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The Effects of a Static Magnetic Field on the sensitivity of *Escherichia coli* to Ampicillin, Streptomycin and Nalidixic Acid

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Abstract

The goal of this study was to determine if we could increase the sensitivity of *E. coli* to antibiotics by exposing it to a static magnetic field (SMF). In this study, we exposed 12 mL aliquots of *E. coli* culture to a 19.5 mT SMF for 300-, 100-, 30-, or 10-minute intervals at 36 °C, then performed a disk diffusion assay using ampicillin, streptomycin and nalidixic acid. We looked for differences in the zones of inhibition (ZI) between SMF exposed and unexposed E. coli to quantify changes in antibiotic sensitivity. We found that exposure to SMF for 300 minutes' results in a significantly larger ZI for ampicillin, and SMF exposure of 30-minutes reduced the ZI of streptomycin. Our results suggest that exposure to a 19.5 mT SMF can change *E. coli*'s susceptibility to ampicillin and streptomycin. This knowledge may be relevant to developing alternative treatments against infectious bacteria.

Keywords — Static Magnetic Field, *Escherichia coli*, Antibiotic Sensitivity

1. Introduction

Exposure to electromagnetic fields affects bacterial cell growth, viability, and proliferation [1]. Rod-shaped bacteria such as *E. coli* are highly susceptible to electromagnetic fields [2]. Static magnetic fields of strengths varying from 450mT to 3500mT were applied to *E. coli* and with the use of both scanning electron microscopy (SEM) and transmission electron microscopy (TEM) cell surface damage was observed [3]. The effects of an SMF were found to be dependent on the magnitude and the duration of exposure [4].

A relationship between the exposure to a SMF and a bacteria's sensitivity to different antibiotics is recognised [5, 6]. This relation between magnetic fields and antibiotic sensitivity can play a key role for treatment of antibiotic resistant bacteria. Medicine is facing a crisis as more virulent strains of bacteria are becoming resistant to common antibiotics [7]. *Salmonella enterica* subsp. *enterica serovar Hadar* grown in a liquid nutrient broth was exposed to a SMF (200 mT) during its growth phase for 12 and 24 hour

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periods, which increased its sensitivity to the antibiotic gentamicin [5]. This result was not observed with all antibiotics and a mechanism of how an SMF interacts with a bacterium to increase its sensitivity to specific antibiotics has not been established. In our study, we used antibiotics with a particular mode of interaction to infer which biological mechanisms a SMF affects to stimulate increased response to specific antibiotics.

We chose the three antibiotics ampicillin, streptomycin and Nalidixic acid because of their different mode of action. Ampicillin is part of the β-lactam family of antibiotics, and inhibits peptidoglycan synthesis in bacteria (a critical component of the outer membrane of bacteria). Thus, cell surface damage caused by a SMF can increase *E. coli*'s susceptibility to ampicillin. Streptomycin, like gentamicin is an aminoglycoside antibiotic and inhibits translation by binding to the 30s ribosomal subunit [8]. We chose streptomycin because of its similarity to gentamicin thus we can determine whether the effects of from an SMF is correlated to the mode of action of aminoglycosides. Lastly Nalidixic acid is a quinolone which inhibits DNA gyrase resulting in inhibition of nucleic acid synthesis [9].

The primary objective of our study is to learn if the application of a weak SMF (19.4-19.5 mT) can result in an increased sensitivity to ampicillin, streptomycin or Nalidixic acid. By exposing *E. coli* to a 19mT SMF for the durations of 300, 100, 30 and 10 minutes our secondary objective becomes to pinpoint at what duration of exposure is needed to observe a significant increase in antibiotic sensitivity. We hypothesize that an increase in antibiotic sensitivity to ampicillin and streptomycin will be observed after *E. coli* has been exposed to a SMF of 19.4-19.5 mT for the greater time periods of 300 and 100 minutes but not for 30 and 10 minutes because it does not allow enough time for the SMF to significantly alter the *E. coli*.

