Antibacterial Effects of Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 ON Klebsiella pneumoniae Producing Extended-Spectrum β-Lactamase (ESBL)

Yuani Setiawati¹, Wiwin Retnowati², Achmad Basori³

¹ Department of Pharmacology and Therapy Medical Faculty of Universitas Airlangga
² Department of Microbiology Medical Faculty of Universitas Airlangga
³ Department of Pharmacology and Therapy Medical Faculty of Universitas Airlangga
Correspondent author: yuanismile@yahoo.com

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| Keywords: | Background: Klebsiella pneumoniae as an opportunistic pathogen can cause nosocomial infection. The main concern on this bacterium is directed on the extended-spectrum β-Lactamase (ESBL)-producing Klebsiella pneumoniae. The therapy of ESBL-producing Klebsiella pneumoniae infections is very limited because of its multidrug resistance. **Materials:** It had been found new local isolates Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 in mangrove East Coast of Surabaya. These isolates have potential to produce antibiotics (Retnowati, 2008). **Methods:** This study was aimed to prove these isolates may inhibit the growth of ESBL-producing Klebsiella pneumoniae. The test of antibacterial activity of Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 using the modification Agar print method against clinical isolates of ESBL-producing Klebsiella pneumoniae. The diameter of inhibition zone (mm) formed shows activity of these isolates. The profiles of antibacterial activity of Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 were different in terms of period required to reach peak activity, duration of activity and inhibition zone diameter produced during 10 days of observation. **Results:** Streptomyces sp-MWS3 reached the peak activity most rapidly on day 3 with the largest inhibition zone diameter of 9 mm in ESBL-producing Klebsiella pneumoniae. **Conclusion:** There were significant differences in inhibition zone diameter between Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 against ESBL-producing Klebsiella pneumoniae. |

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**Introduction**

A lot of nosocomial infections in hospitals that increase morbidity and mortality rates make nosocomial infections an important clinical problem. One of the germs that causes nosocomial infection is Klebsiella pneumoniae. Klebsiella pneumoniae is the second leading cause of Gram-negative nosocomial bacteremia after *Escherichia coli* (Podschun and Ullmann, 1998). Klebsiella pneumoniae is a Gram-negative rod bacterium that is mostly found in the mouth, upper respiratory tract, skin, intestine, urinary tract, and genitals (Jawetz *et al.*, 2004). As an opportunistic pathogenic germ, Klebsiella pneumoniae can cause severe illnesses such as septicemia, pneumonia, urinary tract infections, and soft tissue...
infections, especially in immunocompromised individuals who are being treated in hospital (Hostacka, 2000). It is estimated that Klebsiella pneumoniae causes 8% of all nosocomial bacterial infections in America and Europe and is among the eight most important infectious pathogens in American hospitals (Podschun and Ullmann, 1998).

The main concern for this bacterium is the extended-spectrum β-lactamase (ESBL)-producing Klebsiella pneumoniae which is associated with the spread of nosocomial infections (Podschun and Ullmann, 1998). According to the Centers for Disease Control and Prevention, ESBL-producing Klebsiella pneumoniae causes 8% outbreaks of nosocomial infections (Podschun and Ullmann, 1998), in Europe 14-16% ESBL was found among Klebsiella pneumoniae clinical isolates, whereas in Asia the incidence was 25-40% (Choi et al., 2009). In Indonesia, especially at Dr. Soetomo Hospital Surabaya, the ESBL incidence rate was 20.1% for E. coli and 27.9% for K. pneumonia in 2005 (Lestari, 2009).

