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THE EFFECT OF MAGNETIC FIELD ON ANTIBIOTIC INHIBITION FOR *Escherichia coli* AND *Bacillus* sp.

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ABSTRACT

This study was aimed to test the growth of bacteria *Escherichia coli (E. coli)* and *Bacillus* sp. which were exposed to magnetic field. In the first stage of the study, the effect of magnetic fields on the growth of *E. coli* and *Bacillus* sp were observed. The futher study was aimed to evaluate the effect of magnetic field on antibiotic sensitivity againts the growth of *E. coli* and *Bacillus* sp. The magnetic fields treatments were 0.0 mT (control), 0.1 mT, 0.2 mT, 0.3 mT which were exposed for 10 hours for each treatment. Five antibiotics (trimethoprim, ampicillin, nalidixic acid, streptomycin and chloramphenicol) were used for each bacteria. The result showed that the magnetic field did not influence the colony growth of E.coli, but in *Bacillus* sp. was seen the increasing of colony area in magnetic field of 0.1 mT and 0.2 mT compared with control treatment. Antibiotic of trimethoprim, nalidixic acid, and ampicillin increase the growth inhibition of *E. coli* when the bacteria have been exposed to 0.1 mT; 0.2 mT; 0.3 mT magnetic field for 10 minutes. The inhibition by streptomycin and chloramphenicol antibiotic on *E. coli* did not affected by magnetic field exposure. The inhibition of *Bacillus* sp by trimethoprim and ampicillin increased when the bacteria have been exposed to 0.2 mT and 0.3 mT magnetic field for 10 minutes. The inhibition of nalidixic acid, streptomycin and chloramphenicol to *Bacillus sp*. did not affected by the magnetic field exposure.

Key words: antibiotic, *Bacillus* sp., *Escherichia coli*, inhibition zone, magnetic field

ABSTRAK

Penelitian ini bertujuan mengetahui pengaruh medan magnet dalam pertumbuhan bakteri Escherichia coli (E. coli) dan Bacillus sp. Pada tahap pertama penelitian dilakukan penyelidikan pengaruh medan magnet terhadap pertumbuhan bakteri bakteri E. coli dan Bacillus sp. Selanjutnya dilakukan penyelidikan pengaruh medan magnet terhadap sensitivitas antibiotik pada pertumbuhan E. coli dan Bacillus sp. Perlakuan kuat pemaparan medan magnet yang diberikan yakni 0 mT(kontrol); 0,1 mT; 0,2 mT; 0,3 mT masing-masing selama 10 jam. Perlakuan pemberian antibiotik ada 5 jenis yakni trimetoprim, ampisilin, asam nalidiksat, streptomisin, dan kloramfenikol. Dari hasil penelitian diketahui bahwa luas koloni E. coli lebih kecil dari pada koloni Bacillus sp. Medan magnet tidak memengaruhi pertumbuhan koloni E. coli. Sedangkan bahwa medan magnet 0,1 mT dan 0,2 mT meningkatkan pertumbuhan koloni Bacillus sp. dibandingkan dengan perlakuan kontrol. Pada perlakuan medan magnet 0,3 mT tidak berbeda dengan kontrol. Pada antibiotik trimetoprim, ampisilin, dan asam nalidiksat menghambat pertumbuhan bakteri E. coli yang diperbesar oleh pemaparan medan magnet 0,1; 0,2 dan 0,3 mT selama 10 menit, sedangkan daya hambat antibiotik streptomisin dan kloramfenikol pada E. coli tidak dipengaruhi oleh paparan medan magnet tersebut. Antibiotik trimetoprim dan ampisilin menghambat pertumbuhan bakteri Bacillus sp. yang diperbesar oleh pemaparan medan magnet 0,2 dan 0,3 mT selama 10 menit, sedangkan daya hambat antibiotik asam nalidiksat, streptomisin dan kloramfenikol pada Bacillus sp. tidak dipengaruhi oleh paparan medan magnet tersebut.

