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The diagnostic potential of MicroDTTect compared to conventional culture of tissue samples in orthopedic infections

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Summary

Objective. The evolution of new prosthetic and osteosynthesis devices has led to better outcomes and therefore more frequent surgical indications. As a consequence, an increased incidence of complications such as infections or aseptic loosening in orthopedic and trauma surgery are being recorded in general population. MicroDTTect is a quick and simple system that is useful to detect low grade or bio-film related infections. The aim of this study was to evaluate the reliability of MicroDTTect in detection of orthopedic infections compared to conventional culture of tissue samples.

Methods. The population enrolled was composed of 13 patients undergoing surgery for prosthesis or osteosynthetic device failure or loosening. The MicroDTTect system and traditional culture of tissue samples were applied to identify the pathogens and compared with each other.

Results. MicroDTTect had a higher sensitivity compared to conventional culture of tissue samples. Two cases resulted positive while the traditional culture sample showed a false negative result. In addition, with MicroDTTect a polymicrobial infection was identified, while with traditional methods was misdiagnosed.

Conclusions. We showed that treatment of suspected implant infections using the MicroDTTect device improves microbiological diagnosis with more sensitive results, leading to a more accurate treatment.

Key words: orthopedic infections, periprosthetic infections, MicroDTTect

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Introduction

The evolution of new prosthetic and osteosynthesis devices has led to better outcomes and therefore increasingly frequent surgical indications. This is accompanied by an increased incidence of complications related to the implants and to the surgical intervention.

Among complications, chronic post-operative bacterial infections play an important role associated with primary implants (1-4%) and revision surgery (30%) [1-4].

Chronic infections due to low-virulence organisms can be difficult to diagnose and challenging to treat, leading to high morbidity rates and high healthcare costs [5]. Clinical symptoms, inflammatory markers, and scintigraphy may help in identifying chronic infections, but the diagnostic benchmark is considered to be intra-operative clinical diagnosis and pathogen identification, in order to administer tailored antibiotic treatment [6-8].
It should be taken into consideration that implant-related infections are biofilm-related [9,10] and traditional sampling techniques may not be so reliable to detect biofilm-embedded bacteria from implant surfaces and peri-implant tissues, leading to possible false negative results in culture, and to a misdiagnosis of aseptic loosening, in particular when recent antibiotic treatment or low-grade infections place doubt on clinical signs. Sonication of the explanted implants and culture of the sonication fluids has been considered superior over other methods to disrupt the bacterial biofilm [10-14].
This procedure is, however, not used in all the laboratories given its purchase cost and difficulty of use.
Some authors proposed an alternative technique using dithiothreitol (DTT) to detach biofilm from explanted implants with good results [15,16]. DTT, in fact, is a chemical agent that reduces disulfide bonds in peptides, and can thus alter the matrix of biofilm releasing bacteria without affecting their viability. Accordingly, bacterial culture is possible and permits identification and antibiotic susceptibility tests.
Recently, a commercial device containing a DTT solution has been developed, namely MicroDTTect (4i for infection, Monza, Italy) (Fig. 1). This device is a sterile closed system that avoids multiple manipulation decreasing the risk of contaminations, and is quick and easy to use.
The purpose of the study was to evaluate the reliability of MicroDTTect in prosthetic and implant failures in detecting chronic or silent infections compared to the conventional culture of tissue samples.

Materials and methods

A population of 13 patients undergoing surgery for suspected septic complications or aseptic failure was enrolled. Nine patients underwent prosthesis revision, 3 patient antibiotic cement spacer removal, and 1 plate removal. The demographic and implant characteristics are detailed in Table I.
Both the MicroDTTect system and the collection method used in our institute (culture of tissue samples) were applied to identify the pathogens in the study group. We evaluated the number of positive and negative samples to compare MicroDTTect methodology with tissue sample culture.
Prosthetic implants were aseptically collected and immediately placed in the MicroDTTect collection device (Fig. 2) avoiding unnecessary manipulations (Fig. 3). In parallel, periprosthetic tissue samples (PPT) were collected and immediately placed in plastic sterile containers. Samples were transported as soon as possible to the laboratory for microbiological analysis.

