, and it could be excluded (EXC, 8, 15, 44, 52) that the commensal pathogen detected by culture was a noncausative wound contamination. In 12 other wounds (12/53, 22.6%), one or more additional pathogens were detected by FISHseq (ADP, 16, 20, 22, 24, 31, 37, 38, 41, 48, 49, 50, 59). In five wounds, it was also recognized that the pathogen detected was a nonplanktonic bacterial life form (NPF, 10, 34, 42, 50, 60). Standard culture diagnostics correctly indicated the presence and respective absence of

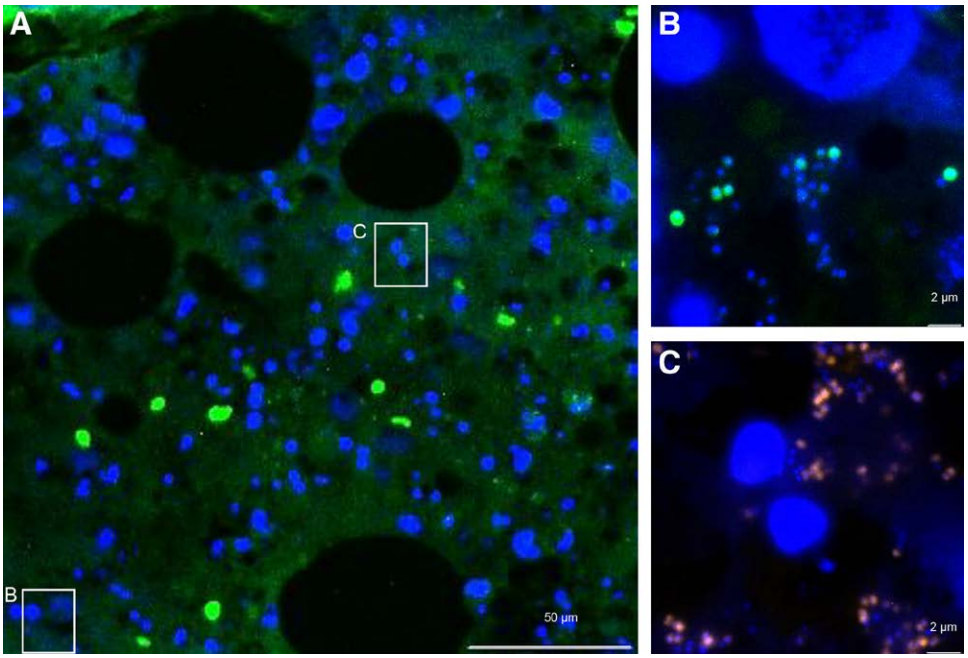

Fig. 2. FISH of wound tissue culture positive for *Staphylococcus aureus*. A, Overview shows host cell nuclei, stained with DAPI (blue); the tissue background appears in green. At higher magnification (B and C), FISH-positive bacteria are visible in microcolonies, which are detected by the *Staphylococcus* genus-specific FISH probe STAPHY (green, B). Another microscopic field of the same sample shows *Finegoldia magna* with the species-specific FISH probe FMAG (orange, C).