Kata kunci: antibiotik, Bacillus sp., Escherichia coli, zona jernih, medan magnet

INTRODUCTION

Indonesia keeps to solve health problems including antimicrobial resistance. Anti-microbial resistance is marked by the emergence of bacteria resistant to antibiotic treatment or as super bacteria (superbug). The bacteria can growth in the intestines of livestock. The super bacteria can live in humans through food, the environment (water, air, soil), or direct contact between animals and humans. Usage of unwise and irrational of antibiotics in the livestock, fishery, agriculture and public health sectors triggers the emergence of antimicrobial resistance.

Many results study have proved that there has been an increase in resistance in some regions of Indonesia. *Staphylococcus aureus* (*S. aureus*) from broiler in Yogyakarta region showed resistance to methicillin, penicillin, tetracycline, erythromycin, gentamicin, and oxycycline. Molecularly there was 34.8% detected gene encoding methicillin resistant *Staphylococcus aureus* (MRSA) (mecA gene) in isolated *S. aureus* isolates (Khusnan *et al*., 2016).

Like bacteria in usual, the growth of resistant bacteria is also influenced by environmental conditions. One such environmental factor is exposure to magnetic fields. The magnetic field is the area around the magnet that can still be affected by the magnetic force (Sudarti, 2010). Segatore (2012) state that the exposure of magnetic fields for 20 mT, 50 Hz at *Escherichia coli* (*F. coli*) and *Pseudomonas aeruginosa* for 4 hours, 6 hours and 8 hours of incubation cause to a significant decrease in cell count compared to controls. Magnetic field exposure of 700 mG, 10 Hz can also cause significant decrease in the number of *E. coli* bacterial cells (Taqavi, 2012). Magnetic field exposure at *Bacillus* sp. on 40 mT, 50 Hz causing inhibition of cell growth (Ibraheim and Darwish, 2013).

In this study, we will know how magnetic fields affect bacterial growth. in order to find out where the growth inhibition is happened. We combine treatments of magnetic field and different antibiotic inhibitory mechanisms against the bacteria *E. coli* and *Bacillus* sp. We used specific antibiotics that have mode of action is difference, namely: (1) trimethoprim which act to disturb the essential enzyme of the folat metabolism, (2) ampicillin which act to disturb the making of cell wall and cell membrane permeability, (3) nalidixic acid which act to disturb the gyrase DNA enzyme, (4) streptomycin which act to disturb the making of mucopeptide, and (5) chloramphenicol which act to disturb the peptide transferase.

MATERIALS AND METHODS

This study consist of 2 stages of experiment. The first stage of the study to test the effect of magnetic field on the growth of *E. coli* and *Bacillus* sp. The bacteria *E. coli* and *Bacillus* sp. were grown in a liquid nutrient medium, then the bacterial culture was incubated for 10 hoursat 37° C. The culture was exposed to 0 mT (control); 0.1 mT; 0.2 mT; and 0.3 mT magnetic field for 10 min. After that it was inoculated into a solid medium of nutrient agar and incubated overnight. The area of colonies formed was then calculated by gravimetric.

The second stage of study was arranged factorial with 4 magnetic field exposure (0 mT; 0.1 mT; 0.2 mT; and 0.3 mT) and 5 types of antibiotics (trimethoprim, ampicillin, nalidixic acid, streptomycin and chloramphenicol) for each bacteria (*E. coli* and *Bacillus* sp.). Each treatment was repeated 3 times therefore the total probability unit was 120 units of experiment. Parameters measured in this study was the inhibitory zone diameters of bacteria that have been exposed to to magnetic fields with several steps as follows, (1) Magnetic field exposure to starter liquid culture bacteria. Bacteria of *E. coli* and *Bacillus* sp. were inoculated into a separate 100 mL Erlenmeyer containing 10 mL of sterile nutrient broth (NB) media. The cultures were incubated at shaking incubator 100 rpm for 10 hours at 37° C. After incubation, the culture was exposed to 0 mT (control), 0.1 mT; 0.2 mT; and 0.3 mT magnetic field for 10 min. (2) Bacterial inoculation. Inoculation of bacterial cultures to solid media using bacterial cultures which have been exposed to magnetic field according the treatment. Bacterial culture inoculated in Nutrient agar medium in Petri dish using Kirby-Bauer method. Each treatment was repeated 3 times. (3) Assay of antibiotic inhibition. Assay of antibiotics on bacterial culture media used antibiotic disks for example, 10 µg ampicillin, 10 µg streptomycin, 30 µg chloramphenicol, 5 µg trimethoprim, and 30 µg of nalidixic acid. Then, an antibiotic disk was placed on a petri dish containing bacterial cultures using sterile tweezers, then bacterial culture incubated at 37° C for 24 hours. The inhibition zone results were observed. The measurement of inhibition zone diameter is determined by using the sliding term.

