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 fields. In the first stage of the study, the effect of magnetic fields on the growth of E. coli and Bacillus sp were observed. The further study was aimed to evaluate the effect of magnetic field on antibiotic sensitivity against 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 2 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 affect 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

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 grow 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 doxycycline. 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 peptidyl transferase.

MATERIALS AND METHODS

This study consists 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 hours at 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 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 to 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 bacteria, the cell wall is composed of peptidoglycan, a repeating unit of alternating N-acetylglucosamine and N-acetylmuramic acid that are covalently linked together by short beta-1,4-linkages. These beta-1,4-linkages are responsible for conferring rigidity and strength to the cell wall. In contrast, Gram-positive bacteria have a thicker peptidoglycan layer that is further stabilized by a glycolipid component known as teichoic acid. These differences in cell wall structure are likely responsible for the observed differences in growth inhibition between E. coli and Bacillus sp. under the influence of magnetic fields.

<table>
<thead>
<tr>
<th>Magnetic field (mT)</th>
<th>E. coli (cm²)</th>
<th>Bacillus sp. (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0 mT</td>
<td>0.25abcd</td>
<td>7.94abcd</td>
</tr>
<tr>
<td>0.1 mT</td>
<td>0.24abcd</td>
<td>8.89abcd</td>
</tr>
<tr>
<td>0.2 mT</td>
<td>0.23abcde</td>
<td>9.58abcde</td>
</tr>
<tr>
<td>0.3 mT</td>
<td>0.21abcde</td>
<td>7.83abcde</td>
</tr>
</tbody>
</table>

* Different superscripts within the same column indicates significant different (P<0.05)

Figure 1. Colonies growth of Escherichia coli and Bacillus sp. which were exposed to 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 Givens 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 states 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 against *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; 0.3 mT) and treated with several antibiotics produce various inhibition zone among the treatments. The inhibition zone of trimetoprim 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 chloramphenicol.

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 against *E. coli* bacteria after exposed to different magnetic fields**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Inhibition zone diameter (cm) after exposed with magnetic field’s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0 mT</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.8a</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1.1a</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>0.9a</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1.0a</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.6a</td>
</tr>
</tbody>
</table>

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

**Figure 2. Inhibitory activity of several antibiotics against *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 mucopontide 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 cococcus 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**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Inhibition zone diameter (cm), after exposed with magnetic field’s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.0 mT</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>2.8a</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1.3a</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>2.1a</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1.3a</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>2.0a</td>
</tr>
</tbody>
</table>

Different superscripts within the same row indicates significant different (P<0.05)

**Figure 3.** Inhibition zone diameter created by *Bacillus* 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 (2.8 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 were 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 folate 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 Li et al. (2015), magnetic field exposure of 4 mT, 50 Hz for 14 hours increased the sensitivity of bacteria for antibiotics like amikacin, norfloxacin, rifampicin, 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.

REFERENCES


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