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Getting it right first time: The importance of a structured tissue sampling protocol for diagnosing fracture-related infections

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ABSTRACT

Introduction: Fracture-related infection (FRI) is an important complication following surgical fracture management. Key to successful treatment is an accurate diagnosis. To this end, microbiological identification remains the gold standard. Although a structured approach towards sampling specimens for microbiology seems logical, there is no consensus on a culture protocol for FRI. The aim of this study is to evaluate the effect of a structured microbiology sampling protocol for fracture-related infections compared to ad-hoc culture sampling.

Methods: We conducted a pre-/post-implementation cohort study that compared the effects of implementation of a structured FRI sampling protocol. The protocol included strict criteria for sampling and interpretation of tissue cultures for microbiology. All intraoperative samples from suspected or confirmed FRI were compared for culture results. Adherence to the protocol was described for the post-implementation cohort.

Results: In total 101 patients were included, 49 pre-implementation and 52 post-implementation. From these patients 175 intraoperative culture sets were obtained, 96 and 79 pre- and post-implementation respectively. Cultures from the pre-implementation cohort showed significantly more antibiotic use during culture sampling ($P=$ 0.002). The post-implementation cohort showed a tendency more positive culture sets (69% vs. 63%), with a significant difference in open wounds (86% vs. 67%, $P=$ 0.034). In all post-implementation culture sets causative pathogens were cultured more than once per set, in contrast to pre-implementation. Despite stricter tissue sampling and culture interpretation criteria, the number of polymicrobial infections was similar in both cohorts, approximately 29% of all culture sets and 44% of all positive culture sets. Significantly more polymicrobial cultures were found in early infections in the post-implementation cohort ($P=$ 0.048). This indicates a better yield in the new protocol.

Conclusion: A standardised protocol for intraoperative sampling for bacterial identification in FRI is superior than an ad-hoc approach. It has a positive effect on both surgeon and microbiologist by increasing awareness about the problem at hand. This resulted in more microbiologically confirmed infections and more certainty when identifying causative pathogens.

Introduction

Fracture-related infections (FRIs) are important complications following surgical fracture management. Not only do patients suffer from increased morbidity and prolonged hospital stay, they have to deal with increased healthcare costs and decreased quality of life [1–3].

Infection around fractures is suspected with the presence of clinical signs and symptoms and, if indicated, on additional laboratory tests and diagnostic imaging [4,5]. Early FRIs tend to be easily recognisable by classic inflammatory symptoms such as redness, pain, warmth, and swelling [6]. Later infections may present more subtly, with delayed fracture healing and persisting tenderness as the only indication of a lingering infection [7]. This can make

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diagnosis more challenging, particularly as these infections are often caused by low-grade or difficult-to-culture organisms [8,9].

Laboratory tests alone are insufficient for diagnosing FRIs [5,6,10]. Diagnostic imaging can aid in the diagnosis and provide information on status of fracture healing and extent of the infection, especially when chronic. However, studies on diagnostic accuracy of medical imaging for FRI are hampered by small patient series and insufficient data [11,12]. The only incontestable gold standard to confirm the diagnosis of FRI is the presence of pathogens in deep surgical wounds [4,5,13].

Apart from establishing the presence or absence of FRI, an accurate microbiological diagnosis is crucial for an effective treatment. Treatment strategies rely heavily on microbiological findings, so it is of paramount importance that the right pathogens be identified [14]. First, targeting the right pathogen is important for effectiveness of the antibiotic treatment. Second, prescription of unnecessary broad-spectrum or incorrect antibiotics may lead to increased antimicrobial resistance [15].

Although the literature agrees that multiple specimens for microbiology should be taken to improve sensitivity and specificity, there is no agreement on the method to obtain these samples [9,16]. Number of recommended cultures, but also source and technique used to obtain the specimen vary between studies and largely focus on prosthetic joint infection [6,16,17].

To introduce uniformity in sampling techniques and handling of samples taken intraoperatively from suspected infection sites, a standardised sampling protocol for FRIs was recently implemented in our hospital. This new approach was based on a tissue sampling protocol developed in the Bone Infection Unit, Oxford, UK for prosthetic joint infections [16] and subsequently applied to osteomyelitis and fracture-related infections [9,18]. The aim of this study is to evaluate the effect of a structured microbiology sampling protocol for fracture-related infections compared to ad-hoc culture sampling.