ESBL (extended-spectrum β-lactamase) is β-lactamase which has the ability to cause Penicillin resistant bacteria, and can be the first, second, and third generation cephalosporins, and Aztreonam (but not Cefamycin and Carbapenem) by hydrolyzing the antibiotic, and can inhibited by β-lactamase inhibitors such as clavulanic acid (Paterson and Bonomo, 2005). ESBL is produced by the mutation of the native β-lactamase coding gene found in Gram negative bacteria, especially Escherichia coli and Klebsiella pneumoniae (Pfaller and Segreti, 2006). A number of these mutated enzymes (ESBL) can hydrolyze broad-spectrum β-lactam antibiotics to produce inactive products (Pitout and Laupland, 2008). Therapy to overcome the bacterial infection of the ESBL-producing Klebsiella pneumoniae is very limited because it is multidrug resistant, especially against β-lactam antibiotics. Carbapenems such as Imipenem or Meropenem are used as the drug of choice for this bacterial infection, but since 1997 it has been found that is less sensitive to Imipenem (Bradford, 1997). Therefore, it is necessary to explore new antibiotics as alternative drugs, for example from the aminoglycoside group.

Aminoglycosides consist of two or more amino sugar groups that are bound by glycosidic bonds in the hexose nucleus. Hexose or aminosikitol is streptidin (in Streptomycin) or 2-deoxystreptamine (Goodman and Gillman, 2008). Aminoglycoside class antibiotics can diffuse through the outer membrane porine water channel of Gram-negative bacteria into the periplasmic space, then by an active transport process across the cytoplasmic membrane. After entering the cell, aminoglycosides bind irreversibly to the 30S ribosome and inhibit protein synthesis that cause damage to the cytoplasmic membrane and are followed by cell death (bactericidal) (Rang et al., 2007).

Various types of antibiotics used in the health field, one of which can be produced by Streptomyces sp. (Prescott et al., 2003). Streptomyces are Gram-positive fungal-like bacteria, which can produce several secondary bioactive metabolites, namely antibiotics used as antibacterial, antiviral, antiparasitic, anti-tumor, and immuno-suppressant drugs (Borodina et al., 2005). One species of Streptomyces sp. can produce more than 2-3 antibiotics naturally (Hoopwood, 1999). The types of antibiotics produced by Streptomyces sp. are
aminoglycosides (Streptomycin, Neomycin, Kanamycin, Lidodimycin, Paramomycin, Ribostamycin, Butyrosin, Amikacin, Isepamycin, Sepamycin, Gentamycin, Tobramycin, Netilmycin and Spectinomycin), Macrolides (Erythromycin, Oleandomycin, Spiramycin, Tetraacyclomycin, Actinohordin, Daunorubisin, Tilosin, etc), Tetracycline, Antihracycline, aromatic (Cloramphenicol), heterocyclic (Polyoxin), alicyclic (Cyloheximide), polypeptide (Viomycin, Actinomycin) dan beberapa jenis lainnya (Hoopwood, 1999).

Streptomyces sp. can live in a variety of habitats namely tropical forests, peat forests, high plains, volcanic areas, composting, farm land, agricultural land, waters, and mangrove areas (Madigan, et al., 2002; Korn and Jurgen, 2002). Indonesia has the world's largest tropical mangrove forest over Brazil (1.3 million ha), Nigeria (1.1 million ha), and Australia (0.97 million ha) which is 3.5 million ha or around 18-23% of the total world mangrove forests (Noor et al., 1999).

Research conducted by Retnowati in 2008 found the new local isolate Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 that potential as producer of antibiotics, which is based on qualitative assay bioautografi was able to produce aminoglycoside class antibiotics. These isolates have been shown antibiotic activity against Gram-positive test bacteria Staphylococcus aureus ATCC 25923, Bacillus subtilis and Gram-negative bacteria Escherichia coli ATCC 25 922, Pseudomonas aeruginosa ATCC 27853, Salmonella typhimurium (Retnowati, 2008).