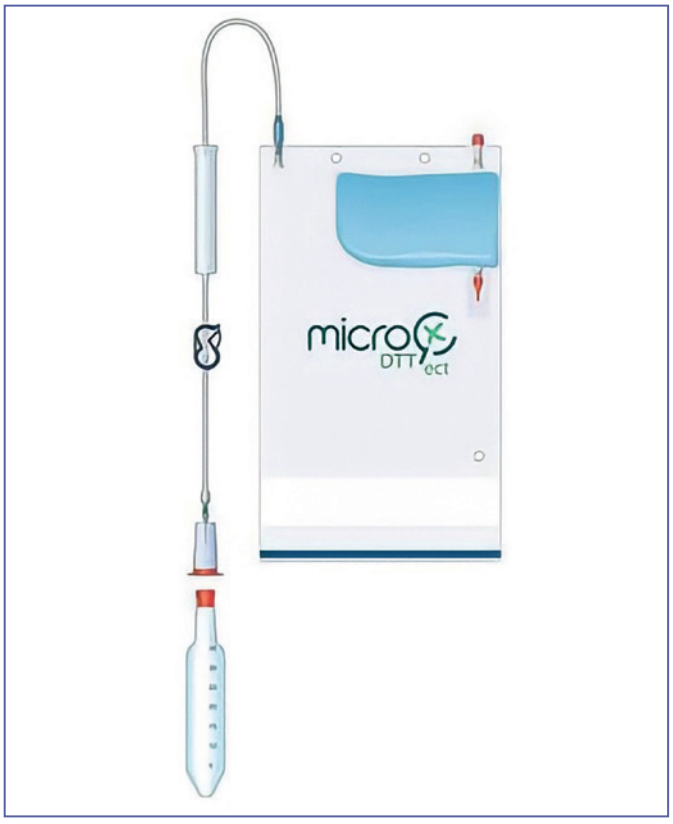

Figure 1. MicroDTTect device.

Table I. Population characteristics.

Males (%)	5 (38.5%)
Females (%)	8 (61.5%)
Age in years, mean ± SD	69 ± 5
Total hip arthroplasty (%)	5 (38.5%)
Total knee arthroplasty (%)	3(23%)
Total shoulder arthroplasty (%)	1 (7.7)
Hip spacer (%)	1 (7.7%)
Knee spacer (%)	1 (7.7%)
Shoulder spacer (%)	1 (7.7%)
Ulnar plate (%)	1 (7.7%)

The MicroDTTect procedure was performed according to the manufacturer's instructions. The MicroDTTect device was placed on a shaker for 15 min. to allow the DTT to detach bacteria and biofilm from the prosthetic material. The DTT suspension was aseptically collected, centrifuged for 10 min. at 3000 x g and the supernatant except for 1 mL was discarded. The pellet was resuspended in the remaining DTT solution. 100 uL aliquots of DTT-treated sample were seeded onto sheep blood

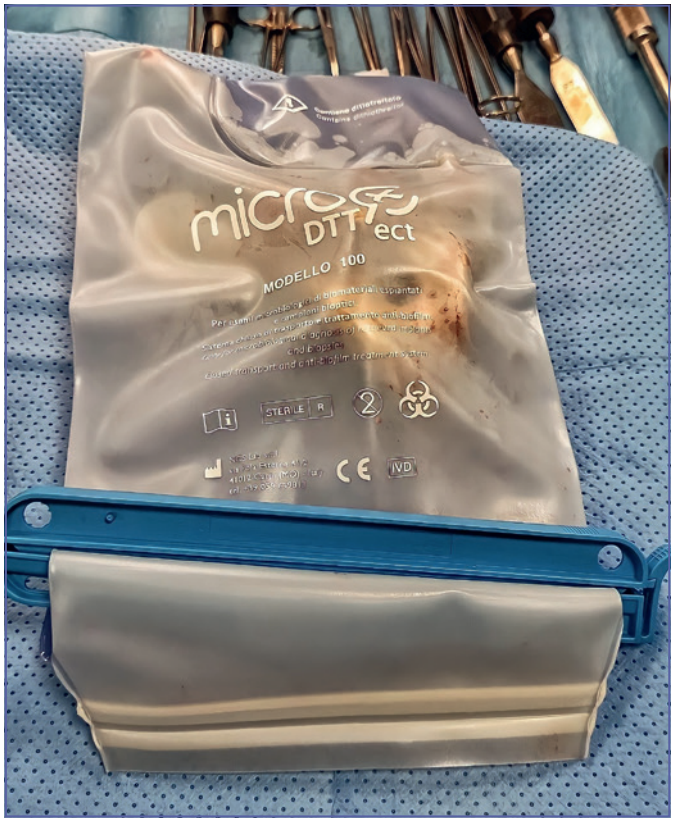

Figure 2. Explanted prosthesis in the MicroDTTect bag.

