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

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ABSTRACT

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 pneumonia*. 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 periode 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*.

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 immuno-compromised 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 aminosiklitol is streptidin (in Streptomycin) or 2-deoxistreptamine (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, Lividomycin, Paramomycin, Ribostamycin, Butyrosin, Amikacin, Isepamycin, Sepamycin, Gentamycin, Tobramycin, Netilmycin and Spectinomycin), Macrolides (Erythromycin, Oleandomycin, Spiramycin, Tetracenomycin, Actinohordin, Daunorubisin, Tilosin, etc), Tetracycline, Anthracycline, aromatic (Cloramphenicol), heterocyclic (Polyoxin), alicyclic (Cycloheximide), polypeptide (Viomysin, 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 30ºC 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 30ºC. 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 108 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 108 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

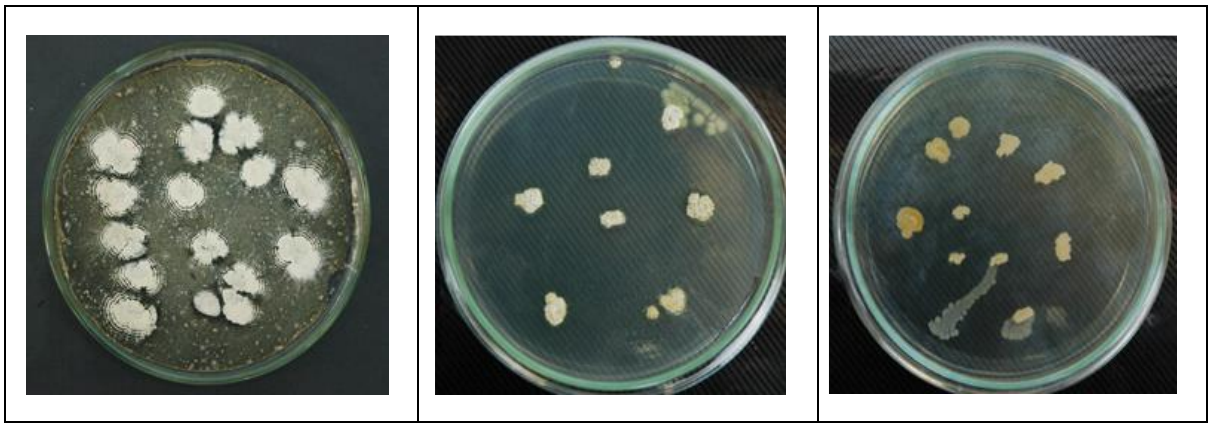
Antibacterial activity test was carried out by attaching the result of Agar print of Streptomyces sp. to the tested bacteria for 10 days. As a positive control was used 10 mg µL Streptomycin sulfate (25 mG in 100 mL) which was placed in wells (0.8 cm diameter; 3 mm height) on the tested bacteria. Replication 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% ($\alpha = 0.05$). 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.



Streptomyces sp-MWS1 *Streptomyces* sp-MWS3 *Streptomyces* sp-MWS6

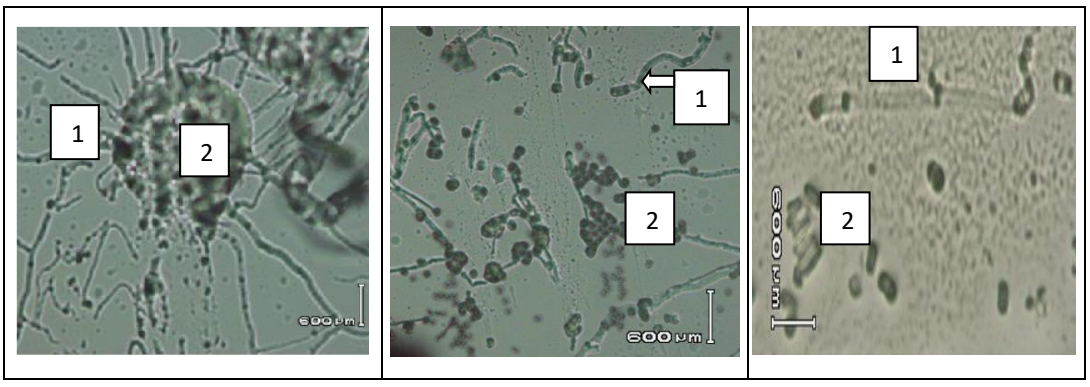
Figure 1. Streptomyces sp. colony on the ISP-4 media culture

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***		
	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.