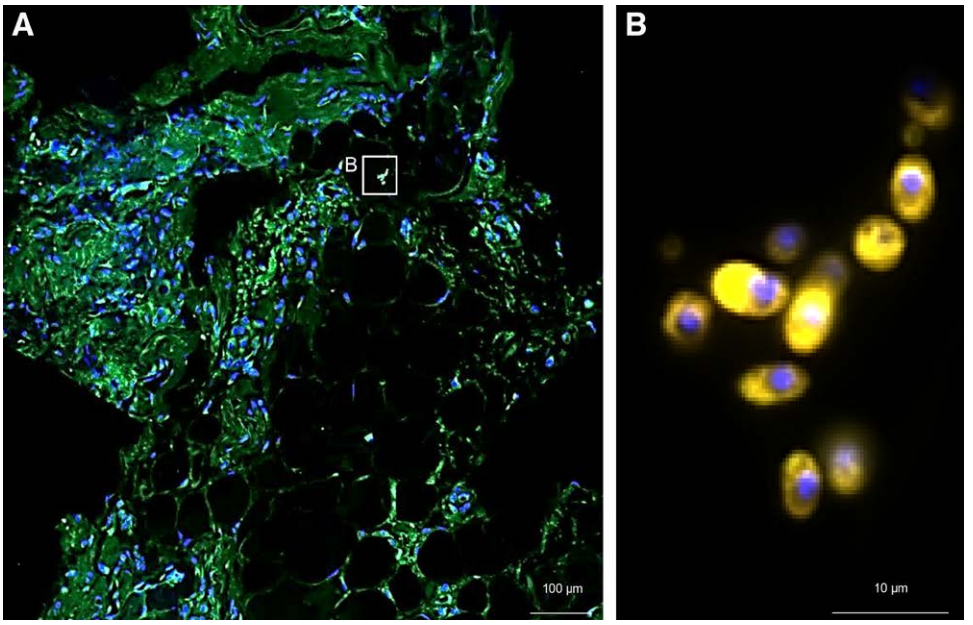

Fig. 3. FISH of negative wound tissue culture. A, Overview shows host cell nuclei, stained with DAPI (blue); the tissue background appears in green. At higher magnification (B), a FISH-positive microcolony is visible, which is detected by the *Candida* genus-specific FISH probe CAND10 (orange).

bacteria in 46 of the 60 wounds (76.7%). According to the FISHseq results, culture-based pathogen detection was likely to be false positive in two cases, and false negative in 12 patients. Applying the criterion formulated by McNally[34] of at least two positive tissue samples for a definite diagnosis of infection would have been possible with conventional microbiological diagnostics (CUL ≥ 2, positive culture in two or more samples with the same microorganism) in only 29 cases (48.3%).

Detection of Biofilm and Microcolonies

Of the 53 infected wounds, nonplanktonic bacterial life forms were detected in five (9.4%) wounds (Table 3). Biofilm was identified in three wounds and microcolonies in two wounds (Figs. 1 and 2). The causative bacterial species were *Staphylococcus epidermidis* in one case and *Staphylococcus aureus* in three cases, with *Klebsiella oxytoca* also detected in the wounds in one case and *Pseudomonas aeruginosa* in another. In one wound, the bacterial pathogen could not be identified with certainty by FISHseq, but the spatial arrangement could be characterized as a microcolony. In this case, *Candida* (Fig. 3) was also involved in the microcolony—the only case of fungal detection in all 118 samples.

DISCUSSION

In this study, we used FISH and FISHseq as an adjunct to routine microbiological and molecular biological diagnostics and for the detection of biofilms in implant-associated infections. Despite an extensive literature research, we could not find any other study that analyzed the frequency of biofilms in septic wounds using both cultural microbiology and FISHseq. The most important finding is that nonplanktonic growth forms occur only in a small proportion of early- or late-onset implant-associated infections. Moreover, the additional use of molecular biology assay techniques (FISHseq) showed additional value, so that a considerably higher proportion of bacterial species could be identified.

The diagnosis of early- or late-onset peri-implant infections is still a challenge in modern surgery.[34–38] Here, routine culture tests are still considered the gold standard for microbiological diagnostics. However, the sensitivity of routine microbiological culture for the detection of implant-associated infections reported in the literature varies between 60.8% and 73.6%,[39,40] our results are slightly above this range at 81.1% (n = 43/53). Overall, this shows that in approximately 20%–40% of infections, the bacterial pathogens cannot be detected at all. It is now obvious that, to increase the detectability of bacterial pathogens, it seems necessary to use all available diagnostic techniques of microbiology,[41] especially if noncultivable, nonplanktonic bacteria are expected in the wounds.[42]

Molecular biological methods such as polymerase chain reaction (PCR) can further improve microbiological diagnostics by detecting bacterial DNA. The sensitivity of PCR in the diagnosis of peri-implant infections is reported to be 50%–100%.[43–47] However, PCR cannot answer the question of the extent to which the DNA detected by PCR belongs to the respective vital microorganism causing the infection, as it also detects DNA from dead microorganisms.[15] Thus, the rate of false-positive results due to contamination by noncausative pathogens or DNA detection of avital bacteria can be up to more than 50%.[48] In this study, tissue samples were therefore additionally examined with FISHseq, which can be used to microscopically identify bacteria in both their planktonic and sessile forms in the biofilm via specific microbial rRNA-targeted probes. In addition to the