In general, one species of Streptomyces sp. can produce more than 2-3 antibiotics, so Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 may be able to produce antibiotics (one of which is aminoglycosides) which are expected to inhibit the growth of Gram-negative Klebsiella pneumoniae that produce extended-spectrum β-lactamase (ESBL). During this time the development of new drugs can be pursued by: 1) random screening, 2) modification of chemical structures, and 3) application of pathophysiology (Goodman and Gilman, 2008). Based on these descriptions, research on the antibacterial effect of Streptomyces sp. soil isolates East Coast Surabaya mangrove ecosystem against Gram-negative bacteria Klebsiella pneumoniae that produce extended-spectrum β-lactamase (ESBL) need to be done so it can be used as a new alternative treatments for therapy.

**Material And Methods**

This study was an experimental laboratory research using the posttest only control group design. The sample of this study is the diameter of the inhibition zone formed due to the antibacterial activity of Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 to clinical isolates extended-spectrum β-lactamase (ESBL)- producing Klebsiella pneumoniae, with a replication number of six.

**Preparation of Streptomyces sp.**

One ose was taken from stock cultures of Streptomyces sp. Agar slant and transferred to liquid medium ISP-4 10 mL, then shaken using shaker at 150 rpm (rotation per minute) at a temperature of 30°C for 2-4 days. Taken with 2.5 mL micropipette and transferred to 25 mL of liquid medium ISP-4 and shaken at 150 rpm at 30°C. 2 mL was
taken by micropipette and put in a sterile tube. Thereafter, the turbidity of the solution containing the Streptomyces sp. was adjusted to the turbidity standard Mc.Farland 0.5 turbidity standard (1.5 X 10^8 CFU / mL). Then 1 mL was taken and put into a petri dish which contains 20 mL of ISP-4 Agar medium that has been liquefied at 45ºC, then homogenized. Wait until it solidified. Incubated at 28ºC. From media that already contains Streptomyces sp. was printed with a diameter of 0.8 cm and height 3 mm. Taken every 24 hour period for 10 days, to be put on the test media for potential tests (Retnowati, 2008)

**Preparation of bacterial inoculum test**

The tested bacteria that have been incubated at 37ºC for 24 hours was added 2 mL of sterile phosphate buffer solution pH 7, then shaken until the entire colony on the surface to be released and suspended in phosphate buffer solution pH 7 and put in a sterile tube. Thereafter, the turbidity test solution containing the tested bacteria was adjusted to the standard turbidity of Mc.Farland 0.5 (1.5 X 10^8 CFU / mL). After appropriate turbidity, 1 mL of tested bacteria in the liquid medium was inserted into the 20 mL of media Mueller Hinton (Alexander and Strete, 2001).

**Antibacterial Activity Test Using Agar Print Modified Diffusion Method**

Antibacterial activity test was carried out by attaching the result of Agar print of Streptomyces sp. to the tested bacteria for 10 days. As a positive control was used 10 mg µL Streptomycin sulfate (25 mG in 100 mL) which was placed in wells (0.8 cm diameter; 3 mm height) on the tested bacteria. Replication test the antibacterial activity of Streptomyces sp. was carried out 6 times. The diameter of inhibition zone (mm) generated was measured with shove. Positive results was indicated by inhibition zone around the culture colonies in petri dishes which meant that the isolate Streptomyces sp. able to produce antibiotics that can inhibit the growth of tested bacteria (Isnaeni, 1998).

**Data Analysis**

The diameter of inhibition zone (mm) was produced for 10 days at 24-hour intervals was statistically analyzed using one-way Anova analysis with a confidence level of 5% (α = 0.05). If the obtained results have a significant effect (significant) among treatments, the LSD (Least Significance Difference) test will be conducted to determine which treatments have significant antibacterial activity.

**Result and Discussion**

In general, Streptomyces sp. has a characteristic soil odor, the surface of the colony which first appeared on the second day is relatively slippery, but then on the fourth day it forms a kind of woven mycelium that can reveal the granular. Figure 1. shows the form of Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6.
The three different species isolates of *Streptomyces* sp. have different antibacterial activity and macroscopic characteristics in the form of colonies, colony colors, colony textures, drops and exudate colors, as well as colony growth which can be seen in Table 1.