2. Materials and Methods

2.1. SMF Apparatus

Our SMF-generating setup consisted of eight independent vertically-positioned 15 cm long copper solenoids (3720 turns per metre), each connected to a Xantrex XT 15-4 power source using cables and alligator clips. The setup is illustrated in Figure 1. The power supply produced a current of 4.16-4.18 A and voltage of 8.5-8.7 Volts. We calculated the produced SMF to be between 19.4 and 19.5 mT using the formula:

$$B = \frac{\mu_0 N I}{L} = \frac{\left(4\pi \times 10^{-7} N/A^2\right)\left(3720 \frac{turns}{m}\right)(4.16A)}{0.15m}$$

For each coil, we positioned a 250 mL Erlenmeyer flask such that the bottom of the flask was 5 mm above top end of each solenoid. Each flask was positioned on the south pole of the SMF.

2.2. Experimental Groups

2.2.1 SMF exposed *E. coli*

We exposed 12mL of a liquid culture of *E. coli* to a 19.5 mT SMF by suspending it above a copper solenoid in an Erlenmeyer flask (Figure 1). The liquid culture was suspended

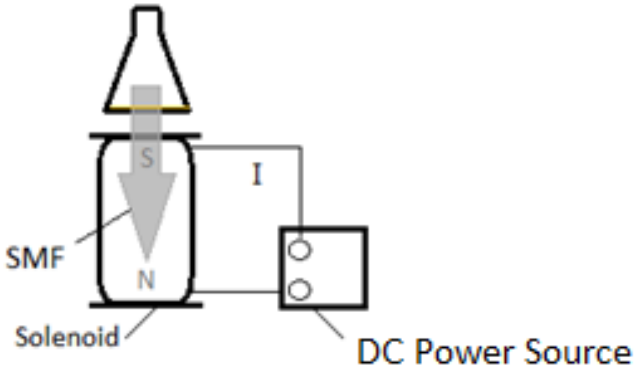

Figure 1: *SMF Apparatus. A diagram of how a 19.5 mT SMF was produced and applied to E. coli. The Erlenmeyer flask was suspended 5 mm above a solenoid that was attached to a DC power source to produce SMF. Each flask was positioned above the South pole of the SMF.*

in Erlenmeyer flasks rather than test tubes to create a thin uniform film of bacteria with a greater surface area to maximize oxygen availability to the bacteria. We tested exposure times of 300-, 100-, 30-, and 10-minutes, using fresh new liquid cultures (LB) for each time period. For each exposure time 8 replicates were tested simultaneously, with each flask suspended above its own solenoid independent from the others.

2.2.2 SMF unexposed *E. coli*

Liquid culture of *E. coli* that was not exposed to the 19.5 mT SMF was prepared in the same way as exposed *E. coli* and kept in 12 mL volumes in Erlenmeyer flasks. For each exposure time (300-, 100-, 30, and 10-minutes) we put 8 replicates in a water bath for the duration of the SMF exposure time.

2.3. Controlling for temperature

Preliminary experiments informed us that the resistance in the solenoid and circuit produced enough heat to increase the temperatures of the test broths to 36 °C. Thus we decided to place the unexposed groups in a Fischer Scientific ISOtemp 210 (product of USA) water bath of 36 °C for same amount of time the *E. coli* was exposed to the SMF (300-, 100-, 30-, or 10-minutes).

2.4. Testing antibiotic sensitivity

We quantified the sensitivity of *E. coli* to an antibiotic using disc diffusion assay and comparing the zone of inhibition (ZI) of the SMF exposed *E. coli* to the SMF unexposed

E. coli. We prepared a plate (diameter: 100 mm, height: 15 mm) for each replicate with 20-mL of BD® Mueller-Hinton II Agar (Lot#: 4216798, France). Immediately following the SMF exposed or unexposed time trial, 100μL of the liquid *E. coli* culture from each flask was spread onto a separate agar plate with a sterile glass rod. After the bacteria was applied, we placed a filter paper disc- cut from VWR® Grade Blotting Paper (catalogue #28298-020, Canada) using a Staples® Adjustable Hole Punch (Canada). We prepared solutions of ampicillin, streptomycin, and Nalidixic acid (all from Sigma Aldrich®, USA) for use in the disk diffusion assay at concentrations of 10 mg/mL. Four disc were placed on each plate with either 10μL of ampicillin, streptomycin, Nalidixic acid or distilled H_2O pipetted onto a disc. We incubated the plates face down for approximately 20 hours, enough time for the *E. coli* to grow into a visible lawn on the plate. We then measured the maximum diameters of the ZIs using a caliper.