Streptomyces sp-MWS1 *Streptomyces* sp-MWS3 *Streptomyces* sp-MWS6

Figure 2. Streptomyces sp. microscopically (400X magnification)
Note: 1. Hifa 2. Spores

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

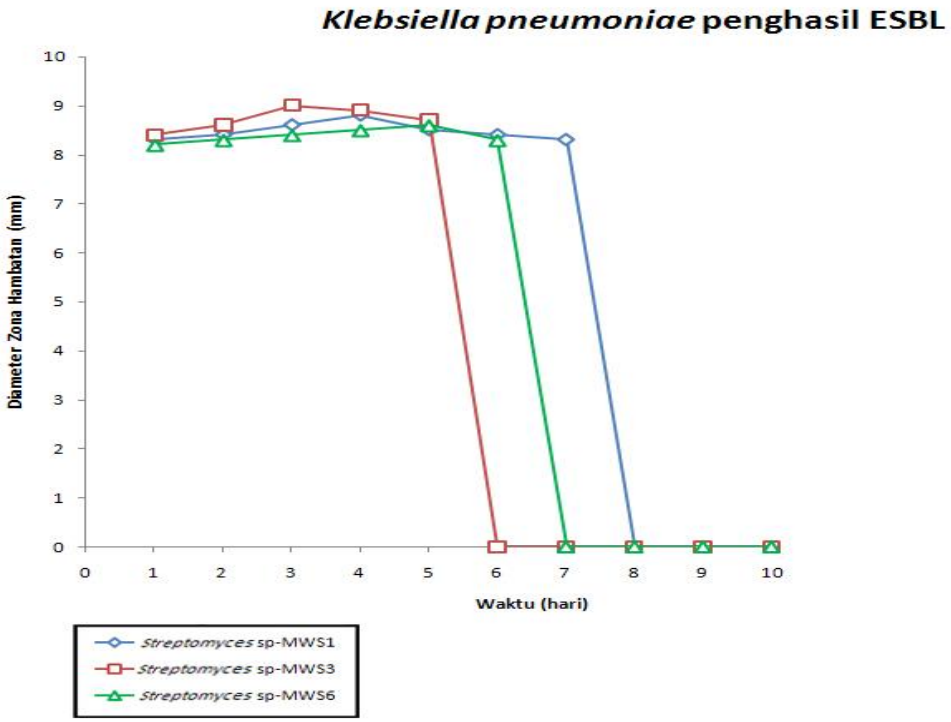


Figure 3. Profile antibacterial activity test *Streptomyces* sp-MWS1, *Streptomyces* sp-MWS3, *Streptomyces* sp-MWS6 of the ESBL- producing *Klebsiella pneumoniae*

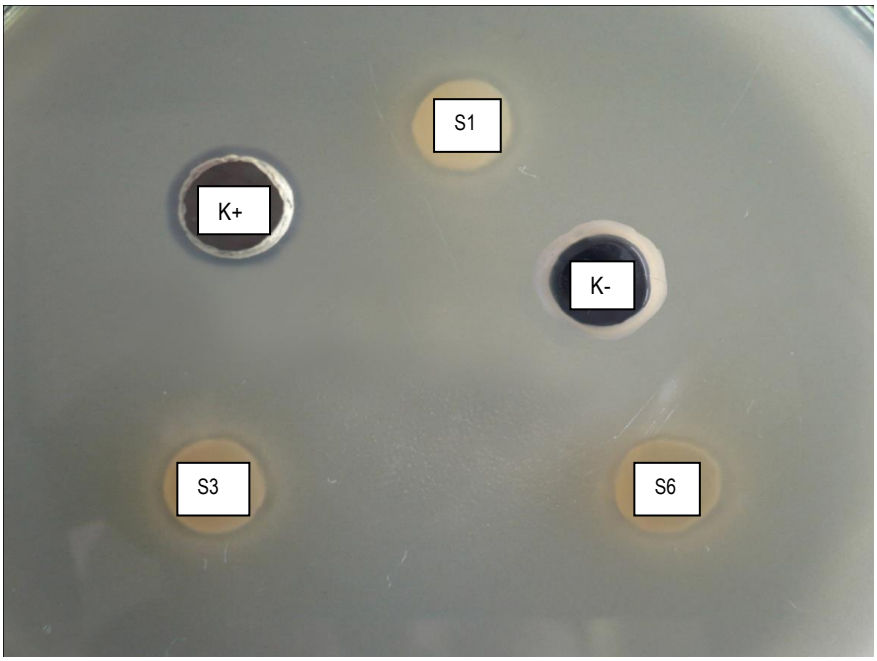


Figure 4. Test results of the antibacterial activity of *Streptomyces* sp. (1.5 X 108 CFU/ mL) against ESBL-producing *Klebsiella pneumoniae* with Agar print modified diffusion method.