Data Analysis

Analysis of data using analysis of variance (ANOVA) and continued with least signification difference (LSD) test at significant level of 5% real level.

RESULTS AND DISCUSSION

Effect of magnetic field on bacterial growth of *E coli* and *Bacillus* sp.

There are significant size differences between two bacterial isolates. Bacteria; colony of *E. coli* was relatively smaller than bacterial colony of *Bacillus* sp. Magnetic field exposure treatment did not affect the growth of *E. coli* bacteria. However, the exposure of 0.1 mT and 0.2 mT magnetic fields to *Bacillus* sp. increase cell growth compared to the control treatment, The magnetic field treatment of 0.3 mT is similar tos the control (Table 1 and Figure 1).

Different growth observed between these two bacteria probably due to differences in cell wall structure of two genera of bacteria. In cell wall of Gram-negative

Table 1. Influence of magnetic field on colonies growth of *Escherichia coli* and *Bacillus* sp.

Magnetic field	Area of bacterial colony (cm^2)	
	E. coli	*Bacillus* sp
0.0 mT	$0.25^{(a)}$	$7.94^{(a)}$
0.1 mT	$0.24^{(a)}$	$8.89^{(b)}$
0.2 mT	$0.23^{(a)}$	$9.58^{(b)}$
0.3 mT	$0.21^{(a)}$	$7.83^{(a)}$

[a,b]Different superscripts within the same column indicates sigificant different (P<0.05)

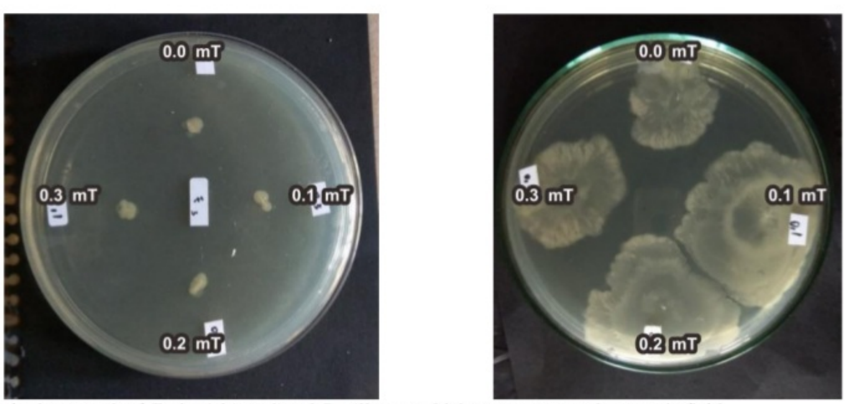

Figure 1. Colonies growth of *Escherichia coli* and *Bacillus* sp. which were exposured magnetic field

bacteria has three layers, while in Gram-positive bacteria only contain 1 layer. Three layers at Gram-negative cause nutritional strengths absorbed into cells fewer compared to Gram-positive bacteria. Growth of *E. coli* colony cells smaller than Bacillus bacteria. According to Giverso *et al*. (2015), bacterial colonies that live on the surface in aligned with continuous growth. There are several parameters that affect bacterial colonies for example: chemicals, substrate interactions, and the ability of diffusion of nutrients into the cell.