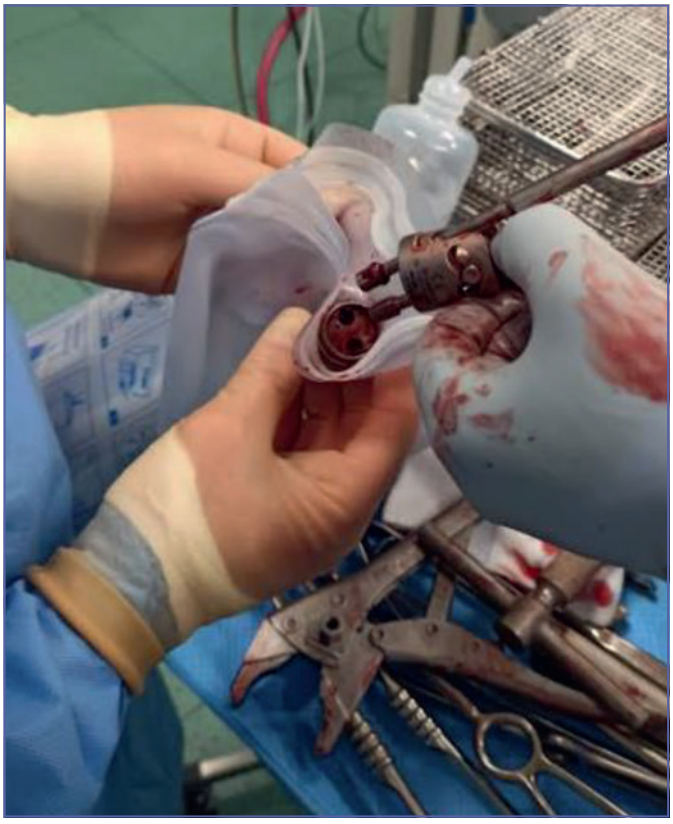

Figure 3. Avoiding manipulation of the explanted implant.

agar (COS), McConkey agar (MCK), mannitol salt agar (MSA), Sabouraud agar (SGC), chocolate agar (PVX), Schaedler agar (SCS) (bioMerieux, Marcy l'Etolie, France), Bi-state blood culture bottle (BCB) (Autobio diagnostics, Zhengzhou, China) and thioglycollate broth (TB) (Liofilchem, Roseto, Italy). PPT were cultured in BCB and TB. COS, MCK, MSA and SGC were incubated for 48 h at 37°C; PVX was incubated for 48 h at 37°C in 5% CO2 enriched atmosphere; SCS was incubated for 48 h in anaerobic conditions; BCB and TH were incubated for 7 days at 37°C and observed daily: positive broths were sub-cultured on agar plates (COS, MCK, MSA, SGC, PVX, SCS) for 48 h.When cultures were positive, identification was performed at the phenotypic level by Vitek2 Compact (bioMerieux, Marcy l'Etolie, France).

Results

MicroDTTect had a higher sensitivity compared to conventional culture of tissue samples. In fact, bacteriological growth was observed in 5 of 13 (38.5%) implant samples using the MicroDTTect system, while PPT samples were positive by culture for only 3 of 13 patients (23%). Two implants resulted positive with MicroDTTect device and negative with traditional culture of tissue samples, respectively, for *Escherichia coli* and Staph*ylococcus hominis ssp Hominis*. Furthermore, one implant resulted positive for 2 microorganisms, *Proteus mirabilis* and *Enterococcus faecium*, while positive for only *Proteus mirabilis* in traditional tissue samples (Tab. II).
Agreement between the two procedures was found in 10 of 13 patients (77%), 8 of them resulting sterile, 2 resulting infected from the same bacteria (one implant was infected from *Serratia marcenses* and one implant from *Staphylococcus hominis ssp Hominis* and *Staphylococcus lugdunensis*).
There was no microorganism identified only in tissue samples.

Discussion

The quality of the implants used in orthopedics and trauma surgery and the surgical techniques and approach are improving clinical results and satisfaction of patients and surgeons. As a result, an increasing number of implant surgeries are being performed. Unfortunately, the increase in the surgical management is inevitably accompanied by an increasing incidence of peri-implant infections [1,2].
Diagnosis of orthopedic infections can be challenging and is always based on a combination of clinical, biological, and microbiological findings. However, when a chronic infection has

Table II. Isolation of microorganisms with MicroDTTect and tissue samples.

	Removed implant	Tissue sample results	MicroDTTect results
1	Total hip arthroplasty (THA)	Sterile	Sterile
2	THA	Sterile	Sterile
3	THA	*Staphylococcus hominis ssp Hominis, Staphylococcus lugdunensis*	*Staphylococcus hominis ssp Hominis, Staphylococcus lugdunensis*
4	Knee antibiotic spacer	*Proteus mirabilis*	*Proteus mirabilis, Enterococcus faecium*
5	Total knee arthroplasty (TKA)	Sterile	Sterile
6	Ulnar plate	Sterile	Sterile
7	TKA	Sterile	Sterile
8	THA	Sterile	Sterile
9	THA	Sterile	*Escherichia coli*
10	TKA	*Serratia marcenses*	*Serratia marcenses*
11	Hip antibiotic spacer	Sterile	Sterile
12	Shoulder antibiotic spacer	Sterile	Sterile
13	Total shoulder arthroplasty (TSA)	Sterile	*Staphylococcus hominis ssp Hominis*

to be treated, the clinical signs and laboratory results might be dubious, especially when recent empiric antibiotic therapy has been administered leading to false negative results.