2.5. Liquid Culture Preparation

We prepared 128 test tubes, each containing 6 mL of BD® Mueller-Hinton broth (Lot#: 4216798, France). Each broth was then inoculated with *Escherichia coli ATCC 11303*. This was done by dragging an inoculating loop across a 1x2mm lawn of the bacteria, which was then dipped into one of our test tubes containing 6 mL of broth, and spun between the fingers for two seconds in order to dislodge the bacteria. This process was repeated for each of the 128 test tubes. The test tubes for the 10-, 30-, and 100-minute trails were incubated on a Barnstead MaxQTM orbital shaker for 18.25 hours at 180 rpm and a temperature of 37 °C. The broths used for the 300-minute trial was incubated for a 40-hour period at a temperature setting of 27.5 °C because the lab was inaccessible, thus we reduced the temperature for this extended time to have similar concentration to the other time trials. After, the cultured broths were transferred to a 250 mL Erlenmeyer flasks, such that two 6-ml test tubes were poured into each flask resulting in a volume of 12 mL of liquid *E. coli* culture per flask.

2.6. Statistical analysis

To determine any significant differences between the ZIs of SMF exposed and unexposed *E. coli* a 2-sample unpaired *t*-test was done ($\alpha = 0.05$). All *p*-values less than 0.05 is reported in our results, *p*-values greater than 0.15 were not reported within our results. In addition to the unpaired *t*-test, the average ZI of each time trial was taken and plotted with 95% confidence intervals.

3. Results

We wanted to determine the effects on antibiotic sensitivity of *E. coli* after exposure to a 19.5 mT SMF. We exposed the SMF to liquid *E. coli* culture to varying time periods of 300-, 100-, 30- and 10-minutes. We performed a disc diffusion assay and used the diameter of the zone of inhibition (ZI) to quantify the sensitivity of *E. coli* to ampicillin, streptomycin, and Nalidixic after SMF exposure. We compare the ZI of SMF exposed bacteria to the ZI of unexposed SMF *E. coli*.

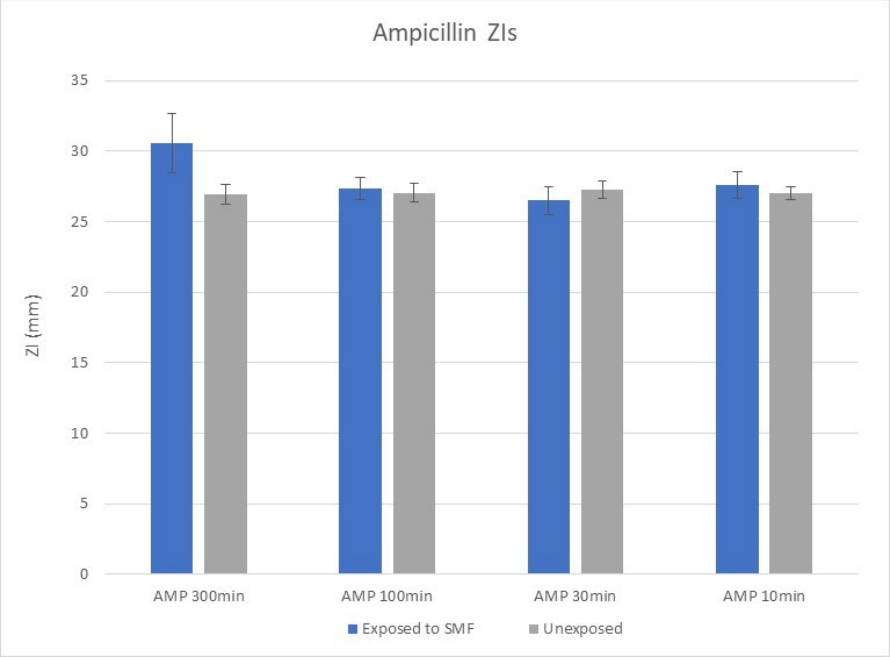

Figure 2: *The average Zones of Inhibition (ZI) of ampicillin at the different time trials. A comparison is made between E. coli that was exposed and unexposed to a SMF. 95% confidence intervals are presented to compare data; a significant difference was observed between the ZIs of ampicillin (300-minute SMF exposure) and streptomycin ZIs (30-minute SMF exposure).*

