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Research Article

# Sensitization of Biofilms to Antibiotics Via Low-Frequency Alternating Magnetic Fields for Safer Implant Therapies

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## Abstract

**Introduction:** An increasing proportion of surgically implanted knee and hip joints become infected with bacterial biofilms, which are notoriously resistant to antibiotic therapies. Despite the use of aggressive intravenous antibiotic treatment, revision surgery is required in many cases to remove the infected prosthetic and treat the life-threatening infection. Such revision surgery is physically demanding for the surgeon, is expensive, and can result in significant tissue loss, necessitating prolonged patient recuperation and recovery.

**Methods:** In this article, we report our progress on the development of a coil-based AC Electromagnetic Field (EMF) device that produces a controlled Alternating Magnetic Field (AMF) to enhance antibiotic efficacy against biofilms. To optimize treatment, we sought to establish whether lower-frequency EMF-generated AMFs, which reduce unwanted tissue heating, could improve biofilm susceptibility to antibiotics. Biofilms were grown on various solid substrates to model surgical implants. After exposure to AMFs and antibiotic treatment, the number of bacterial cells in the biofilms were enumerated by Colony-Forming Units (CFU) or optical density.

**Results:** Two AMF generators were designed to operate between 30 and 60 kHz and assembled. *Staphylococcus aureus* (MRSA M2) biofilms propagated on various solid substrates were subjected to AMF which heats the substrates by induction via eddy currents. A 30-second increase in temperature to 45 °C was sufficient to increase bacterial sensitivity to vancomycin and other antibiotics. CFU decreased by up to 4 $\log_{10}$ following combined AMF and vancomycin treatment.

**Conclusions:** This EMF-based low-frequency AMF modulation strategy shows promise as a basis for developing non-invasive treatment of biofilm infections and may serve as a prophylactic measure following surgical implantations.

**Keywords:** Antibiotic resistance; Biofilm infection; Electromagnetic field; Surgical revisions

**Abbreviations:** AMF: Alternating Magnetic Field; °C: Degrees in Celsius; CFU: Colony-Forming Units; DNA: Deoxynucleic Acid; EMF: Electromagnetic Field; kHz: Kilohertz; kW: Kilowatt; MOSFET: Metal-Oxide Semiconductor Field-Effect Transistor; MRSA: Methicillin-Resistant *Staphylococcus aureus;* OR: Operating Room; PBS: Phosphate-Buffered Saline; THA: Total Hip Arthroplasty; TKA: Total Knee Arthroplasty

## Introduction

The development of novel technologies for major joint repair, such as robotic assistance and better adhesion materials, has greatly improved the quality of life for many patients with chronic pain and/or mobility problems [1,2]. In the US, the numbers of hip and knee replacements exceed 500,000 and 790,000 per year [1]. Globally, these numbers are on the order of 1M hip and 3.6M knee replacements. Hip and knee replacement failures can be caused by mechanical failures, dislocation, instability, allergy to metals, and infection [3-5]. Hip and knee revision surgery is a complex procedure performed to replace a joint that has become damaged, infected, or otherwise failed [6,7]. Revision surgeries can be time-consuming and expensive as they require high degrees of effort to remove the infected implant, which is often attached by bone cement [8,9]. During the removal process, significant bone and soft tissue loss can occur as a result of the removal of infected material and physical trauma. The incidence of PJIs (peri-prosthetic joint infection) following primary THA and TKA ranges from 0.5% to 2.4% [10,11]. In revision surgeries, this rate can increase up to 20%, with PJIs accounting for approximately 21.4% of THA and 33.1% of TKA revision cases [12,13]. As of 2020, PJIs incurred costs exceeding $1.62 billion, with individual case management averaging $90,000 [12,13]. Efforts to treat infected implants with intravenous antibiotics are often unsuccessful due to the nature of biofilm in the infection [14,15]. A bacterial biofilm is a structured community of live and dead bacterial cells that adhere to the implant surface and to each other, forming a protective matrix that limits antibiotic penetration and activity. Within the biofilm, bacteria can exchange antibiotic resistance genes – sometimes regulated by quoarum sensing – further enhancing their survival [16-21].

Initial efforts to treat infected implants involve aggressive antibiotic treatment and/or irrigation of the infected joint with saline and antibiotics. Often, the biofilm nature of the infection resists these types of treatments, even when the dose of antibiotic is increased to one thousand times the minimum inhibitory concentration [15,20]. As a last resort, removal of the infected joint during revision surgery may be required [7,15,22-27]. Revision surgery for hip or knee implants involves dislocating the joint and using various specialized instruments to remove the prosthesis and any bone cement, often resulting in unavoidable damage to bone and surrounding soft tissue [7]. Tools such as osteotomes, saws, implant-specific extraction devices, drills, reamers, and ultrasonic cement removal tools are employed to separate the implant from the remaining bone, bone cement, and/or other fixation material. This forceful and tedious process can lead to bone erosion, osteolysis, and trauma to muscles, tendons, and ligaments, which weaken joint stability and complicate re-implantation [28,29]. The complexity of the procedure, which can last from 90 minutes to over 8 hours, increases surgical costs and risk factors. Complications in revision surgeries are more frequent than in primary surgeries, occurring in 3.0-11.2% of cases [30]. Patients may experience chronic pain, reduced mobility, bruising, scar tissue formation, prolonged recovery, and psychological and financial burdens, further exacerbating long-term health challenges [9,31].