Methods

Patient identification and ethical aspects

A cohort study was conducted in a single Level-1 trauma centre in the Netherlands, comparing a retrospective pre-implementation cohort with a prospective post-implementation cohort. Both cohorts included intraoperative cultures from patients with suspected or confirmed FRI, both early (<6 weeks) and late (>6 weeks) following the index operation. In both cohorts during all revision surgery of surgical debridements intra-operative samples were taken. Sampling for another indication then suspected or confirmed FRI were excluded. The first retrospective cohort included samples obtained between January and December 2014, based on a previous study [19] and served as a historical control group before implementation of the standardised sampling protocol. Possible subjects were retrospectively identified by screening surgical registries. Subjects from the second cohort were prospectively included from July to December 2017, starting at the implementation of the new standard-of-care sampling protocol for suspected or confirmed FRI, and thus became the research cohort. All patients with suspected infection following osteosynthesis were eligible for inclusion. Only intraoperative culture results from before and after implementation of the new sample protocol were included in the study to ascertain comparability of circumstances. The intraoperative cultures obtained during each revision operation were recorded for culture-based analyses, and are referred to in this paper as the 'culture set'. Patients from whom no intraoperative cultures were obtained were excluded from analysis. Baseline characteristics of both patients and cultures were collected from the electronic patient files (e.g. age, gender, affected bone, initial soft tissue damage, early/late infection, wound characteristics, etc.).

This study was reviewed and a waiver was provided by the local Medical Ethics Review Committee (METC), no: 18-147/C. All study procedures were performed in accordance with relevant guidelines and regulations. Patient-related data was processed and analysed anonymously.

Sampling approaches

Pre-implementation

There was no standardised sampling method for microbiological cultures. Ad-hoc intraoperative cultures were obtained. Number of cultures, method (e.g. tissue sample, swab, fluid, sonification), and use of antibiotics during sampling were decided by the treating surgeon. Antibiotic use was defined as prophylactic antibiotics before surgery or therapeutic antibiotics continued during perioperative sampling, or started before the first sampling moment.

Post-implementation

Samples for microbiological cultures were obtained following a standardised protocol. First, antibiotics during or prior to the sampling were avoided, preferably for at least two weeks. Preoperative antibiotic prophylaxes were withheld until all culture samples were obtained. A minimum of five deep-tissue or fluid samples were collected intraoperatively at the area of suspected FRI. Tissue samples were only obtained from infection-suspected tissue adjacent to the fracture, preferably from the implant-bone interface (e.g. muscle, periost, callus). Bone samples were only obtained when sequesters or loose infected bone fragment were present peri-operatively. Superficial or skin tissue or fluid samples were not considered for bacterial identification. Due to their low sensitivity, swabs were not allowed [20]. Sinus tracts were disregarded because of their low diagnostic value [21]. To minimise the risk of cross-contamination all samples were obtained with separate, sterile surgical instruments using a no-touch technique. After the sample was obtained it was placed directly into separate sterile specimen containers with clean instruments by the surgeon and transported to the microbiology laboratory (Fig. 1) [16]. By limiting the exposure to the operating room environment the contamination risk is limited [22]. Osteosynthetic implants were sent for sonication when appropriate. The microbiology request form was redesigned to emphasise that the patient had a suspected or confirmed FRI. Data on whether antibiotics had been administered prior to sampling and whether osteosynthetic implants remained in situ was mandatory clinical information that needed to be completed on the microbiology request form. First cultures sets were always sampled without antibiotics. When redebridement was nec-

Fig. 1. The sampling set.

essary during the therapeutic antibiotic treatment, the antibiotics were continued peri-operatively.

Microbiological procedures

Procedures within the microbiology laboratory did not change between the retrospective pre- implementation and prospective post-implementation periods.

Bone specimens were cultured in brain heart infusion broth with added hemin and nicotinamide adenine dinucleotide (X and V factors (BHXV)), if possible in thioglycolate enrichment broth (thio) incubated aerobically for 7 days, and if turbid subcultured in blood agar (BA) and chocolate agar (GC) (both 5% CO_2) or BA and brucella blood agar (BBA) (anaerobic incubation). Tissue specimens other than bone were homogenised using a bead-beater protocol.