**Table 1.** Macroscopic characteristics of *Streptomyces* sp. producing antibiotics in isolation of the mangrove ecosystems of the East Coast of Surabaya

| Karakterisasi          | Isolat Streptomyces | Isolat Streptomyces | Isolat Streptomyces |
|------------------------|----------------------|----------------------|----------------------|
|                        | sp-MWS1              | sp-MWS3              | sp-MWS6              |
| Colony shape           | Circular, convex surface | Circular, convex surface | Circular, convex surface |
| Coloni colour          | White                | Yellowish white       | Yellowish white       |
| Colony texture         | Not translucent, dry, turbid | Not translucent, dry, turbid | Not translucent, dry, turbid |
| Exudate drops & color  | Npne                 | None                 | None                 |
| Colony growth          | Spore growth is thick & fast | Spore growth is rather thick & slow | Spore growth is not thick & slow |

Microscopically, *Streptomyces* sp. has slender hyphae. When mature, the air mycelium forms a chain consisting of three to many spores. The image of *Streptomyces* sp. can be seen microscopically in Figure 2.
The results of all isolates gram staining showed the same color, purplish blue, which means Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 were Gram positive bacteria.

The activity of Streptomyces sp-MWS1, Streptomyces sp-MWS3, Streptomyces sp-MWS6 isolates in inhibiting the growth of extended-spectrum β-lactamase-producing Klebsiella pneumoniae were expressed as inhibition zones. The magnitude of the formed inhibition zone is shown in Figure 4.

Table 2. The mean diameter of the inhibition zone are formed from the antibacterial activity test of Streptomyces sp-MWS1, Streptomyces sp-MWS3, Streptomyces sp-MWS6 against ESBL producing Klebsiella pneumoniae with Agar print modified diffusion method.

|                      | The mean diameter of the inhibition zone against ESBL producing Klebsiella pneumoniae (mm) of different day |
|----------------------|---------------------------------------------------------------------------------------------------------|
|                      | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  |
| **Streptomyces sp-MWS1** | 8.3 | 8.4 | 8.6 | 8.8 | 8.5 | 8.4 | 8.3 | 0   | 0   | 0   |
| **Streptomyces sp-MWS3** | 8.4 | 8.6 | 9   | 8.9 | 8.7 | 0   | 0   | 0   | 0   | 0   |
| **Streptomyces sp-MWS6** | 8.2 | 8.3 | 8.4 | 8.5 | 8.6 | 8.3 | 0   | 0   | 0   | 0   |

Figure 3. Profile antibacterial activity test Streptomyces sp-MWS1, Streptomyces sp-MWS3, Streptomyces sp-MWS6 of the ESBL-producing Klebsiella pneumoniae
Figure 4. Test results of the antibacterial activity of *Streptomyces* sp. (1.5 X 10^8 CFU/mL) against ESBL-producing *Klebsiella pneumoniae* with Agar print modified diffusion method.

S1: *Streptomyces* sp-MWS1  
S2: *Streptomyces* sp-MWS3  
S3: *Streptomyces* sp-MWS6  
K+: positive control (10 µL streptomycin sulfate 250 ppm)  
K-: negative control

There are significant differences in inhibition zone between *Streptomyces* sp-MWS1, *Streptomyces* sp-MWS3, and *Streptomyces* sp-MWS6 against ESBL-producing *Klebsiella pneumoniae* for 7 days of observation with value of p ≤ 0.05 based on the Oneway Anova test, which is seen in the following table 3.