Emerging approaches for treating these biofilms, such as Alternating Magnetic Fields (AMF), offer a promising non-invasive strategy by inducing eddy currents in metal implants to generate heat, effectively disrupting biofilms. However, conventional AMF techniques require heating implants to 50–80°C, risking tissue damage and uneven heating due to the skin effect at high frequencies [32,33]. Our proposed solution aims to overcome these limitations by utilizing an AC Electromagnetic Field (EMF) device that produces lower-frequency AMF in the range of 50 kHz to achieve more uniform heating while maintaining a safer target temperature of 45°C, potentially preventing tissue damage entirely. By selectively heating only the implant surface to 45°C and allowing heat to dissipate before subsequent pulses, we propose a cyclic heating strategy that gradually increases the entire implant surface temperature to 45°C without exceeding thresholds for tissue injury. Lower frequencies allow for deeper penetration of eddy currents, reducing surface overheating at ends and stress points and enables faster, more even heat distribution. This controlled, non-invasive approach has the potential to serve as both a treatment and a preventative measure as tissue damage may be reduced, improving patient outcomes while avoiding the risks associated with conventional surgical interventions. Thus, it is essential to verify that lower frequency AMF can enhance antibiotic susceptibility at lower temperatures.

In this article, we describe the design and use of an electrical coil device that increases the temperature of a metallic implant. The alternating flow of electricity creates a low-frequency alternating electromagnetic field that raises the temperature of various metals through induction heating. We show that bacteria grown on solid substrates as biofilms are relatively insensitive to vancomycin and other antibiotics. However, when the surface temperature of the solid substrate is increased to greater than 45 °C using low

frequencies, the bacteria become significantly more sensitive to antibiotic treatment.

## Materials and Methods

### Bacterial Cultures

MRSA M2 [34,35] was propagated in TSB-II broth from frozen stock cultures. Coupons were placed in large, deep petri dishes (~20 cm in diameter). Each coupon (several per dish) was submerged in 100 mL of TSB-II broth containing M2 Methicillin-Resistant *Staphylococcus aureus* (MRSA) at a concentration of $1 \times 10^7$ CFU/mL, ensuring a liquid depth of approximately 1 cm. The samples were incubated at 37°C for various times to allow various degrees of bacterial attachment or biofilm formation. Following incubation, each coupon was removed, washed for the indicated number of times in Phosphate-Buffered Saline (PBS) via inversion in a 50 ml tube, and transferred to a new petri dish containing fresh TSB-II broth. Coupons were then incubated at 37°C overnight (~16 hours). After incubation, the coupons were removed, and washed again in PBS, and bacterial cells were recovered by scraping the coupon surface with a sterile cell scraper (biofilm) or from the supernatant (planktonic progeny). The scraper was then rinsed/agitated in 3 mL of TSB-II to collect detached bacteria. Serial dilutions of the bacterial suspension were prepared and plated onto Tryptic Soy Agar (TSA) for enumeration. Where indicated, antibiotics were added at specified concentrations during Alternating Magnetic Field (AMF) treatment and subsequent culturing.

### Antibiotics

For bacterial susceptibility testing the indicated antibiotics were obtained from the following sources and calibrated to be within an effective dose range that was biologically relevant under the indicated culture conditions.

Multiple antibiotics were chosen; Ampicillin (Fisher Scientific 50-213-247), Vancomycin (Millipore-Sigma PHR1732-4X250MG), Cefepime (Thermo Scientific, J66237.03), Kanamycin (Fisher Scientific BP906-5), Tobramycin (Thermo scientific 455430050), Ertapenem (Erythromycin) (Fisher Scientific 50-997-767), Chloramphenicol (Fisher Scientific BP904-100), and Sulfamethoxazole (Sulfadimidine) (Fisher Scientific AAJ6656522).

### Coupons

The following surfaces were used to propagate biofilm and or investigate the physical properties of the magnetic fields. Silicone Rubber, Grade 5 (6AL-4V), Titanium Slide , and Polypropylene Slide Coupons from BiosurfaceTechnologies Corporation, Montana. 430 and carbon steel from Cut2SizeMetals, IN, USA.

### Measuring Elevation in Temperature with AMF Exposure

Titanium (Ti) coupons were placed in 20 mL of PBS or culture media within a 50 mL tube and allowed to equilibrate to room temperature (23˚C). AMF treatment was applied at specified conditions for varying durations. The temperature of the medium was monitored using a traditional mercury thermometer (following a lag period) and an Infrared (IR) laser thermometer (instantaneous measurement), with temperature readings confirmed by tactile assessment. During AMF exposure, tubes were positioned consistently inside the coil to maintain uniform field application. Temperature variation across repeated trials was less than 1˚C, ensuring consistency in experimental conditions

### Bone Cement

Simplex 2 bone cement (Stryker Corporation, USA) was compounded as per the manufacturer's instructions and applied by hand to 430 steel coupons of 19 x 19 x 2 mm dimensions. The resulting bone cement casing was 2 mm thick above the steel surface on average.

### Statistical Analysis

One-way ANOVA with Tukey's or two-way ANOVA with Sidak's multiple comparison tests were performed on CFU data taken at varying conditions to assess significance as shown in figure legends. Asterisks (*) are used to indicate p-values of data that are significantly different.

