Short Communication

Sonication of stainless steel wire as a tool for microbiological diagnosis of diabetic foot infection

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Abbreviations

KwM: Kirscnher wire method.

CM: Conventional method.

Abstract. We hypothesized that biofilm production occurs on stainless steel when incubated with tissue specimens in thioglycolate broth media (TBM). In a diabetic foot infection (DFI) cohort, applying the Kirschner wire and conventional methods were more sensitive than applying only the latter (CI 90%; 0.167 *versus* 0.375).

Keywords: Diabetic foot infection; Sonication Method; Microbiological diagnosis

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Increasing evidence presented in the literature confirms that biofilms are ubiquitous in diabetic foot ulcers (DFUs) and suggests that they participate in delayed wound healing (Gomes et al., 2017; Johani et al., 2017; Versey et al., 2021). DFI is the most common, severe, and costly complication of diabetes mellitus, with a high risk of mortality and morbidity. Clinically, biopsy tissues are the most reliable samples for revealing a biofilm-producing microorganism in deep tissues, but presently, there is no clear evidence on how to diagnose biofilm-related wound infection (Lipsky et al., 2020). In addition, the culture yield can be harmed in the presence of a low quantity of planktonic and sessile pathogens in tissue due to the competitiveness among the microorganisms,

which makes one or more species prevail, or by using ATM (Hibbing et al., 2010; Young et al., 2017). Therefore, it is not enough to construct an accurate diagnostic method but to know how to interpret it in light of the circumstances and about pathogens' behavior in their sessile forms.

Given the negative impact of biofilms, biofilm disruption may be crucial to improving microbiological outcomes since biofilms can identify sessile pathogenic microorganisms (Kalan and Brennan, 2019; Pouget et al., 2020; Trampuz et al., 2007). However, to date, there is no accurate microbiologic diagnostic method regarding biofilm disruption in DFI (Schultz et al., 2017). Here, we postulated that the sonication of a stainless steel wire after incubation with tissue samples into a TBM bottle could disrupt microorganisms, improving the microbiological diagnosis of DFI. A stainless steel wire was arbitrarily chosen for this investigation since the affinity for stainless steel is a hallmark of several microorganisms involved in DFI (Andrade et al., 1998; Bremer et al., 2002; Gomes et al., 2015; Jia et al., 2017; Malhotra et al., 2019; Selow et al., 2015; Tsikopoulos et al., 2021).

We conducted a prospective unicentric study involving DFI patients who underwent surgical debridement between April 2018 and September 2021 at a private tertiary hospital in Volta Redonda, Rio de Janeiro, Brazil. The inclusion criteria for this cohort were as follows: age ≥ 16; acute (erysipelas OR cellulitis OR necrotizing fasciitis OR gas gangrene); chronic DFI defined according to IWGDF 2019 guidelines (Lipsky et al., 2020); and > 1 CFU/ml of any microorganism on sonication. In addition, we excluded participants hospitalized before six months from discharge, patients harmed by any inaccurate application of both methods, and, if apparent, individuals who had their samples contaminated in the operating room.

The vascular surgeon operated on all cases. Soft tissue or bone fragments were collected and inoculated into three TBM bottles for the execution of the conventional method (CM). Other specimens with a sterile and smooth stainless steel Kirschner wire (IOL- Implantes LTDA; 1.0-2.0 x 50.0 mm) were inoculated into three other TBMs to perform the K-wire method (KwM). Of note, collecting multiple samples to improve culture yield is not recommended by the International Working Group on the Diabetic Foot (IWGF) 2019 guidelines (Lipsky et al., 2020).

The TBM harboring a K-wire was sonicated as soon as they arrived at the laboratory earlier in an ultrasonic bath using a Soniclean (Sanders Medical) at a frequency of 40 kHz at 35°C inside a rigid container made of polypropylene. First, the KwM bottles were vortexed for 30 seconds, sonicated for 1 minute, and then vortexed again. This sequence befell as soon as they arrived at the laboratory. At the end of the first 24 hours and the 5th and 10th days of incubation (at 35°C), the microbiologist sowed the tissue fragments of CM and the sonicated fluid (after vortexing and sonicating as described earlier) in aerobic horse-blood agar plates (BD® Columbia Agar with 5% Horse Blood), utilizing a laminar flow

hood. The attached infographic explains the KwM (**Figure 1**). The VITEK 2 system identified the isolates according to international standards and the definitions of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) ("The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters, version 10.0, 2020," n.d.).