The average ampicillin ZI of the 300-minute trial is significantly larger in the SMF exposed *E. coli* than the SMF unexposed *E. coli* with a p-value of 0.0043 (Figure 2). We found no significant differences between the SMF exposed and unexposed *E. coli* for the 100-, 30-, and 10-minute time trials (all p-values > 0.1).

The average streptomycin ZI for the 30-minute time trial was significantly smaller in the SMF exposed *E. coli* than the SMF exposed *E. coli* with a p-value of 0.0026 (Figure 3). But no statistical significant difference was observed for any of the other exposure times, all p-values obtained were greater than 0.1, thus not reported in this paper.

There was no significant difference in the ZI's of Nalidixic acid measured in any of the SMF exposure times in comparison to *E. coli* unexposed to a SMF (Figure 4). All p-values for each time trial was greater than 0.1 and thus not reported in our paper. Additionally, no ZI was observed for the negative control discs with distilled H_2O.

4. Discussion

Disc diffusion assay can be used to indicate a bacterium's susceptibility to an antibiotic. An antibiotics concentration is inversely related to the distance from the disc. From the paper disc antibiotic diffuse outward, with the concentration dropping as the distance from the disc increases [10]. A larger ZI in the SMF exposed bacteria indicates that a lower concentration of antibiotic is required for a bactericidal effect on the *E. coli*. Our data suggests that *E. coli*, after an exposure of 300 minutes to a 19 mT SMF, is more sensitive to ampicillin than *E. coli* that was not exposed to any SMF (Figure 2).

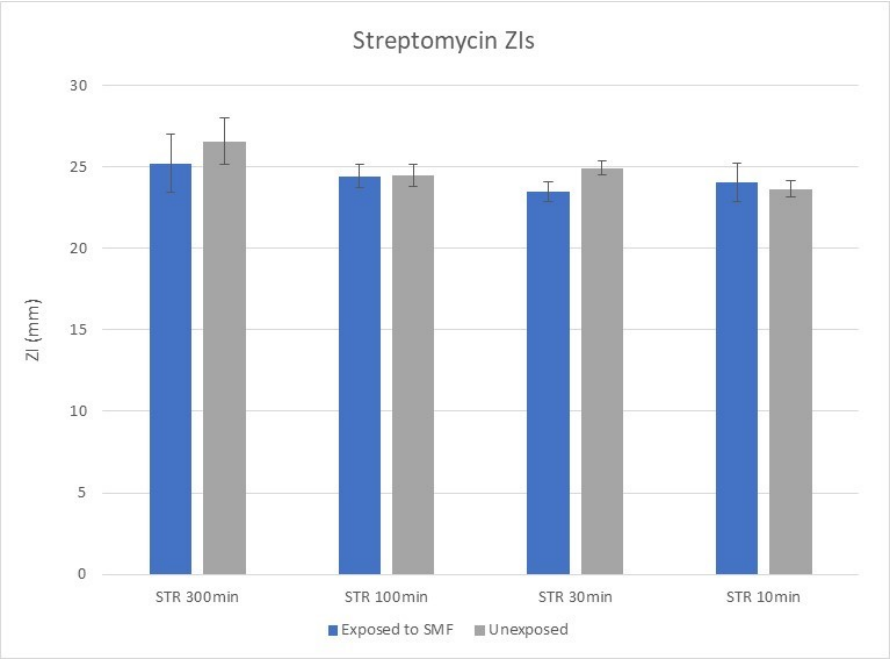

Figure 3: *The average Zones of Inhibition (ZI) of streptomycin at the different time trials. A comparison is made between E. coli that was exposed and unexposed to a SMF. 95% confidence intervals are presented to compare data; a significant difference was observed between the ZIs of ampicillin (300-minute SMF exposure) and streptomycin ZIs (30-minute SMF exposure).*