## Results

### Growth of Biofilm Bacterial Cultures

Small metal bacterial growth surfaces, termed "coupons" and measuring 19 x 25.4 x 2 mm composed of 430 stainless steel were used to culture biofilms. *Staphylococcus aureus* strain M2 was originally isolated from an infected human femur. M2 is a Methicillin-Resistant *Staphylococcus aureus* (MRSA) USA400 strain that produces a-toxin and the Sag SEC and can cause lethal sepsis and cardiac infections. Bacterial culture coupons composed of stainless steel 430 were submerged under varying culture media formulations in 100- 200 mm Petri dishes (demonstrated in Figure 1A). The cultures were inoculated with bacteria and incubated at 37 °C for 2 hours (Figure 1B ) or 6 hours (Figure 1C) and then cultured at 37 °C. Every two days, the plates were swirled and the liquid and planktonic bacteria were removed with a pipette and fresh media added. On the day of experiment with the AMF, the coupons were washed extensively with sterile PBS to remove planktonic forms and the biofilm forms were harvested using a small plastic scraper. Bacteria were enumerated by serial dilution and plating on agar media. Coupons were also subject to the addition of 1mg per ml of vancomycin (Figure 1D). The coupons were washed at room temperature and vancomycin was added to fresh media. After 16 hours of further growth at 37°C, the bacteria were removed by scraping and enumerated. These results are consistent with previous data showing adherence of MRSA M2 to coupons and forming stable biofilm surfaces and that 1 mg per ml of vancomycin is minimally effective.

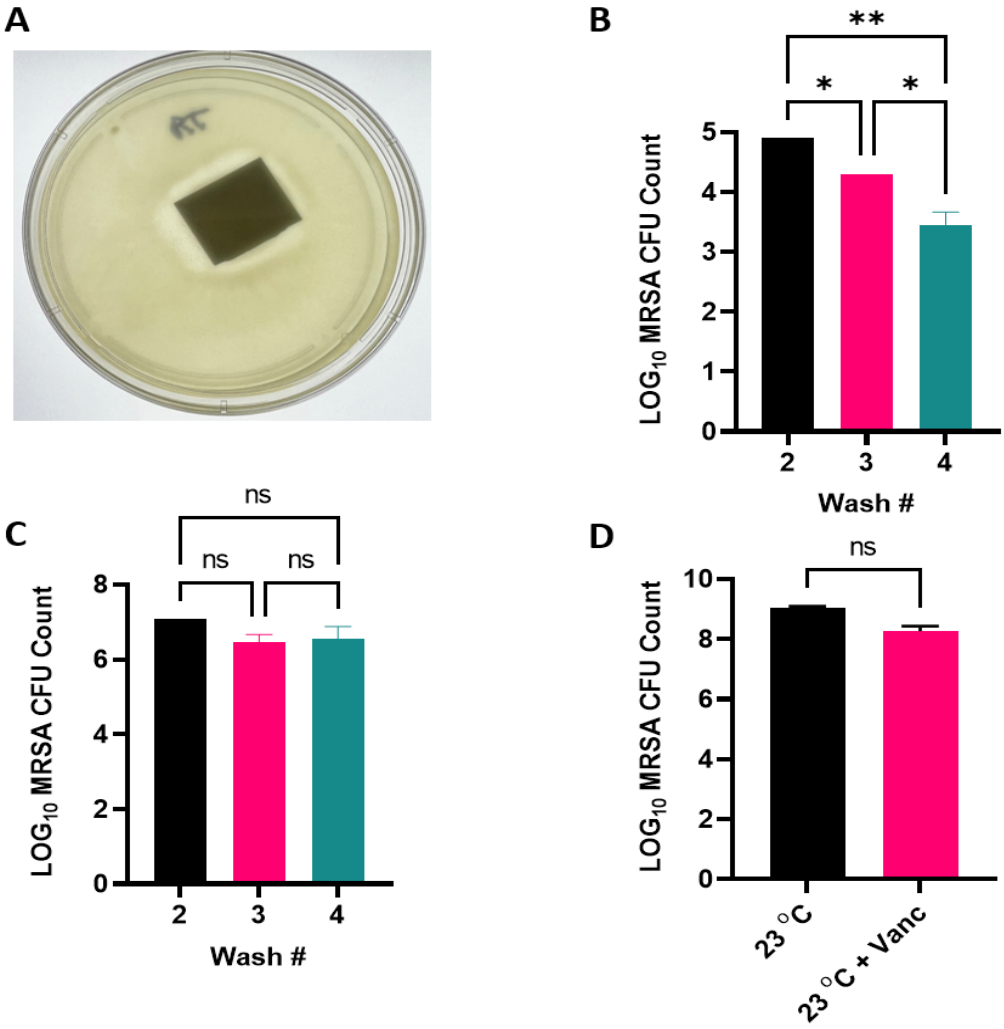


**Figure 1:** MRSA attaches to metal coupons and forms biofilms. **A)** A *Staph. aureus* M2 (MRSA) culture after 1 week in TSB broth with media replaced every 2 days, washed at room temperature and replated overnight. **B)** A histogram of MRSA CFU on metal coupon after 2 and 6 hours with washing in PBS. **C)** MRSA CFU per coupon following 6 hours of attachment with washing. **D)** Culture as in A, washed at 23 °C and incubated with 1 mg/ml vancomycin- biofilm was harvested and enumerated. One-way ANOVA with Tukey's multiple comparison test was used to determine significance of difference between CFU counts from various groups. * indicates $p < 0.05$, ** $p < 0.01$, n.s. = not significantly different.