Biofilm formation in chronic infections is considered as a major cause of the insufficient sensitivity of classical culture approaches using tissue samples. Sonication has shown to notably increase the sensitivity of microorganism identification by detachment of bacteria biofilm covering the orthopedic implant, and many Authors documented the superiority of sonication in comparison with tissue culture, with a lower sensitivity for the latter one (ranging from 61 to 76%) with respect to sonicated implants (77-95%) [17].

Previous studies [13,14,16] have shown that a chemical agent, namely DTT, is a reliable alternative to sonication for microbiological diagnosis of orthopedic infections and may be even more sensitive than sonication towards *S. epidermidis*, which is often involved in peri-implant infections.

As sonication devices are not available in all laboratories, it was decided to undertake this study with the aim of testing the efficacy and reliability of a commercial device that takes advantage of the properties of DTT to disrupt bacterial biofilm, called MicroDTTect.

Other authors have investigated the properties of MicroDTTect in daily clinical practice. Calori et al. [16], for example, reported a higher sensitivity of MicroDTTect in analyzing both prosthetic and osteosynthetic devices compared to a control group. However, the control group, was composed of samples collected with flocked swabs, which are not considered a suggested method for periprosthetic infections [18,19].

Kolenda et al. [19] described a higher sensitivity of MicroDTTect compared with tissue sample cultures, but in their study only prosthetic joint infections were investigated.

In our study, periprosthetic, periosteosynthetic device, and pericement spacer infections were investigated when there was failure and loosening of the implant. As sustained by Kolenda et al. [20] and Sambri et al. [14] in their studies, low-grade and chronic infections can be difficult to detect and DTT treatment and MicroDTTect may be useful especially in cases when peri-implant infection is not suspected preoperatively.

Although with a low level of evidence, our data confirms the results of Calori [16] and Kolenda [20]. MicroDTTect had greater sensitivity compared to conventional culture of tissue samples. In two cases, in fact, there was a positive result (for *Escherichia coli* and *Staphylococcus hominis ssp Hominis* respectively) with MicroDTTect, while negative with traditional culture of tissue sample. In one patient, MicroDTTect allowed us to detect a polymicrobial infection due to *Proteus mirabilis* and *Enterococcus faecium*, while only *Proteus mirabilis* was isolated with conventional tissue culture (Tab. II).

MicroDTTect is a quick and easy device to use, with minimal manipulation needed and with a closed system that guarantees safe and sterile transportation of the material to the laboratory, thus reducing the risk of bacterial contamination.

In addition, Romanò et al. [17] also suggested that the use of the MicroDTTect device may be cost-effective. In fact, although the immediate direct costs are increased, the extra costs generated by diagnostic inaccuracy of traditional tissue culture may increase indirect costs due to time required for sample treatment, useless or unnecessary medical treatments, and possible medical claims. As the Author reported in his manuscript, for every 100 patients treated each year the total cost of a wrong diagnosis with a tissue culture would amount to € 1.6 mln; with sonication the total potential costs would be reduced to respectively € 1.4 mln and with MicroDTTect to € 0.7 mln [17].

The limitations of the study are the small number of the cases analyzed. Thus, studies on a larger population are needed as well as a clinical trial to validate the use of the device.

Conclusions

In conclusion, the use of MicroDTTect device in implant loosening management improved microbiological diagnosis with

more sensitive results by allowing identification of additional bacteria compared to traditional culture.
High morbidity rates and high healthcare costs are related to chronic infections, and more accurate and quicker identification of the pathogen may increase the appropriateness and efficacy of antibiotic therapy. This could and reduce the severity of sequelae, recovery time, and costs of treatment while improving patient's quality of life.

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Conflict of interest statement
None of the Authors has any financial and personal relationships with other people or organizations that might pose a conflict of interest in connection with the submitted article.

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Authors' contributions
AG, JR, AF, MDM: have made substantial contributions to the conception and design of the work; AG, JR: have been involved in acquisition of data and drafting the manuscript; MB, FF: have contributed with analysis and interpretation of data; FF, VC: revised the manuscript critically and have given final approval of the version to be published. All Authors read and approved the final manuscript.

Ethical consideration
All patients were treated according to the ethical standards of the World Medical Association's Declaration of Helsinki and were invited to read, understand and sign the dedicated informed consent form.

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