However, magnetic field treatment also gives different effects on bacterial growth. In gram-positive bacteria, the magnetic field treatment gives a significant effect. In the gram-positive bacteria peptidoglycan protein content is much more than the gram-negative bacteria (Carr, 2016). The presence of the proteins increased the proliferation of bacterial cells after exposure of magnetic field at 0.1 mT and 0.2 mT. This is supported by Sumardi *et al*. (2018), which stated that exposure of magnetic fields for milk proteins can increase the activity of protease enzymes. The increasing of enzyme activity means there is an increase in bacterial growth. However, the increase in growth can have a negative impact. The bacterial cell divides then the enzyme will work fast and the cell is in a sensitive condition. As a result, cells will be more sensitive to antibiotics than normal cell.

Antibiotic Inhibitory Assay on *E. coli*

Inhibitory activity of several antibiotics againts *E. coli* after exposed to a strongly different magnetic field is shown in Figure 2. Figure 2 indicated that exposure of E.coli to magnetic field (0.0 mT; 0.1 mT; 0.2 mT; and 0.3 mT) and treated with several antibiotics produce various inhibition zone among the treatments. The inhibition zone of trimetropin was significantly (P<0.05) larger when *E. coli* exposed to all magnetic field compared to control (unexposed), while for ampicilin, the inhibition zone was smaller than control (Table 2). On the use of nalidixic acid, exposure of *E. coli* to 0.2 mT and 0.3 mT significantly increase the inhibition zone compare to those exposed to control (0.0 mT) and 0.1 mT magnetic field. Table 2 also showed that magnetic field did not have any effect on the inhibition zone of streptomycin and chloramfenicol.

The present result indicates that the magnetic field exposure (0.1 mT; 0.2 mT; and 0.3 mT) for 10 minutes could inhibit the essential enzyme inside the folate

Table 2. Diameter of the inhibitory zone of antibiotics againts *Escherichia coli* bacteria after exposed to different magnetic fields

Antibiotic	Inhibition zone diameter (cm) after exposed with magnetic field's			
	0.0 mT	0.1 mT	0.2 mT	0.3 mT
Trimethoprim	0.8^a	1.0^b	1.0^b	1.0^b
Ampicilin	1.1^a	1.0^b	1.0^b	1.0^b
Nalidixic acid	0.9^a	0.9^a	1.1^b	1.1^b
Streptomycin	1.0^a	1.0^a	1.0^a	1.0^a
Chloramphenicol	0.6^a	0.7^a	0.7^a	0.7^a

^{a,b}Different superscripts within the same row indicates significant different (P<0.05)

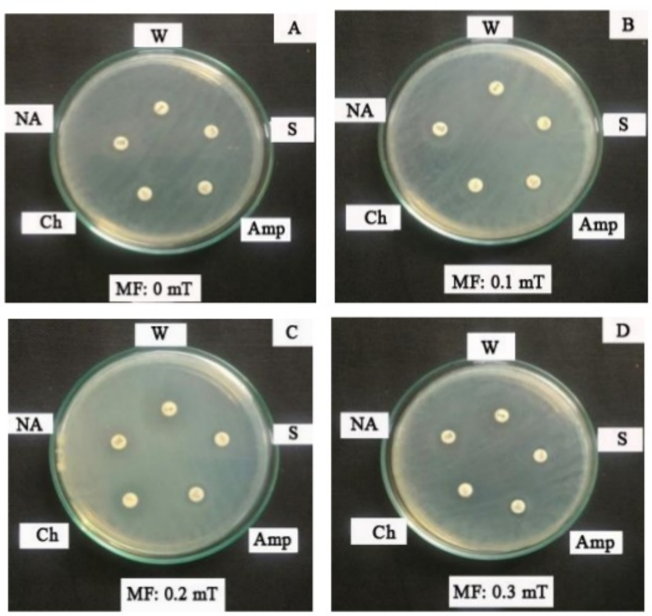

Figure 2. Inhibitory activity of several antibiotics againts *E. coli* after exposed to different strong magnetic fields. A= Magnetic field exposure 0 mT (Control), B= Magnetic field exposure 0.1 mT, C= Magnetic field exposure 0.2 mT, D= Magnetic field exposure 0.3 mT, W= Trimethoprim antibiotic, AMP= Ampicillin antibiotic, NA= Nalidixic acid antibiotic, S= Streptomycin antibiotic, C= Chloramphenicol antibiotic