**Construction of an AMF-Generating Electric Coil**

MRSA biofilms are known to form on implants (Figure 2A) [15,20] For the construction of an electrical device to generate an AMF, we purchased a 1000W ZVS low voltage induction heating board module flyback driver heater 12V-48V designed for heat treatments and jewelry working (Cuifati, China). We then modified it by replacing the MOSFET, adding a liquid cooling system, and a DC power supply. This is referred to as System 1. Coils of varying number of turns and diameters were assessed for performance. A three-turn coil of approximately 10 cm diameter was selected for use with stainless steel coupons. System 2 is a more integrated system and was purchased as a single unit. It is a 15kw 30-80kHz 220V system with an integrated liquid cooling system made by US Solid, Inc. and

is designed primarily for welding. Figure 2B presents a cartoon of an AMF system in which a power control module provides precise metering of electricity which flows through a copper coil. The number of coil turns and diameter can be varied to adjust the induction heating of a metal device within the coil. Figure 2C shows theoretical differences in heating characteristics of a high frequency AMF field operating at ~500 kHz versus a low-frequency AMF field at 1-80 kHz. The high frequency system (middle panel) produces rapid heating which is increased as the diameter of the implant decreases. The lower frequency system (right panel) produces more even heating of the surface of the implant with reduced temperature spikes at the ends of the implant. By increasing the amperage of the electricity flowing through the coil, the low-frequency AMF field can be adjusted to increase heating more rapidly depending upon the composition of the implant.

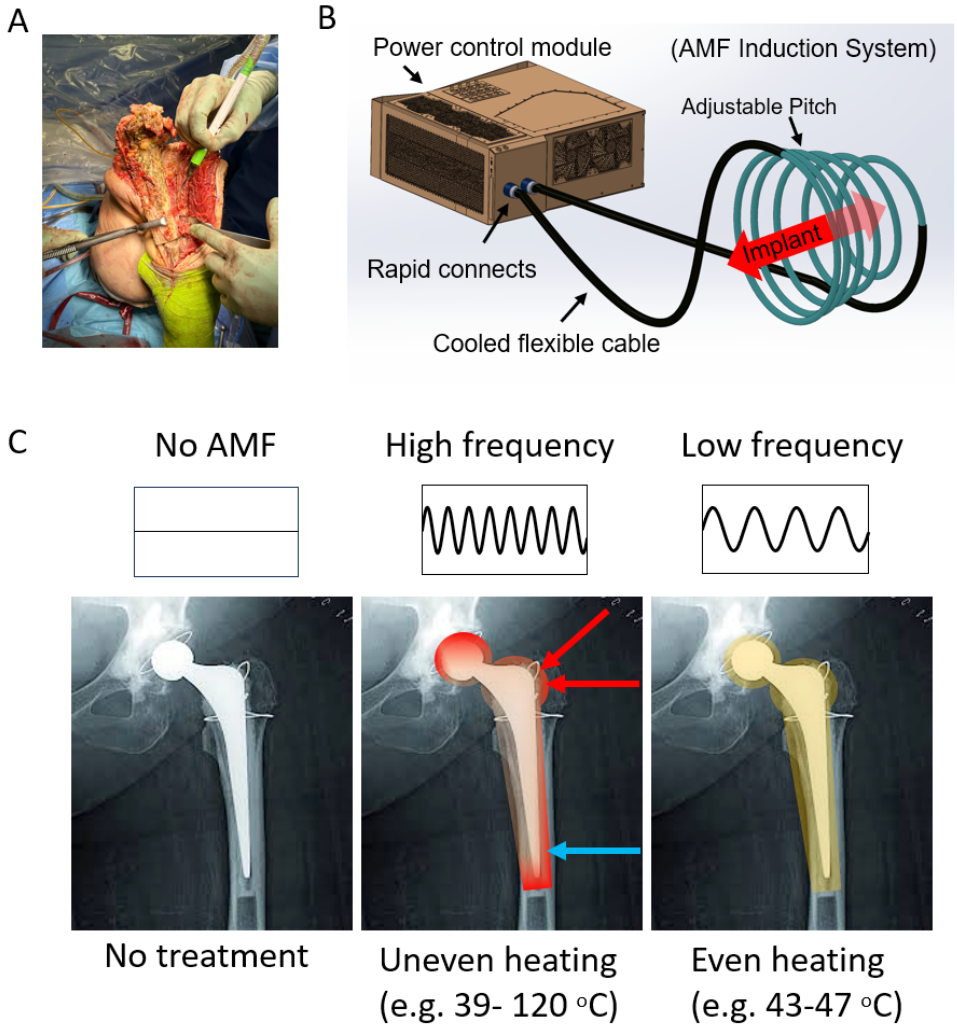


**Figure 2:** Revision of a necrotic implant. **B)** Alternating-Magnetic Field (AMF) system, further described in text. **C)** Artistic comparison of the effects of no AMF, a previously described high frequency 530 kHz AMF, and the newly developed ultra-low/low 1kHz-80kHz frequency which allows for uniform heating avoiding uneven heating on stress risers- Radiograph modified from Reilingh et al. [30], CC BY 4.0.

### Induction Heating Enhances Antibiotic Susceptibility

430 stainless steel coupons upon which MRSA biofilms had been grown were submerged in 20 mL PBS in a 50 mL tube with an integrated thermometer probing the temperature of the liquid at the surface of the metal coupon. AMF time was calibrated to temperature for each setup where heating was observed (Figure 3A for example). An infrared thermometer, mercury thermometer and tactile assessment were used during calibrations and IR thermometer and tactile assessment were used during MRSA experiments. Figure 3A shows a typical heating curve. As can be seen, heating was both gradual and linear with respect to time following a small lag. Figures 3B and 3C demonstrate the remarkable effect of the AMF on MRSA M2 colonies. Figure 3B shows planktonic progeny from the biofilm while Figure 3C shows the effect on the biofilm component of the same coupons. In both cases, modest heating by the AMF significantly increased MRSA to clinically relevant doses of antibiotics which is consistent with previously reported results [15,32]. The AMF needed for a 100-fold reduction in MRSA biofilm was consistent with a surface temperature of around 45 °C.