Homogenised tissue specimens were cultured in BA (4 days, aerobically), GC (3 days 5% CO_2) McConkey agar (McC) (2 days, aerobically) and BBA (14 days, anaerobically), as well as in BHXV (7 days aerobically). Pus samples were cultured in BA (4 days, aerobically), GC (3 days 5% CO_2) McC (2 days, aerobically) and BBA (7 days, anaerobically).

Osteosynthetic materials were submerged in sterile sodium chloride 0.9% (w/v) by the operating surgeon in a sterile 'sonication jar' in the operating room. Upon arrival in the laboratory, sonication jars are sonicated for 1 min at maximum power (Bandelin BactoSonic). Uncentrifuged sonication fluid is cultured in BA and GC (4 days 5% CO_2), in BBA (7 days, anaerobically) and in thio (14 days aerobically).

Growth of different colonial morphologies was identified using MALDI-TOF MS (MBT Smart, research use only (RUO DB 6903) and security-related (SR) databases, Compass software, Bruker, Germany). Susceptibility testing was performed on isolates using Phoenix automated susceptibility testing (enterobacteriaceae, staphylococci, enterococci) or disk diffusion and/or E-test (all other isolates according to EUCAST methodology (disk diffusion) and manufacturer's instructions (Etest)). MIC values and disk diffusion growth inhibition zone diameters were interpreted according to EUCAST criteria [23].

Study outcomes

The primary outcome measure was the number of positive cultures. Incidences of the different pathogens were described. Incidences of polymicrobial and staphylococcal infections were compared. The definition of a causative pathogen was different in the pre- and post-implementation cohort:

Pre-implementation cohort: All reported isolates from tissue samples, swabs or fluids cultures were defined as causative pathogens. Culture of a single pathogen was regarded as significant and included in treatment considerations. This was in line with the clinical practice at that time.

Post-implementation cohort: Phenotypically indistinguishable microorganisms cultured from a minimum of two separately obtained samples from the same culture set were regarded as causative [5]. Again, swab samples were considered insufficiently reliable and were not allowed. Intraoperative fluid, tissue, and hardware cultures were considered as relevant cultures. Polymicrobial infection was defined as $\geq$2 pathogens cultured from at least two (out of five) specimens obtained in the same operation.

Differences between the causative pathogens and the incidence of polymicrobial infections between the two cohorts were analysed.

As a secondary outcome parameter, adherence to the protocol was described in the post-implementation cohort. Deviation from protocol was defined as sampling of less than five relevant cultures according to the method described above.

Statistical analysis

Patient characteristics and microbiological aspects of both cohorts were descriptive. Baseline characteristics were compared between the two patient cohorts. Differences in culture set results between the two protocols were analysed with the Pearson Chi-square test for categorical variables and the Mann-Whitney *U* test for non-parametric continuous data. *P*-values < 0.05 were considered significant. Analyses were conducted using SPSS 24 (IBM SPSS-statistics, New York, NY).

Results

Study population

In total 102 patients were eligible for inclusion in the study; 49 patients included in the retrospective 2014 pre-implementation cohort, which underwent the old sampling approach, and 52 patients included in the prospective 2017 post-implementation cohort, which underwent the new sampling protocol. Seventy-one patients were male, median age was 52 years(range 9–92). Tibial fractures were most often affected, at approximately 45%. In 43% of the patients it involved open fractures with varying degrees of soft-tissue injury. Plates were the hardware most often involved (51%).

The 102 patients underwent 175 separate operations in which samples for microbiology were obtained (sampling moments); 96 from the pre-implementation and 79 from the post-implementation cohort. Comparison based on culture set characteristics showed significantly more culture sets obtained from the radius and ulna ($P = 0.012$) pre-implementation and comparable soft-tissue injuries, but slightly more grade IIIb open fractures post-implementation($P = 0.347$). In both cases this could be attributed to prolonged infection in selected cases (two and three patients respectively), with multiple revision surgeries and thus multiple culture sets (Table 1).

Table 1
Baseline characteristics.