We formulated two possible explanations for the significantly larger ZI for ampicillin after 300-minute exposure to an SMF in comparison to the control groups. Damage from the SMF decreased the ability of the *E. coli* to defend itself from incoming ampicillin thus increasing its susceptibility to ampicillin. After *E. coli* is exposed to a SMF, SEM and TEM images showed cell surface damage [4]. SMF exposure has been shown to induce a stress on a bacterium [11, 12]. A second possibility is a change in membrane symmetry, ion concentration, pH and other biological changes from a SMF exposure [12, 13, 14]; could have resulted in an increased efficiency of ampicillins mode of action or membrane penetration. Increased uptake of ampicillin would result with more antibiotic entering the cells therefore increasing *E. coli*'s sensitivity to it. If the application of an SMF to a bacterium can increase the influx of an antibiotic into the cell, this knowledge can be used to develop a strategic treatment against resistant bacteria. To confirm these changes further studies to determine the relationship between SMF exposure times and ZIs with greater exposure times, such as 400- and 600- minutes. A progressive trend following 300 minute SMF exposure time will confirm these differences. We further recommend for future studies to be done to test any changes in antibiotic susceptibility on resistant bacteria.

No differences in ZI's between SMF exposed and unexposed *E. coli* were observed for ampicillin at shorter exposure times of 100-, 30-, and 10-minutes (Figure 2). Ji et al. (2009) determined a time dependence on the effects of a SMF on *E. coli* viability, they found the number of colony forming units (used to quantify viability) dropped exponentially as the exposure time increased to a 450 mT SMF. Our results suggest that 100-, 30-, and 10-minutes isn't a long enough exposure time for a 19 mT SMF to cause

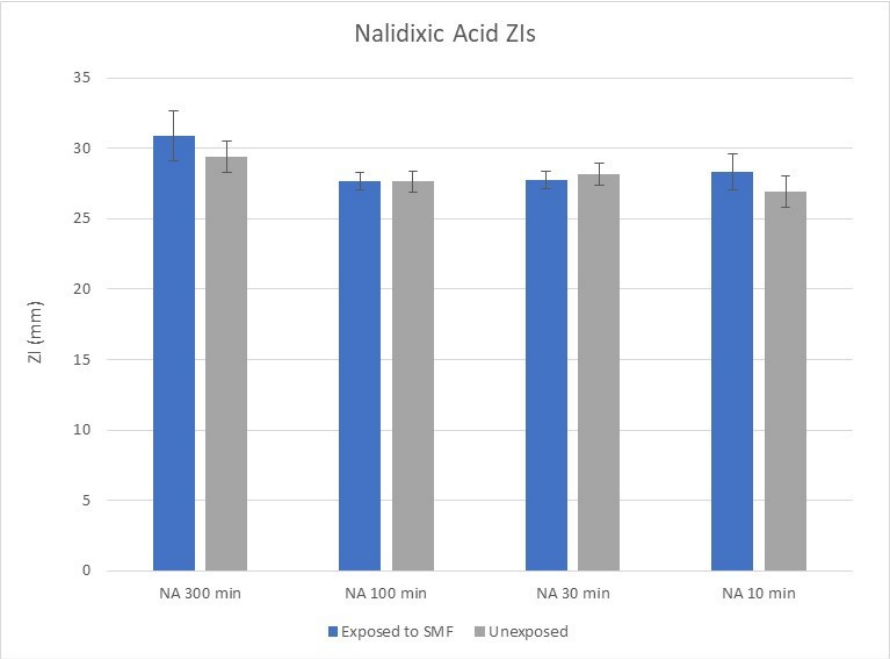

Figure 4: *The average Zones of Inhibition (ZI) of Nalidixic acid at the different time trials. A comparison is made between E. coli that was exposed and unexposed to a SMF. 95% confidence intervals are presented to compare data; a significant difference was observed between the ZIs of ampicillin (300-minute SMF exposure) and streptomycin ZIs (30-minute SMF exposure).*

any observable effect on the *E. coli*'s susceptibility to ampicillin.