Table 3. Oneway Anova test results on the differences in the antibacterial activity of *Streptomyces* sp-MWS1, *Streptomyces* sp-MWS3, and *Streptomyces* sp-MWS6 against ESBL-producing *Klebsiella pneumoniae*

| Observation Day | Streptomyces | N  | Inhibition Zone Growth (mm) | Oneway Anova |
|-----------------|--------------|----|-----------------------------|--------------|
|                 |              |    | x              | SD          | Min | Max  | F   | p   |
| 1               | sp-MWS1      | 6  | 8.300          | .0894       | 8.2 | 8.4  | F=5.000 | p=0.022 |
|                 | sp-MWS3      | 6  | 8.400          | .1414       | 8.2 | 8.6  |
|                 | sp-MWS6      | 6  | 8.200          | .0894       | 8.1 | 8.3  |
| 2               | sp-MWS1      | 6  | 8.400          | .1095       | 8.3 | 8.5  | F=15.000 | p=0.000 |
|                 | sp-MWS3      | 6  | 8.600          | .0894       | 8.5 | 8.7  |
|                 | sp-MWS6      | 6  | 8.300          | .0894       | 8.2 | 8.4  |
| 3               | sp-MWS1      | 6  | 8.600          | .1414       | 8.4 | 8.8  | F=35.000 | p=0.000 |
|                 | sp-MWS3      | 6  | 9.000          | .0894       | 8.9 | 9.1  |
|                 | sp-MWS6      | 6  | 8.400          | .1414       | 8.2 | 8.6  |
| 4               | sp-MWS1      | 6  | 8.800          | .1414       | 8.6 | 9.0  | F=21.667 | p=0.000 |
|                 | sp-MWS3      | 6  | 8.900          | .0894       | 8.8 | 9.0  |
|                 | sp-MWS6      | 6  | 8.500          | .0894       | 8.4 | 8.6  |
| 5               | sp-MWS1      | 6  | 8.500          | .0894       | 8.4 | 8.6  | F=3.750  | p=0.048 |
|                 | sp-MWS3      | 6  | 8.700          | .1414       | 8.5 | 8.9  |
|                 | sp-MWS6      | 6  | 8.600          | .1414       | 8.4 | 8.8  |
Streptomyces sp. produce antibiotics potentially. Among the ten types of antibiotics, all can be produced by the genus Streptomyces (Korn, 2002). In general, one species of Streptomyces sp. can produce more than 2-3 antibiotics, so Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 are able to produce antibiotics (one of these is aminoglycosides) which can inhibit the growth of Gram-negative Klebsiella pneumoniae that produce extended-spectrum β-lactamase (ESBL). Retnowati in 2008 found the new local isolate Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6, which is based on qualitative assay bioautograpfi was able to produce aminoglycoside class antibiotics. Clinically aminoglycosides are often used to treat severe infections by Gram-negative bacteria (Vakulenko and Mobashery, 2003). Aminoglycosides as an antibacterial agent have concentrated bactericidal activity (concentration dependent), post-antibiotic effects (postantibiotic effect), and synergism with other antibiotics (Katzung, 2018).

The use of selective media ISP-4 (International Streptomyces Project) is very supportive for the growth of Streptomyces sp. and suppress the growth of other bacteria. Streptomyces sp. have a growth of colonies that appear slowly and attach to Agar with 2-4 days at room temperature, circular, opaque, dry, turbid, and spores such as powder granules (Madigan et al., 2002). Identification of Streptomyces sp. includes macroscopic observation (growth of the colony, the presence or absence of exudate drops, the shape of the colony, the edge of the colony, the color of the colony, and characteristic odors such as soil), microscopic, and physiological. From the overall characteristics it appears that there is a special characteristic possessed by each Streptomyces sp.