	Culture set-based			
	Protocol implementation			
	Pre ($n = 96$)	Post ($n = 79$)	*P*	Missing
Gender, male	65	61	0.123	0
Age, years (range)	50 (18-92)	57 (9-83)	0.201	0
Body part			0.012	0
Humerus	3	4		
Radius/ulna	21	4		
Sternum	0	1		
Pelvic ring/acetabulum	5	12		
Femur	15	10		
Tibia/fibula	41	42		
Foot	11	6		
Soft-tissue injury				
Closed	61	49	0.525	2
Open, Gustilo classification			0.347	7
Gustilo I	4	5		
Gustilo II	6	5		
Gustilo IIIa	9	5		
Gustilo IIIb	6	12		
Gustilo IIIc	4	2		
Fixation type			0.231	0
Plate	45	44		
Screws	6	4		
Intramedullary nail	20	21		
K-wire/Tension Wire	5	2		
External fixator	14	3		
Prosthesis	1	0		
No fixation device	5	5		

Data are presented as number of cases or median (range).

Table 2
Data are presented as number of cases or median (range).

	Protocol implementation			
	Pre ($n=96$)	Post ($n=78$)	P	Missing
Infection type			0.799	1
Early (<6 w after index surgery)	53	44		
Late (>6 w after index surgery)	43	33		
Soft-tissue status at time of surgery for suspected infection			**0.026**	1
Closed	44	41		
Dehiscent	21	23		
Draining fistula	10	10		
VAC in situ	20	4		
Antibiotics given during sampling	53	25	**0.002**	
Median number of samples	1 (1-7)	n/a	**n/a***	0
Median number of relevant samples	1 (0-5)	5 (2-9)	**<0.001**	0

n/a not applicable; VAC = vacuum dressing.
Data are presented as number of cases.
* All samples were relevant (i.e. deep uncontaminated surgically obtained tissue samples from the area of suspected infection) in the post-implementation cohort and thus described only under 'relvant'.

Culture approach characteristics

Culture characteristics per cohort are shown in Table 2. One case from the post-implementation cohort was excluded from further analysis due to gross protocol violation, as only one intraoperative tissue sample was obtained. Early and late infections were equally represented in both cohorts. In both cohorts early and late infections were equally represented (55% pre-implementation vs. 56% post-implementation). In the post-implementation cohort significantly more deep samples were obtained from closed wounds (46% vs. 53%, $P=0.026$). No samples from the wound or fistula itself were obtained. The tendency towards more pre-implementation cultures from vacuum-dressed wounds was largely explained by the patients with prolonged infection discussed above. Secondly, in the post-implementation cohort surgeons were strongly discouraged from culturing wounds covered by VAC dressings, as this merely reflects the superficial bacterial colonisation of the wound and the VAC sponge. As per protocol, there was a difference in the incidence of antibiotics being administered at the moment of culturing, with a significant lower incidence post-implementation. The median number of relevant samples obtained pre- and post-implementation differed significantly: 1(range 0–5) pre-implementation and 5(range 2–9) post-implementation. As stated, pre-implementation 'non-relevant' intraoperative cultures from swabs and sinus tracts were included for further analysis, as this was clinical practice at that time. Median number of all cultures combined obtained in the pre-implementation cohort was 1(range 1–7).

Twenty post-implementation culture sets deviated from the protocol, all based on the number of relevant cultures obtained during each set. Ten sample sets contained four samples, five sets with three samples, and five sets with only two samples. Except for the number of cultures obtained, culture set characteristics did not differ between the sets obtained according to the protocol and the sets that deviated from it.

Microbiology

Bacteria most often found were *Staphylococcus aureus*(23%), Coagulase-negative *Staphylococci*(19%), *Enterococcus* species(8%), *Corynebacterium* species(7%), *Enterobacter* species(7%), and *Pseudomonas aeruginosa*(7%). Slightly more Coagulase-negative *Staphylococci* were found in the post-implemenation cohort. Otherwise causative pathogens were quite similar. The same bacteria were also most often cultured in cases of polymicrobial cultures. Of the 200 bacteria cultured, 134(67%) were found in a polymicrobial culture. The difference in frequency of aerobic and anaerobic bacteria found was largely due to the high number of *S. aureus.* An overview of the identified bacteria is shown in Table 3.