Overall, 31 patients were enrolled. The mean age of the patients was 60.5 ± 13.2 years, with 51.63% and 48.4% having PEDIS (having perfusion, extent, depth, infection, and sensation) severity scores of 3 and 4, respectively. There was no significant difference in the number of pathogens between the methods (**Table 1**). However, a significant statistical result was observed in sensitivity between CM and CM plus KwM **(Table 2)**. The CM detected an additional pathogen in 3 cases. KwM identified an extra nine pathogens in 6 cases (**Figure 1** and **Table S1**). A subanalysis of the patients who took antibiotics (n = 23) was also significant regarding the number of identified pathogens (**Table 3).**

Foremost, it is essential to note that the microorganisms isolated in this research are under previous publications (Ghotaslou et al., 2018; Kwon and Armstrong, 2018; Lipsky et al., 2012; Macdonald et al., 2021; Noor et al., 2017). Concerning the aim of the study, all nine different bacteria isolated in KwM have already been identified in sonicate fluid and stainless steel (Holinka et al., 2011; Piper et al., 2009; Shen et al., 2015; Trampuz et al., 2007). Furthermore, our result concerning the previous use of ATMs follows the findings of several studies regarding implant-associated infections (Holinka et al., 2011; Piper et al., 2009; Shen et al., 2015; Trampuz et al., 2007).

Presently, a biomaterial with no bacterial attachment is unknown. However, it is impossible to state biofilms' presence in the K-wire since no microscopic technique was used since the study was conducted in the hospital's microbiology laboratory, a sector designed to handle routine tests. There are additional limitations to point out: (1) We also sonicated and vortexed the tissue fragments contained in the bottles (with the wire), which is not a validated approach for microbiological diagnosis. *S. aureus*, for example, can evade the immune system surviving in osteoblasts, reduce osteoblast activity, promote necrosis (Wen et al., 2020), and risk missing a considerable count of viable bacteria. Furthermore, vortexing tissues can lead to mechanical shearing of the DNA. Hence, sonicating and vortexing tissue specimens may kill microorganisms and harm the culture yield. (2) Even when using a laminar flow hood, the repeated exposure of opened TBM bottles to the environment in KwM may increase the chance of contamination of the contents. (3) We did not consider *Staphylococcus hominis* as a contaminant, despite its growth in a single TBM bottle in CM. (4) Due to the lack of required materials, anaerobic culture has not been performed. (5) The class and the time course of the drugs were not considered for this preliminary analysis.

Applying CM and KwM may be a useful microbiologic diagnostic tool to improve the microbiological diagnosis of DFI. Furthermore, KwM also detected more pathogens in those who utilized ATMs in the last three months. Thus, despite the small cohort analyzed, it is feasible to state that our results can produce a shred of solid evidence.

Data Availability Statement: Data available on https://figshare.com/ (10.6084/m9.figshare.14762559).

Competing interests: The authors declare that they have no conflicts of interest.

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Institutional Review Board Statement: Ethical review and approval were waived for this study for the following reasons:

1. because it is an observational and prospective study that will employ mainly information from medical records, institutional information systems, and other sources of data and clinical information available in the institution,

2. because all data were carried out and analyzed anonymously,

3. because the results resulting from the study will be presented in aggregate form, not allowing the individual identification of the participants, and

4. because it is a noninterventional study and without alterations or influences in the routine and treatment of the research participant, and consequently without adding risks or damage to their well-being.

Informed Consent Statement: Ethical review and written informed consent approval were waived for this study. The reasons are explained in the "**Institutional Review Board Statement.**"

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Table 1. The number of pathogens (CM x KwM).

Variables	n	Min	Max	Md	Media	SD	p
Pathogens (CM)	31	0	4	1	1.65	0.88	0.152
Pathogens (KwM)	31	0	4	2	1.84	0.97	

CM (conventional method); KwM (K-wire method). n (number); Min (minimum); Max (maximum); Md (median); SD (standard deviation) P value obtained via Wilcoxon's test

Table 2. Analysis of CM *versus* KwM (white) and CM *versus* CM/KwM (gray).