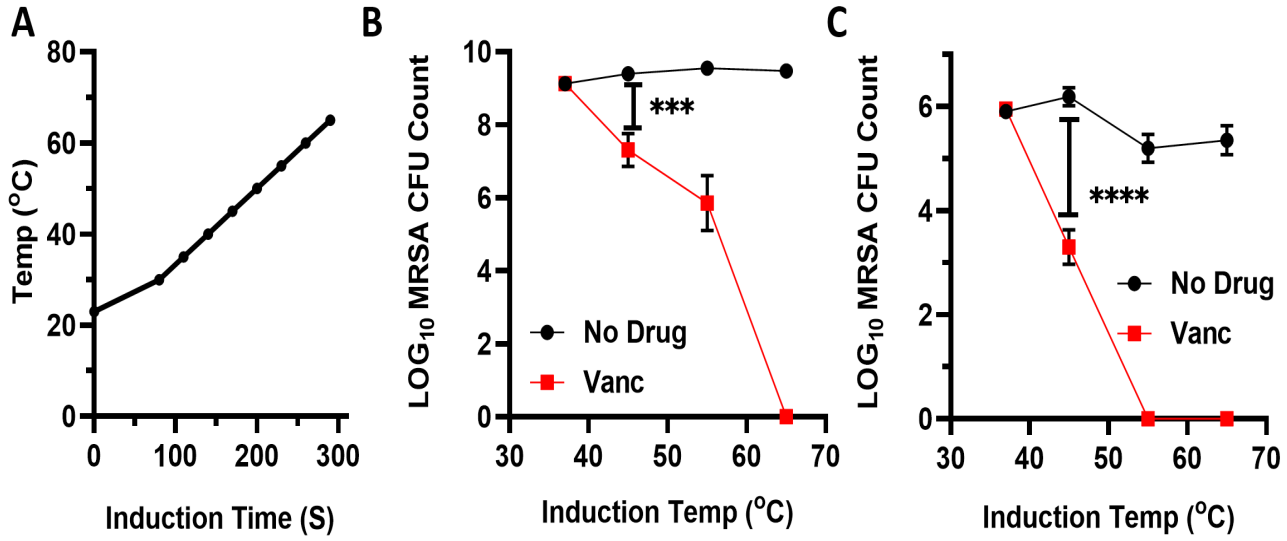


**Figure 3:** AMF renders MRSA biofilms more sensitive to antibiotics. **A)** Temperature over time graph for a 430-steel coupon in 20 ml of TBS broth subject to a standardized AMF field. **B)** Progeny planktonic bacteria from biofilms subject to AMF induction to the indicated temperatures with or without 1 mg/ml vancomycin. **C)** Biofilm survival following AMF induction with or without 1 mg/ml vancomycin. A two-way ANOVA with Sidak's multiple comparison test was done to determine whether CFU counts vary significantly at the 45 °C data points. *** $p < 0.001$; **** <0.0001.

### AMF-Induced Heating Appears Critical for Biofilm Reduction

It has been shown that AMF fields can alter quorum sensing [18,36]. It is therefore possible that the duration of the applied AMF, rather than the induced heating, enhances susceptibility through a pathway independent of heat. To assess this possibility, we performed similar studies on two alternative coupons, silicon rubber and Polypropylene. Figure 4A shows that different materials behave in a predictable way when entered into a magnetic field, namely that non-magnetic materials do not heat up when subjected to AMFs. We extended this principle to biofilm-coated magnetic and non-magnetic materials. In Figure 4B, we observed that the enhancement in susceptibility to vancomycin occurs only in materials that heat up readily in AMF fields. In the case of this study, it appears that the enhancement of vancomycin susceptibility directly correlates with heat. To test heat in the absence of AMF, coupons were placed at 65 °C in vancomycin which matched the temperature at the 300-second AMF time. The observed reduction in biofilm was comparable with heatable coupons in the presence of vancomycin but not those where heat was not generated, or in the absence of vancomycin (Figure 4C). In both instances, heat resulted in enhanced biofilm susceptibility but AMF alone did not. Together, these data indicate that AMF-induced heating is essential for biofilm reduction in these conditions.