The selection of the tested bacteria clinical isolates ESBL-producing Klebsiella pneumoniae is based on consideration because it has a very strong activity and often causes disease. According to Jawetz et al. (2015) that Klebsiella pneumoniae is a Gram-negative, rod-shaped, immobile, encapsulated, facultative anaerobic, lactose fermenting bacterium, known as one of the bacteria that causes pneumonia that can cause serious pyogenic infections and will cause death if not handled properly. Clinically Klebsiella pneumoniae is the most important species of the genus Klebsiella which is known as

|   | sp-MWS1 | 6 | 8.400 | .1414 | 8.2 | 8.6 | F=14942.143 | p=0.000 |
|---|---------|---|-------|-------|-----|-----|-----------|--------|
| 6 | sp-MWS3 | 6 | .000  | .000  | .0  | .0  | F=51667.500 | p=0.000 |
| 6 | sp-MWS6 | 6 | 8.300 | .0894 | 8.2 | 8.4 |
| 7 | sp-MWS1 | 6 | 8.300 | .0894 | 8.2 | 8.4 |
| 8 | sp-MWS3 | 6 | .000  | .000  | .0  | .0  |
| 8 | sp-MWS6 | 6 | .000  | .000  | .0  | .0  |
| 9 | sp-MWS1 | 6 | .000  | .000  | .0  | .0  |
| 9 | sp-MWS3 | 6 | .000  | .000  | .0  | .0  |
| 9 | sp-MWS6 | 6 | .000  | .000  | .0  | .0  |
| 10| sp-MWS3| 6 | .000  | .000  | .0  | .0  |
| 10| sp-MWS6| 6 | .000  | .000  | .0  | .0  |
one of the germs that causes the second Gram-negative nosocomial infection after *Escherichia coli* (Podschun, 1998; Irawan D et al, 2012). As an opportunistic pathogenic bacteria, *K. pneumoniae* can cause severe diseases such as septicemia, pneumonia, urinary tract infections, and soft tissue infections, especially in immunocompromised individuals who are hospitalized and suffer from serious diseases such as diabetes mellitus or chronic obstructive pulmonary disease (chronic obstructive lung disease pulmonary disease), who use ventilator, infusions and get old antibiotic therapy (Hostacka, 2000; CDC, 2012).

ESBL--producing *Klebsiella pneumoniae* is *Klebsiella pneumoniae* which can produce extended spectrum β-lactamase enzymes, which are multidrug resistant especially to β-lactam antibiotics (Wong A and Beringer, 2001; Wan Ho M; 2008; Sharma, 2010). This ESBL-producing *Klebsiella pneumoniae* has high virulence, resistant to various antibiotics and has the ability to spread rapidly, causing a nosocomial outbreak which will increase the burden of hospital costs (Sahly et al., 2003; Ramphal and Ambrose, 2006; Drawz SM and Bonomo RA, 2010).

Print modification diffusion method to be used to know qualitatively the production of antibiotics from *Streptomyces* sp. against tested bacteria by forming inhibition zone around the colony. This method is the best alternative method for screening the antibacterial activity of *Streptomyces* sp. compared to the dilution method, because *Streptomyces* sp. is a bacterium that resembles a fungus, so it can grow on bacterial and fungal media. In the dilution method, antibiotics produced by *Streptomyces* sp. will inhibit the growth of tested bacteria, but on the other hand *Streptomyces* sp. It also carries out cell division continuously, resulting in turbidity, which causes the dilution method to be unreadable (Retnowati, 2008).

Based on the description above, the diffusion method is the best alternative method for screening *Streptomyces* sp. isolates, because it is easy to do and economically, although it has the disadvantage of *Streptomyces* sp. in ISP-4 breeding media fluctuate, so the number of *Streptomyces* sp. which is taken in each treatment is different and the diameter of the formed inhibitor zone cannot be measured quantitatively, so that a lot of replication is needed.

This study uses the standard *Streptomycin* sulfate as one of the aminoglycoside derivatives. *Streptomycin* sulfate as a positive control in the antibacterial activity test of *Streptomyces* sp. to find out whether the tested bacteria used can show a response to the antibiotic, so it is suitable to be used as a tested bacterium.

During the process of antibacterial activity testing used Mueller Hinton Agar as a non-selective medium for the growth of the tested bacteria used. The thickness of the activity test media containing the test bacteria must be the same and homogeneous to avoid the fluctuation in the diameter of inhibition zone (Isnaeni, 1998).