The study demonstrated that a 19 mT SMF does not change *E. coli*'s sensitivity to streptomycin for 300-, 100- and 10- minutes (Figure 3). These results contradict our hypothesis that streptomycin would have an increased bactericidal effect on *E. coli* exposed to an SMF. *Salmonella enterica*, a Gram-negative bacterium, displayed an increase in sensitivity to gentamicin after 12 and 24 hours of exposure to a 200 mT SMF [5]. We hypothesized a larger ZI for streptomycin because of its similarity to gentamicin, but our data shows that there was no change in *E. coli*'s sensitivity to streptomycin after 300-minute exposure to a 19mT SMF. SMF exposure didn't strongly affect *E. coli* susceptibility to streptomycin in the way it did to gentamycin (in Salmonella). This is evidence that SMF induced susceptibility is not correlated with aminoglycoside antibiotics like streptomycin and gentamycin. Additionally, our hypothesis needs to be investigated with the same conditions with gentamycin. It is very probable that the differences in our experimental design would account for this difference between these two studies. A repetition of these experiments done with gentamycin would establish if this is relevant to the antibiotic mechanism.

Our experiment obtained some inconsistent results for the ZI's of streptomycin. After an exposure time of 30-minutes, the unexposed SMF had a significantly larger ZI than the treatment group for streptomycin (Figure 3), possibly indicating an increased resistance to streptomycin when *E. coli* is exposed to a 19.5 mT SMF. This difference in the ZI would be expected to be observed at the higher SMF exposure times for streptomycin as well but this pattern did not occur within our results. A variation in

ZIs is shown in the unexposed samples for streptomycin. These values should remain consistent between the different exposure times. This can be due to the application of streptomycin to the disc, or inconsistent concentrations of streptomycin applied to the discs. This variation is not seen with the other antibiotics used in this study.

No difference in *E. coli*'s sensitivity to Nalidixic acid was observed when comparing the ZIs for all exposure times (Figure 4). This is congruent with previous studies which found that after 12 and 24 hours' exposure to a 200 mT SMF there was no difference in Salmonella enterica susceptibility to Nalidixic acid [5]. Previous studies showing no effect of an SMF on a bacterium's susceptibility to Nalidixic acid [5, 6] verifies the differences observed in the other antibiotics are due to the exposure to the 19.5 mT SMF because no difference was measured in the susceptibility to Nalidixic acid.

From our results, we can conclude that SMFs affect *E. coli*'s sensitivity to certain antibiotics by its interaction with the cell membrane and protein translation because of the changes observed with ampicillins and streptomycin's ZIs. Our study shows that this interaction is dependent on the exposure time to the SMF, other variables such as temperature and SMF magnitude can possibly lower the time required to increase *E. coli*'s susceptibility to these antibiotics. Also, our research shows that this change can be observed with only half the SMF exposure time than previous studies that detect this change after 12 hours to a much stronger SMF (200 mT) [5]; this can be used to impose these changes in a more efficient manner with fewer resources to produce a SMF. Our study highlights the importance of understanding the effect of magnetic fields on bacteria, and could contribute to developing alternative treatment to combat antibiotic resistant bacteria.

5. Conclusion

Our results show that an SMF can have three different effects on *E. coli*'s sensitivity to different antibiotics. *Escherichia coli* had an increased sensitivity to ampicillin after 300-minutes of SMF (19.5 mT) exposure, this can be further investigated to determine if this can be observed in other gram-negative and antibiotic resistant bacteria. The opposite effect was measured after 30-miuntes of exposure to a SMF (19.5 mT) for streptomycin. A decrease in streptomycin sensitivity is inconsistent with the response to other antibiotics of the same class, thus this warrants further investigation of SMF influence on *E. coli* and other bacteria.

Investigating whether exposure to low strength SMFs can produce changes to antibiotic susceptibility in bacteria is useful because of the increasing challenges to treat resistant strains of bacteria. Furthermore, knowing more about low SMF would be useful in medical therapies because high SMF could present more risk to the patient.

6. Acknowledgments

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