standard culture method, FISHseq improved the detection of implant-associated infections. Thus, in almost a quarter of all infected wounds, a pathogen or colonization with additional bacteria could only be detected at all by means of FISHseq. Standard culture and FISHseq were finally able to detect bacterial pathogens in every infection situation (100%). Furthermore, in 5% of all wounds, the bacterial pathogen detected by culture was not detected in the tissue by FISHseq and thus "unmasked" as a highly probable pathogen contamination that did not cause the infection. It should be emphasized that FISHseq is unsuitable as a sole diagnostic tool due to the existing false-negative FISHseq results (n = 4) and the necessary determination of the antibiogram as well as the extent of bacterial resistance status, which so far can only be elaborated by cultural evaluation.[49]

Another meaningful advantage of FISHSeq is the ability to detect the microorganisms causing the infection in their biofilm environment without destroying the biofilm by manipulation. In the present work, we were able to detect the presence of an intact, mature biofilm in only three cases and nonplanktonic growth with a smaller number of colonies (microcolonies) in two other cases in the tissue samples. The ratio was greater in the early infected wounds (three of 16) than in the late infected wounds (two of 37). Overall, nonplanktonic growth was observed in just under 10% of implant-associated infections. This frequency is much lower than in studies of chronic open wounds (eg, diabetic foot syndrome, leg ulcers, pressure ulcers; pooled n = 101/143; 70.6%, range: 59.1%–100%).[50–54] However, a similar frequency to our own study was found in the investigation of 16 early infected wounds by the research group of James et al [one of 16 (6.3%)].[52] However, none of the aforementioned studies originally had closed implant-associated infections; so the results cannot be related to our own patient population. To our knowledge, there is only one article that actually shows the occurrence of biofilm on osteosynthesis implants. However, it only examined the implant and analyzed any colonies on the implant surface in terms of their spatial structure using scanning electron microscopy. The study group showed a biofilm frequency of 50% (three of six implants) in the context of uncomplicated (noninfected) metal removals.[55] In the present study, however, mainly soft tissue samples were examined in clinically clear infections, so that completely different examination conditions were present and the data on frequency of occurrence do not seem comparable. The overall picture shows that there is hardly any information available so far on the frequency of biofilms in early- or late-onset implant-associated infections. In addition, the information provided in previous studies on biofilm frequency in wounds generally varies greatly, which is certainly also due to the very heterogeneous distribution of pathogens in the wound, which holds the scientific basis for the requirement to take at least two tissue samples.[14,56]

The study results are limited by several factors. First, the patient and implant groups as well as the clinical expression of implant-associated infections are highly heterogeneous. Differences between different implants were not examined. Although several surgeons were involved in the diagnostic procedures, all patients were treated uniformly according to established military wound center standards for septic defects. The administration of antibiotics, which in some cases was started preoperatively, certainly had an impact on the detection of bacteria in tissue samples taken intraoperatively. On the other hand, the fact that antibiotic administration was often started before surgery in our study is more in line with the reality of care, as unsuccessfully treated cases or patients with complications are usually transferred to our treatment center with antibiotic therapy already in progress.

CONCLUSIONS

In summary, this study may have been the first to use a larger patient population with implant-associated infections to investigate the presence of nonplanktonic growth microscopically and molecularly and to test the added value of FISHseq. FISHseq demonstrated important added diagnostic value, was able to exclude infection in individual cases despite a high clinical suspicion of wound infection, and in other cases detected commonly commensal pathogens as causative pathogens for infection and identified additional pathogens in a large proportion of wounds. However, the FISHseq method used was only able to detect a nonplanktonic bacterial life form in one tenth of the wounds, which is very rare compared with the reported frequency in open chronic wounds. Despite the additional diagnostic benefit, it must be kept in mind that FISHseq is expensive and certainly not needed for routine microbiological assessment of every wound. However, it can be recommended and should be considered as an additional investigation method for special indications such as refractory, therapy-resistant and difficult-to-treat infection situations and in cases of uncertainty about the causative pathogen. Furthermore, FISH is not limited to a specific surgical specialty. It can be used in all questionable occurrences of biofilms, in soft tissue, bradytrophic tissue, implants, or foreign materials.

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DISCLOSURES

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