In addition to culture set characteristics, no differences were found in microbiological aspects between protocol deviations and non-deviations in the post-implementation cohort. Hence all sets were analysed together, as this represents clinical practice. Microbiology results from culture sets are summarised in Table 4. Inherent to the sampling approach, there were significantly more positive cultures per culture set post-implementation (4 vs. 1). In total, the overall culture set results came back negative in 60 cases, 36 pre-implementation and 24 post-implementation. From the positive cultures in the pre-implementation cohort, more than one culture per set identified the same pathogen in 42% of the culture sets, compared to 100% in the post-implementation cohort ($P<0.001$). Implementation of a sampling protocol for FRI did not lead to decreased numbers of polymicrobial (33% post-implementation vs. 25% pre-implementation) or *Staphylococcus aureus*-positive cultures (27% post-implementation vs. 26% pre-implementation) in the total groups. Sub-analysis of open (dehiscent wounds, fistulas and wounds temporarily covered with vacuum dressings) and closed wounds at the time of culture sampling showed an additional difference in negative culture results, with significantly more negative results found pre-implementation in patients with open wounds ($P=0.034$). No additional differences were found in closed wounds. Sub-analysis of early and late infection showed significantly more polymicrobial infections post-implementation in early infections ($P=0.048$) (Table 4).

Because, higher numbers of positive cultures were obtained per culture set post-implementation, we calculated the ratio of positive cultures per absolute number of samples obtained. Although not significant, this was also higher in the new cohort than in the old cohort, 0.68 vs. 0.59 positive culture per sample obtained. Hence, a higher number of samples obtained after implementation of the sampling protocol did not seem to be the only reason for higher overall positive culture results.

The use of antibiotics during sampling affected the number of positive culture sets in the pre-implementation cohort, but not in the post-implemenation cohort. In the pre-implementation cohort significantly less positive culture sets were found when antibiotics where administered during sampling (45% vs. 84%, $P<0.001$), and also less positive cultures per culture set (median 0 (0–30 vs. 1 (0–6), $P<0.001$. Whereas, both 70% positive cultures sets were found in the post-implemenation cohort, regardless of antibiotic administration.

Table 3
Microbial identification in early and late culture sets.

Microorganism (MO)	Total $n=200$	PolyMO $n=134$	Early $n=144$	Late $n=56$
Staphylococcus aureus	46	18	32	14
CoNS	37	24	30	7
Enterococcus species	15	14	12	3
Corynebacterium species	14	13	12	2
Enterobacter species	13	9	11	2
Pseudomonas aeruginosa	13	9	5	8
Escherichia coli	10	6	5	5
Streptococcus	9	9	7	2
Klebsiella species	5	4	2	3
Finegoldia magna	5	5	4	1
Acinetobacter species	4	3	1	3
Clostridium species	3	2	2	1
Proteus mirabilis	3	3	3	0
Bacteroides fragilis	3	3	3	0
Peptoniphilus harei	3	3	2	1
Propionibacterium species	3	1	2	1
Fusobacterium nucleatum	2	1	1	1
Serratia marcescens	2	0	1	1
Actinobaculum schaalii	1	1	1	0
Cedecea davisae	1	1	1	0
Citrobacter koseri	1	1	1	0
Granulicatella adiacens	1	1	1	0
Kytococcus schroeteri	1	0	1	0
Parvimonas micra	1	0	0	1
Porphyromonas spp	1	1	1	0
Prevotella denticola	1	1	1	0
Rhodococcus equi	1	0	1	0
Lactobacillus	1	1	1	0

The total number is the combined values of early and late infections.
Infections are regarded as early when manifested within 6 weeks after initial fracture fixation surgery, late infections manifested after 6 weeks.
PolyMO is the number of times the bacterium is cultured as part of a polymicrobial culture.

Table 4
Microbiological characteristics of all culture sets.