		KwM		p	CM/KwM		p
		No	Yes		No	Yes	
CM	No	23	5	0.450¶	23	5	0.074*¶
	Yes	2	1		0	3	
Sen	Spe	NPV	PPV	Sen	Spe	NPV	PPV
0.167	0.920	0.333	0.821	0.375	1.000	1.000	0.821

CM (conventional method); KwM (K-wire method) Sen (sensitivity); Spe (specificity); NPV (negative predictive value); PPV (positive predictive value) * P value obtained via McNemar's test ¶ P value obtained via McNemar's test; significant results: * (10%), ** (5%), *** (1%).

Table 3. The number of pathogens in individuals with previous ATM intake*.

Variables	n	Min	Max	Md	Media	DP	p
Pathogens (CM)	23	1	3	1	1.52	0.67	0.037**
Pathogens (KwM)	23	1	3	2	1.74	0.75	

ATM (antimicrobial) *Last three months. n (number); Min (minimum); Max (maximum); Md (median); SD (standard deviation) P value obtained via Wilcoxon's test; significant results: * (10%), ** (5%), *** (1%).
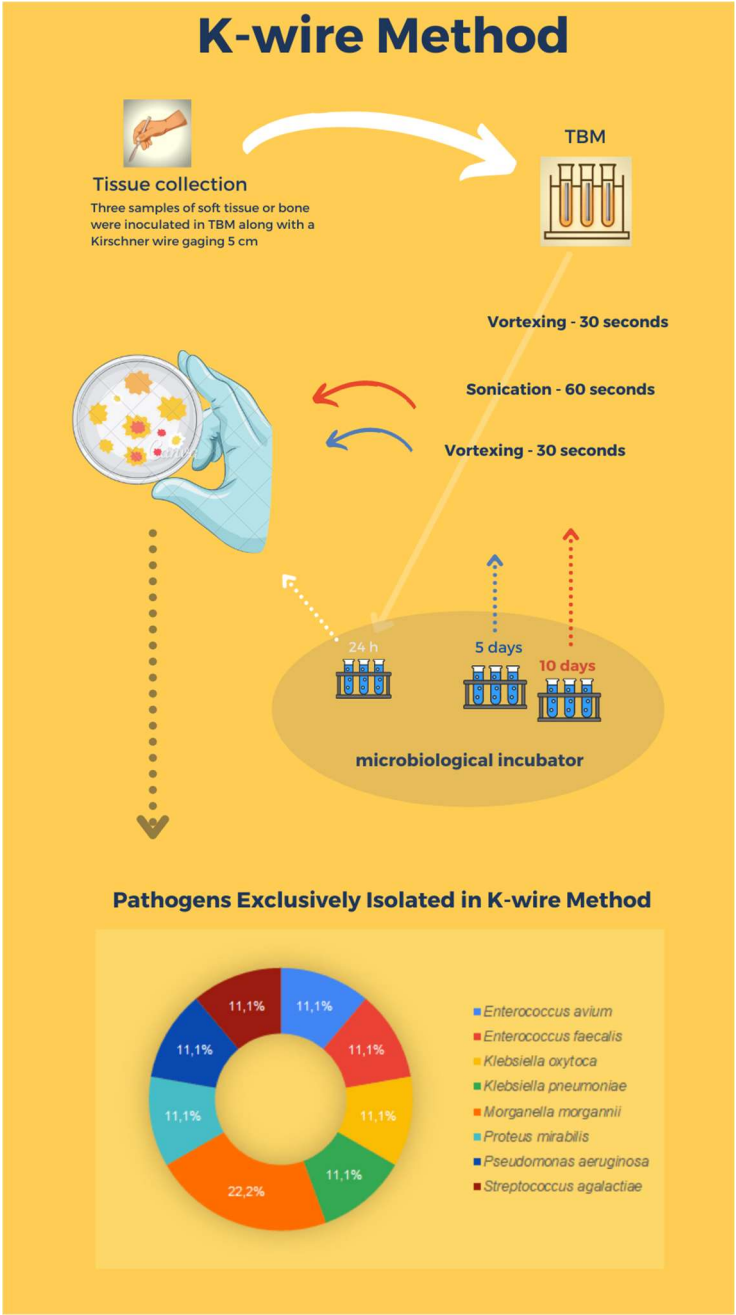
Figure 1. Infographic of KwM.