metabolism in *E. coli*. However the magnetic field , did not affect the protein synthesis did not disturbed the making mucopeptide on bacteria, and did not disturbed the peptide transferase. Exposure *E. coli* to 0.1 mT; 0.2 mT; and 0.3 mT magnetic field for 10 minutes could assist the development of cell wall and cell membrane permeability.

According to Gaafar (2006), the magnetic field exposure of 2 mT, 50 Hz for 16 hours cause *E. coli* bacterial cells more resistant to antibiotic activity in which the inhibition zone diameter for chloramphenicol, amoxicillin, and nalidixic acid.was a smaller compared to control. However, magnetic field exposure with the same intensity for 6 hours cause the *E. coli* bacteria become more sensitive towards amoxicillin and nalidix acid antibiotic.

This is probably due to magnetic field exposure cause the moving energy from the magnetic fields to the ions inside the bacteria and the environment medium. This moving energy caused an increasing speed of ions stream to pass the membrane cell (Ma'rufiyanti *et al*., 2014).

Griffith *et al*. (2001) stated, the significantly exposed bacteria could change the sensitivity of the bacteria towards antibiotic. He also found that *E. coli* bacteria that have been exposed to magnetic fields and the unexposed have different sensitivity towards antibiotic.

Fojt *et al*. (2009) stated that after exposing to the bacteria for an hour, with the intensity of 10 mT, there is no morphological change on bacill bacteria as well as coccus bacteria. This proves that the magnetic field exposure on liquid medium would affect the absorption of nutrients. Therefore it could fasten the growth and metabolism activity of bacteria cell, but it was not changed the morphology of the bacteria.

Results of Antibiotic Inhibitory Assay to *Bacillus* sp.

Diameter of the inhibition zone resulted from a *Bacillus sp*. bacterial inhibitory test after exposed to a strongly different magnetic field against antibiotics is shown in Figure 3. The result illustrated that there was different inhibition zones of antibiotic to *Bacillus* sp. after exposed to all magnetic field treatments (0.0 mT; 0.1 mT; 0.2 mT; and 0.3 mT).

Table 3. Diameter of inhibition zone produced by *Bacillus* sp. after exposed the magnetic field

Antibiotic	Inhibition zone diameter (cm), after exposed with magnetic field's			
	0.0 mT	0.1 mT	0.2 mT	0.3 mT
Trimethoprim	$2.8^{a)}$	2.9^{a}	3.0^{b}	3.1^{b}
Ampicilin	1.3	1.4^{a}	1.6^{bc}	1.8^{c}
Nalidixic acid	2.1^{a}	2.4^{a}	2.3^{a}	2.5^{a}
Streptomycin	1.3^{a}	1.3^{a}	1.3^{a}	1.3^{a}
Chloramphenicol	2.0^{a}	2.3^{a}	2.4^{a}	2.5^{a}

a,b Different superscripts within the same row indicates significant different ($P<0.05$)