Microbiological variable	All cultures					
	Total		Cultures from open wounds		Cultures from early infections	
	Protocol implementation		Protocol implementation		Protocol implementation	
	Pre $n=96$	Post $n=78$	Pre $n=51$	Post $n=37$	Pre $n=53$	Post $n=44$
Median number of positive cultures	1 (0-6)	3.5 (0-8)	1 (0-6)	4 (0-7)	1 (0-6)	4 (0-7)
Negative culture set	36	24	17	5	18	6
Positive culture set	60	54	34	32	35	38
	Positive cultures					
	Pre $n=60$	Post $n=54$	Pre $n=34$	Post $n=32$	Pre $n=35$	Post $n=38$
Same pathogen >1 cultures per set	25	54	12	32	15	38
Median number of pathogens	1 (1-5)	1 (1-6)	1 (1-5)	2 (1-6)	1 (1-5)	2 (1-6)
Median number of species	1 (1-5)	1 (1-6)	1 (1-5)	1.5 (1-6)	1 (1-5)	2 (1-6)
Staphylococcus aureus (mono/polymicrobial)	25	21	14	15	17	15
Polymicrobial infections	24	26	14	17	14	24
Monomicrobial infections	36	28	20	15	21	14
	Monomicrobial cultures					
	Pre $n=36$	Post $n=28$	Pre $n=20$	Post $n=15$	Pre $n=21$	Post $n=14$
Staphylococcus aureus	16	12	10	8	9	7
Other monomicrobial infections	20	16	10	7	12	7

Data are presented as number of cases or median (range).

Discussion

This study aimed to evaluate the effect of a structured sampling protocol for FRI. The new structured sampling protocol performed better at identifying pathogens with more positive cultures. As in all positive culture sets the causative pathogen had to be cultured at least in two different cultures, increased microbiological certainty was achieved. Despite these stricter criteria for causative pathogens no decrease in number of polymicrobial cultures or number of *Staphylococcus aureus*-positive cultures was found post-implementation.

Our hypothesis was that the *ad hoc* (pre-implementation) sampling method is inferior to a structured and standardised protocol (the post-implementation method) and this hypothesis is positively confirmed by the results of this study.

We found no difference in the species of microorganisms cultured between the two cohorts. We expected to find less polymicrobial infection as a result of the stricter criteria for causative pathogens (growth in at least two separate cultures and prevention of cross-contamination with skin flora). This was not the case, with even more polymicrobial infections found in the post-implementation cohort in the early infections. A possible

explanation for this outcome is that the higher number of relevant samples led to increased opportunities to identify pathogens. Meanwhile, more precise sampling and stricter criteria will lower the possibility of identification, thereby partially equalling out the measurable effect of the protocol in this cohort. Overall, we found high numbers of polymicrobial infections (25% and 36% of all cultures pre- and post-implementation respectively). This is on the upper limit of what is described in the literature. The incidence of polymicrobial infection following osteosynthesis is described to be around 15%–27 [6,24]. This may be explained by the setting of the study. Ours being a Level-1 trauma centre, most of the trauma patients admitted to our hospital are severely injured which may contribute to the high number of fractures accompanied by soft-tissue injury (Table 1). Although not proven, one can argue that severe soft-tissue injury leads to more complex pathogen patterns and multiple operations, which can subsequently result in more complex infections. Additionally, severe injury affects the host's immune system, making it susceptible to bacterial infections [25]. Accurate diagnosis of polymicrobial infections is necessary for proper antibiotic treatment, as such infections are associated with worse outcome in orthopaedic patients [26]. However, argued above, a lack of structured sampling with strict microbiological definitions can skew data on polymicrobial infections in other studies. Thus, making it hard to directly compare results.

The difference of the effect of antibiotics on microbiological outcome between the two cohorts can be explained by protocol adherence. In the post-implementation cohort it was encouraged to withheld antibiotics until all samples were taken. We feel that the results from the pre-implemenation cohort reflect the effect of antibiotics because this also includes a significant subgroup of patients who underwent surgical procedures with administration of (prophylactic) antibiotics prior to first tissue sampling. This outcome emphasizes the importance of avoiding antibiotic administration before microbiological diagnosis were possible.