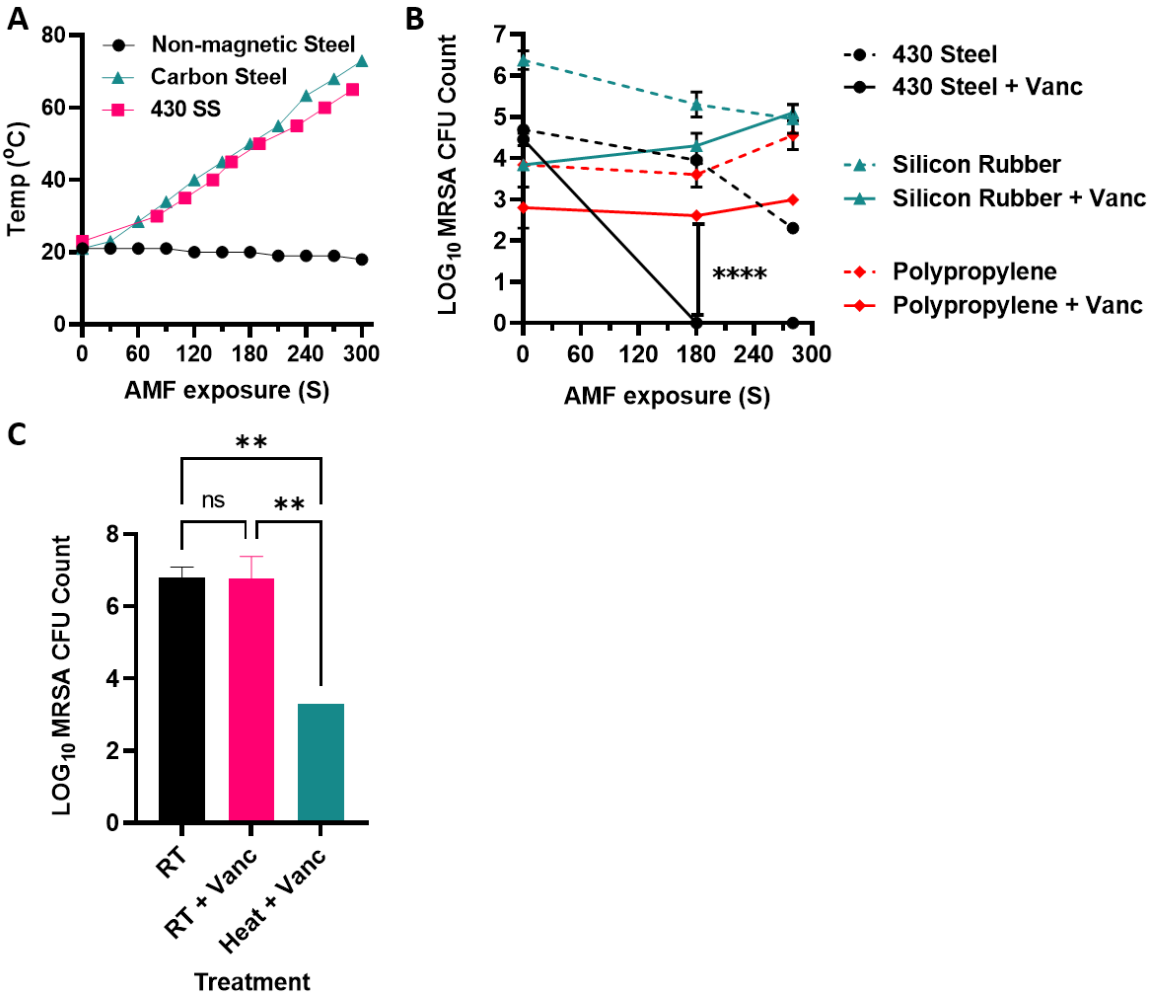


**Figure 4:** MRSA killing is dependent on the magnetic properties of the surface (likely heat). **A)** Temperature over time graph for magnetic and non-magnetic coupons in 20 ml of TBS subject to a standardized AMF field. **B)** Biofilm survival following AMF induction with or without 1 mg/ml vancomycin. **** $p < 0.0001$ significance determined by two-way ANOVA with Sidak's multiple comparison test. **C)** Biofilm CFU following coupon treatment with vancomycin or vancomycin and heat in the absence of AMF. ** $p < 0.01$ significance determined by one-way ANOVA with Tukey's multiple comparison test, n.s. = not significant difference.

**AMF-Enhances the Susceptibility of MRSA to Numerous Antibiotics**

A panel of antibiotics spanning multiple classes was tested to assess their efficacy against AMF-treated MRSA fields (Figure 5). The β-lactam class was represented by ampicillin, ertapenem and cefepime, which inhibit bacterial cell wall synthesis by binding to penicillin-binding proteins. Vancomycin was again included which is known to also disrupt cell wall synthesis through preventing peptidoglycan crosslinking. Aminoglycosides, which inhibit bacterial protein synthesis at the ribosome, were represented by kanamycin and tobramycin. Chloramphenicol inhibits the 50S ribosomal subunit, blocking peptide bond formation. Sulfamethoxazole, a sulfonamide, which inhibits folate synthesis by acting as a competitive antagonist to dihydropteroate synthase, thereby impairing bacterial nucleotide biosynthesis was also included. As in previous experiments, coupons were subject to AMF to increase their temperature to 45 °C. Most of the antibiotics tested exhibited some enhancement of inhibition as determined by measuring the absorbance at 600 nm of the 1-mL volumes of buffer into which the biofilm cultures were scraped from the coupons. The results presented here highlight the observed susceptibilities and resistances among the tested bacterial strains.

**A**

Table 1

Increased MRSA susceptibity to additional antibiotics

| | | OD600 | |
|---|---|---|---|
| **Antibiotic** | **Conc. (µg/mL)** | **- AMF** | **+ AMF** |
| Ampicillin | 100 | 1.12 | 0.76 |
| Eretapenum | 10 | 1.078 | 1.026 |
| Cefepime | 75 | 1.043 | 0.391 |
| Vancomycin | 1 | 0.96 | 0.361 |
| Kanamycin | 100 | 1.28 | 0.776 |
| Tobramycin | 50 | 0.538 | 0.035 |
| Chloramphenicol | 3.4 | 0.315 | 0.131 |
| Sulfamethoxacole | 1 | 1.116 | 0.844 |