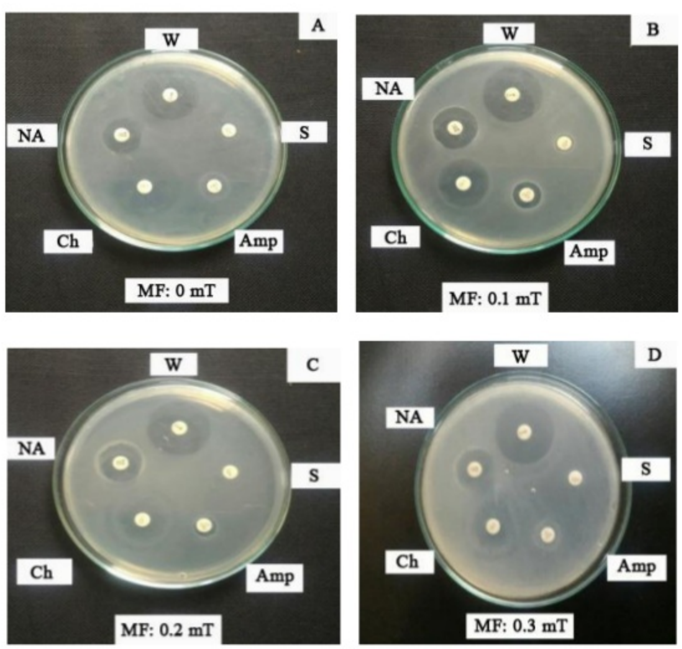

Figure 3. Inhibition zone diameter created by *Baccillus* sp. bacteria after magnetic field's exposure with different strength towards antibiotic. A= Magnetic field exposure 0 mT (control), B= Magnetic field exposure 0.1 mT, C= Magnetic field exposure 0.2 mT, D= Magnetic field exposure 0.3 mT, W= Trimethoprim antibiotic, AMP= Ampicillin antibiotic, NA= Nalidixic acid antibiotic, S= Streptomycin antibiotic, C= Chloramphenicol

Table 3 showed that the inhibition zone of the trimethoprim antibiotic in control was smaller (1.8 cm) than those were exposed to magnetic field of 0.1 mT; 0.2 mT; 0.3 mT (2.9 cm; 3.0 cm; 3.1 cm). Similarly, the inhibition zone of ampicillin, nalidixic acid, and chloramphenicol antibiotics also smaller compared to those exposed to magnetic field of 0.1 mT; 0.2 mT; 0.3 mT (1.4 cm; 1.6 cm; 1.8 cm).

From analysis of variance showed that the inhibition zone diameter of *Bacillus* sp. for streptomycin, nalidixic acid, and chloramphenicol antibiotic did not differ significantly among the treatments. However for trimethoprim and ampicillin antibiotic, exposure of *Bacillus* sp. to 0.2 mT and 0.3 mT magnetic field was significantly difference compared to control and 0.1 mT magnetic field.

The result of the study showed that the magnetic field exposure of 0.2 mT and 0.3 mT for 10 minutes on *Bacillus* sp. could inhibit metabolism i.e. the essential enzymes of folat metabolism, and disturb the making of cell wall and cell membrane permeability. A magnetic field exposure did not affect the gyrase DNA enzyme and the protein synthesis, and not disturb the building of mucopeptide on bacteria and not disturb the peptide transferase on 0.1 mT; 0.2 mT; 0.3 mT for 10 minutes.

According to Ibraheim and Darwish (2013), magnetic field exposure of 4 mT, 50 Hz for 14 hours increased the sensitivity of bacteria for antibiotics like amikacin, norfloxacin, rifampin, and ciprofloxacin. According to Li *et al*. (2015), magnetic field exposure of 300 mT for 10 minutes on *Paenibacillus* sp. increased the growth, but strong exposure of magnetic field (500 mT) decreased the growth.

CONCLUSION

Magnetic field exposure did not affect the cell growth of *E. coli*, whereas for *Bacillus* sp., 0.1 mT and 0.2 mT of magnetic field exposure increase the cell growth. Exposures of magnetic fields also give different antibiotic inhibitory effects. The inhibition of trimethoprim, ampicillin, and nalidixic acid increased against *E. coli* for all of magnetic field treatments, while antibiotics of streptomycin and chloramphenicol did not show an effect. For *Bacillus* sp., the inhibition of trimethoprim and ampicillin antibiotics increased when exposed to the magnetic field of 0.2 mT and 0.3 mT for 10 minutes, while the antibiotics of nalidixic acid, streptomycin and chloramphenicol did not show an effect.

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