In our study, we observed a significant difference between the number of positive cultures per operation. We also observed a trend towards more positive culture sets under the structured protocol, with significantly more positive culture sets in open wounds. We feel that the difference in positive cultures is a result of the protocol as a care-package. Multiple relevant tissue sampling, avoidance of antibiotics [27] and improved information transfer on the microbiology request form contributed to this result. Atkins et al. [16] recommend a minimum of five protocolised tissue samples for prosthetic joint infections (PJI). They compared several tissue cultures to histological specimens of PJI and found 65% positive cultures in histologically confirmed infections. Based on this data they calculated that five or six cultures should be obtained to reliably diagnose infection. By contrast, Peel et al. [28] and Bémer et al. [24] showed that fewer specimens seeded on more culture media was as effective as five specimens on fewer media. However, as they themselves argue, this method is mostly recommended for subacute or chronic infections and is hard to extrapolate to acute infections because of pathogenicity and growth characteristics of bacteria involved. The overwhelming clinical presentation in acute infections is due to more bacteria, which should simplify bacterial identification and decrease the need for repetitive tissue sampling [24,28,29]. Nevertheless, it takes a couple of days for the bacterial burden to reach maximum levels [30]. So when suspicion of infection is raised early on, bacterial burden may still be relatively low and therefore the proposed method still applies. In order to establish a clear and non-confusing protocol with minimal opportunities for errors we chose a pragmatic recommendation of a minimum of five samples in every type of FRI.

Earlier studies show lower sensitivity and specificity of swab cultures compared to extensive cultures of tissue samples or scrapings from biomaterial surfaces [20,30–32]. And yet, the same studies also show increased numbers of positive cultures with prolonged incubation time for bacterial growth [31,33]. Although the generally recommended incubation time of 7 days is also maintained in our protocol for broth cultures, a prolonged incubation of 14 days is recommended in specific cases [33]. This might be of interest when slow-growing microorganisms are to be expected. The introduction of a customised microbiology request form raised awareness among all parties involved. This resulted in more careful sampling by the surgeon and more thorough culture procedures with different media and prolonged incubation time by the microbiologist. Implant-related infections are known to be associated with anaerobic bacteria (especially in open fractures) [34] as well as frequent infection by *Staphylococcus aureus* [35]. Both pathogens require special attention in interpreting culture data, as anaerobic and aerobic pathogens require different culture conditions, and *S. aureus* can enter a stationary growth phase in biofilms, resulting in impaired bacterial growth. By implementing a structured microbiology request form in the new protocol sample origin (e.g. deep tissue, bone) and the problem at hand (infection near orthopaedic implants) are emphasized. Thereby, triggering the microbiologists to prolong culture time if necessary and minimize 'missed' pathogens.

There are some drawbacks to our study. First we compared two different approaches, with differences in their definition of FRI and causative pathogen. For purposes of this study, to evaluate the effect of a sampling protocol compared to previous clinical practice, this did not form a problem. Unfortunately, this does result in the inability to analyse the individual elements incorporated into the new standardised protocol. Second, in the pre-implementation cohort swabs and tissue samples were obtained from every re-debridement surgery, even VAC-system replacements. Because of stricter sampling criteria in the post-implementation cohort only uncontaminated, deep samples were obtained. This could have lead to more false culturing outcome in the pre-implementation cohort compared tot he post-implementation cohort. Again, because this was customary at that time and (antibiotic) treatment was based on the microbiology results of each sample obtained in the pre-implementation cohort, we used these results specifically to compare old versus new standard of care. As discussed above, chronic and acute infections may differ in the difficulty of bacterial identification. This may skew results. However, the percentage of patients with early versus chronic infections did not differ in the cohorts compared in this study. Last, it is possible that with a prolonged inclusion time and thus more included patients, more (significant) differences in outcome could be found. Remarkably however, is that relatively more infections are included in the post-implementation cohort (same number, short inclusion period). We feel this increase in infectious complication rate is due to improved pre-hospital triage, leading to increased numbers of complex monotrauma patients referred to our hospital over the years [36].

In conclusion, even with stricter criteria for pathogen identification a structured tissue sampling approach for fracture-related infection leads to increased microbiological identification with more certainty of causative pathogens. This ensues from both the improved and standardised sampling technique and the customised microbiology request form. It has a positive effect on both surgeon and microbiologist by increasing awareness about the problem at hand. This results in a more complete and honest overview of the infected tissue, more trustworthy culture results, and consequently a more targeted treatment. Future research should focus on cost effectiveness of such a protocol and possible alternatives in microbial culture or pathogen identification techniques.

Transparency document

The Transparency document associated with this article can be found in the online version.

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