**B**

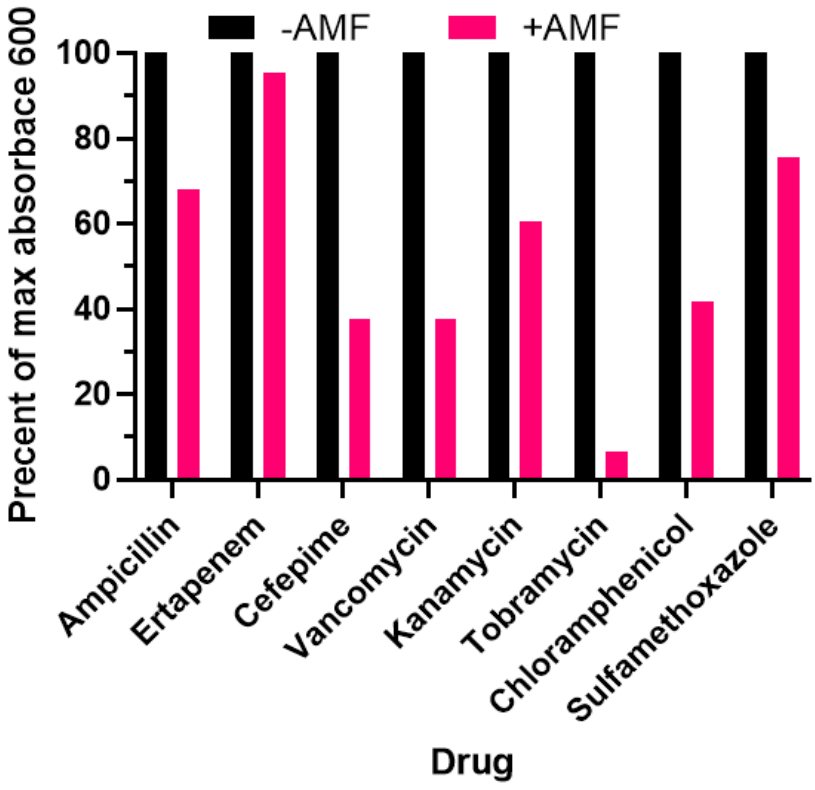


**Figure 5:** Enhanced inhibition of antibiotic classes on *S. aureus* M2 treated with AMF. **A)** Table of $OD_{600}$ of progeny planktonic bacteria from biofilm coupons with and without AMF induction to 45 °C, all in the presence of a semi-efficacious dose of the indicated antibiotic. **B)** A histogram showing the percent reduction in absorbance as a measure of bacterial growth.

### Growth on Bone Cement

To extend the practicality of the AMF treatment, MRSA M2 was grown on non-magnetic/non-inducible bone cement surrounding an inducible/magnetic core. This was designed to mimic the material that often surrounds an implant. As shown in Figures 6A and 6B, inducing the coupon to a temperature of 45 °C once again resulted in an enhancement of susceptibility to vancomycin. The CFU count was reduced approximately 1 log10 with AMF treatment alone and by over 4 log10 with combined AMF and 1 mg/mL vancomycin. This indicates that MRSA in regions proximal to an inducible core may benefit from induction.

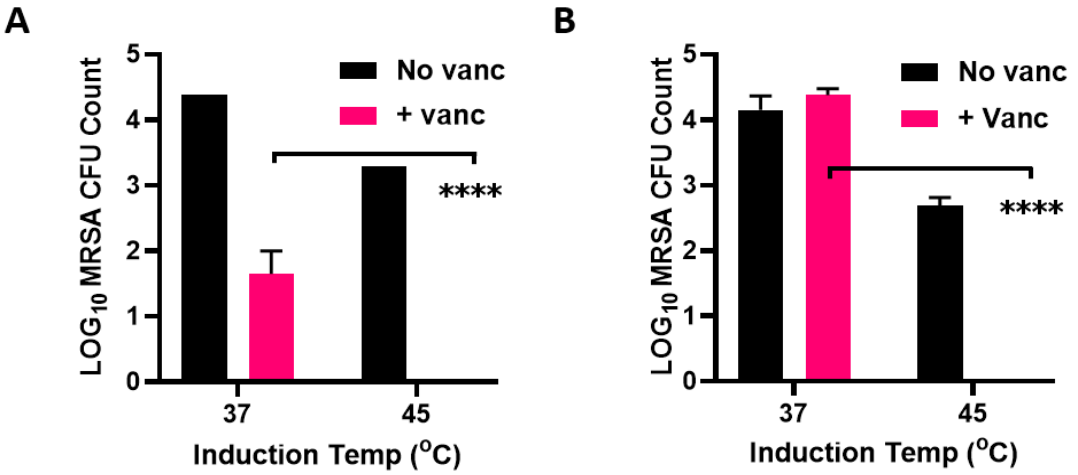


**Figure 6:** *Staph* reduction on proximal regions surrounding magnetic material. MRSA M2 was cultured on bone cement surrounding a magnetic coupon and subject to AMF and antibiotic treatment. A and B) Two independent experiments showing biofilm survival following AMF induction with or without 1 mg/ml vancomycin. **** $p < 0.0001$ significance as determined by two-way ANOVA with Sidak's multiple comparison test.

### Discussion

Biofilm-related Periprosthetic Joint Infections (PJIs) often require local antibiotic concentrations to be 100 to 1000 times above the Minimum Inhibitory Concentration (MIC) to achieve eradication, as systemic antibiotics alone are insufficient due to the persistence of metabolically inactive persister cells within the biofilm [7,15,37] To achieve this treatment, numerous local delivery mechanisms have been developed which provide high concentrations of drugs at the infection site with minimal serum concentrations [7,38-42]. In many cases, these types of aggressive antibiotic therapies fail to resolve the infection. For example, PMMA antibiotic spacers, commonly used in two-stage revisions, fail to fully penetrate biofilms and may contribute to persistent infections due to their non-bioabsorbable nature [37]. Because the biofilm nature of infections severely reduces the efficacy of antibiotic treatment, surgical intervention is often necessary. In revision surgery, the infected implant is removed, damaged tissues are trimmed, and a new joint is installed. This procedure is lengthy, requires advanced surgical skills, and is physically taxing for the surgeon. Often, patients suffer extensive bone and tissue loss, requiring extended rehabilitation to regain function.