In the antibacterial activity test, the antibiotic fermentation process of *Streptomycyes* sp. for 4-6 days so that *Streptomycyes* sp. stationary phase (Retnowati, 2008). In the stationary phase a secondary metabolic product is produced, an antibiotic that can inhibit the growth of other organisms (Wibowo, 1990), so that *Streptomycyes* sp-MWS1, *Streptomycyes* sp-
MWS3, and Streptomyces sp-MWS6 can inhibit Klebsiella pneumoniae producing extended-spectrum β-lactamase (ESBL) starting the 1st observation day.

Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 have different activity against ESBL-producing Klebsiella pneumoniae in terms of the time required to reach the peak activity, the duration of activity and the size of the inhibition zone diameter during 10 days of observation. Streptomyces sp-MWS3 reached the peak of activity fastest on 3rd day with the greatest inhibition zone diameter of 9 mm. Streptomyces sp-MWS1 reached peak activity on 4th day with inhibition zone diameter of 8.8 mm. Streptomyces sp-MWS6 reached the peak of maximum activity on 5th day with inhibition zone diameter of 8.6 mm. According to Retnowati (2008) that the greatest antibiotic production can be produced when Streptomyces sp. reached highest activity peak.

The duration of Streptomyces sp-MWS1 activity against ESBL-producing Klebsiella pneumoniae is 7 days, the duration of Streptomyces sp-MWS6 activity is 6 days, and the duration of Streptomyces sp-MWS3 activity is 5 days. It can be explained that each Streptomyces sp. grown on ISP-4 medium at 1st day to 3rd day have adequate nutrition, while around 4th day generally decreased inhibition zone diameter caused by nutrients in the media is reduced, but Streptomyces sp. still able to survive. Starting around 4th day, the number of dead cells is greater than the number of living cells resulting in decreased inhibition zone diameter and until the 10th day the inhibition zone has not formed. It was means at approximately 6th day to 10th day Streptomyces sp. reached the phase of death.

To determine differences in the antibacterial activity of Streptomyces sp-MWS1 isolates, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 against ESBL-producing Klebsiella pneumoniae for 10 days of observation used a one-way analysis of variance (OneWay Anova). Statistical test results on the diameter of the inhibition zone showed significant differences between Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 against the ESBL-producing Klebsiella pneumoniae with value of p ≤ 0.05. From the LSD test (Least Significance Difference) known difference at inhibition zone diameter on 1st, 7th to 10th day is significant between Streptomyces sp-MWS3, and Streptomyces sp-MWS6. On 2nd day to 6th day, the difference at inhibition zone diameter is significant between the three isolates of Streptomyces sp.

Significant differences at inhibition zone diameter between Streptomyces sp-MWS1, Streptomyces sp-MWS6 and Streptomyces sp-MWS6 can be explained because Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 are different species from each other (Retnowati, 2008) so that the antibiotics produced by the three isolates of Streptomyces sp. can be different, causing the ability to inhibit ESBL-producing Klebsiella pneumoniae differently.

Conclusion

Based on the analysis and discussion above, the following conclusions can be concluded.

1. The profile of the antibacterial activity of Streptomyces sp-MWS1, Streptomyces
sp-MWS3 and Streptomyces sp-MWS6 against ESBL producing Klebsiella pneumoniae turned out to be different from each other in terms of the time needed to reach peak activity, the duration of activity and the magnitude of the inhibition zone produced during ESBL-producing Klebsiella pneumoniae. 10 days of observation. Streptomyces sp-MWS3 reached the peak of the fastest activity that is on the 3rd day with the largest diameter of the inhibition zone which is 9 mm. Streptomyces sp-MWS1 has the longest activity time of 7 days.

2. There is a significant difference in the diameter of the inhibitory zone between Streptomyces sp-MWS1, Streptomyces sp-MWS3, and Streptomyces sp-MWS6 against ESBL-producing Klebsiella pneumoniae.

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