Given the limitations of antibiotic-only treatments, we sought to develop a noninvasive or prophylactic procedure to reduce the incidence and severity of infected implants. Our primary observation is that exposing MRSA biofilm cultures to a low-frequency AC Electromagnetic Field (EMF), also known as an Alternating Magnetic Field (AMF) increases their sensitivity to antibiotics when the temperature of the implant is raised from 37°C to 45°C. However, heating alone (below ~70°C) does not significantly reduce bacterial growth unless combined with antibiotic pressure. This suggests that temperature-induced changes in biofilm structures enhance antibiotic penetration and effectiveness at temperatures that do not cause tissue damage. We believe this AMF-induced biofilm disruption will be a critical feature in prophylactic treatments, is more effective than standard antibiotic therapies, and avoids complex revision surgeries.

While the exact mechanisms underlying this enhanced antibiotic sensitivity are still under investigation, we hypothesize that temperature changes may physically soften the biofilm, reduce bacterial attachment, and enhance diffusion of molecules through the matrix. Additionally, bacterial heat-shock gene activation may lead to increased membrane permeability, further improving

antibiotic efficacy. Quorum sensing modulation could also alter gene expression which could lead to increased sensitivity to antibiotics. Notably, even drugs within the same class may be affected differently, depending on their solubility, import mechanisms, and other biological determinants, as shown in Table 1. Furthermore, AMF-induced cellular responses may trigger counteractive effects, selectively enhancing or suppressing the action of different antibiotics.

Induction heating of the surgical implant could improve antibiotic efficacy and patient outcomes, but AMF performance is highly dependent on frequency selection. High-frequency fields, such as those generated at 200 to 500 kHz, exhibit lower penetration depth, greater field decay, and more pronounced skin-effect heating, whereas low-frequency fields penetrate deeper and provide more uniform heating. In metallic implants, low-frequency AMFs can penetrate up to 5 mm, ensuring even temperature distribution and mitigating the localized heating inconsistencies seen with high-frequency fields. In some instances, features on some implants are calculated to heat up thousands of times more than the rest of the surface when a high-frequency EMF is used- rendering surface treatment with higher frequencies more difficult to control.

For clinical application, an AMF system must be customizable to different implant types and sizes. The system should be deployable in an operating room setting at the time of implantation to mitigate biofilm formation, which can occur within minutes post-surgery. Additionally, because the patient will already be administered antibiotics, AMF treatment can increase antimicrobial efficacy. To ensure compatibility across multiple anatomical sites (knees, hips, ankles, wrists, etc.), a flexible, insulated conductor with liquid cooling appears to be optimal over rigid copper coils. Furthermore, the AMF generator must allow for flexible field strengths and adjustable applicators to accommodate various appendage sizes and implant compositions. The field must be strong enough to reach the implant without excessive decay, while also being precisely controlled to prevent overheating and tissue damage. Finally, the applicator must be sterilizable for use in an operating room setting.

Since an initial report by Costerton et al. in 1994 [18], recent interest in the use of electrical or electromagnetic fields to treat biofilm infections have increased and many promising studies have been published. Studies published in 2017 and 2020 by Pijls et al. demonstrated greater than a 6 $Log_{10}$ reduction in biofilm infection of various bacteria after heating for 3.5 minutes at 60°C. [43,44]. Enrique et al., report the development of a compact induction heating system and demonstrated reduction in *Staph spp.* biofilm of between 0.37 and 1.2 $Log_{10}$ after heating to 70 °C for 3.5 minutes [45]. The group published a follow-up showing bacterial load reduction of between 0.5 and 1.9 $log_{10}$ in infected screws implanted into rabbits [46]. None of the treated femora showed more than a 650 mm wide necrosis margin, suggesting that this could be a relatively safe treatment in humans. In studies that use a relatively high frequency (>500 kHz) AMF system, Shaikh et al., showed that intermittent heating to 65 °C in combination with antibiotic treatment resulted in an up to 1.8 $log_{10}$ reduction in P. aeruginosa biofilm on an implant while heating to 75 °C resulted in a >2.5 $log_{10}$ reduction [47]. Even at these relatively high temperatures, only minor tissue damage was observed [33].

In this publication, we have reported biofilm reductions similar to or greater than those reviewed briefly above using brief heating to 45°C in conjunction with antibiotic treatment. We hypothesize that the low-frequency (50 kHz and below) AMF may be more effective and cause reduced tissue damage. If the AMF systems are to be used prophylactically to prevent biofilm infections and not only to treat ongoing infections, even minor tissue damages may not be acceptable. Additional studies are planned to further investigate the mechanisms of AMF-enhanced antibiotic sensitivity and evaluate the system's performance on biofilm-infected implants in animal models.

## Conclusions

We describe the use of a low-frequency alternating electromagnetic field generator that elevates the temperature of metallic objects by induction heating. Upon increasing the temperature to 45 °C, bacteria grown as biofilms become statistically more sensitive to vancomycin. We propose that this type of device could be used to heat the surfaces of metallic implants to render antibiotic therapy more effective. The non-invasive AMF generator could be used for both acute and chronic infections or, preferably, as a prophylactic treatment prior to detection of biofilm infections.

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