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 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 $p \leq 0.05$ 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 againts ESBL producing- *Klebsiella pneumoniae*

Observation Day	*Streptomyces*	N	Inhibition Zone Growth (mm)				Oneway Anova
			$\bar{x}$	SD	Min	Maks	
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	

6	sp-MWS1	6	8.400	.1414	8.2	8.6	F=14942,143 p=0,000
	sp-MWS3	6	.000	.0000	.0	.0	
	sp-MWS6	6	8.300	.0894	8.2	8.4	
7	sp-MWS1	6	8.300	.0894	8.2	8.4	F=51667,500 p=0,000
	sp-MWS3	6	.000	.0000	.0	.0	
	sp-MWS6	6	.000	.0000	.0	.0	
8	sp-MWS1	6	.000	.0000	.0	.0	
	sp-MWS3	6	.000	.0000	.0	.0	
	sp-MWS6	6	.000	.0000	.0	.0	
9	sp-MWS1	6	.000	.0000	.0	.0	
	sp-MWS3	6	.000	.0000	.0	.0	
	sp-MWS6	6	.000	.0000	.0	.0	
10	sp-MWS1	6	.000	.0000	.0	.0	
	sp-MWS3	6	.000	.0000	.0	.0	
	sp-MWS6	6	.000	.0000	.0	.0	

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 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 bioautografi 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

one of the germs that causes the second Gram-negative nosocomial infection after *Escherichia coli* (Podschun, 1998, Irawan D et all, 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 *Streptomyces* sp. for 4-6 days so that *Streptomyces* 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 *Streptomyces* sp-MWS1, *Streptomyces* 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 s*p-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 $p \leq 0.05$. 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.

Refferences

Alexander SK and Strete D, 2001. *Microbiology: A Photographic Atlas for the Laboratory*. Addison Wesley Longman. Inc.

Borodina I, Preben K, Jens N, 2005. *Genome-Scale Analysis of Streptomyces coelicolor A_3 (2) Metabolism*. Genome Research. 15: 821.

Bradford PA, 1997. Imipenem resistance in *Klebsiella pneumoniae* is associated with the combination of ACT-1, a plasmid –mediated AmpC β-lactamase, and the loss of an outer membrane protein. *Antimicrob. Agents Chemother*. Vol. 41. 563-569.

Bradford PA, 2001. Extended Spectrum β-lactamases in 21 st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat, *Clinical Microbiology Review*. American Society for Microbiology, October 2001, p.933-951.

Centers for Disease Control and Preventions. Klebsiella pneumoniae in healthcare settings. Available from:www.cdc.gov/HAI/organisms/klebsiella/klebsiella.html. Last updated: August 27, 2012

Choi SB, Normi YM, Wahab HA, 2009. Why Hypothetical Protein KPN00728 of *Klebsiella pneumonia* Should Be Classified as Chain C of Succinate Dehydrogenase?. *Protein J*. Vol. 28. 415-427.

Drawz SM, Bonomo RA, 2010. Three Decades of β-lactamase Inhibitors. Clin Microbiol Rev 23: 160-201.

Hopwood DA, 1999. Genetic Contributions to Understanding Polyketide Synthases. *Chemical Rev*. Vol. 97. 2465-2497.

Hostacka A, 2000. The in Vitro Effect of Imipenem and Ofloxacin on Enzymic Activity of Klebsiella Penghasils. *Folia Microbiology*. Vol. 45 (5): 387-390.

Irawan D, Hamidah, Purwati, Triyono, Bramantono, dkk. Profil Penderita Sepsis Akibat Bakteri Penghasil ESBL. J Penyakit Dalam. 2012. Volume 13 No. 1

Isnaeni, 1998. *Mutasintesis Antibiotik Mutan Streptomyces griseus ATCC 10137*, Disertasi, ITB, Bandung.

Jawetz, Melnick, and Adelberg, 2015. *Medical Microbiology*, 27st ed. Appleton and Lange, USA.

Katzung BG, 2018. *Basic and Clinical Pharmacology*, 14th Edition. The McGraw-Hill companies, Inc. United States of America.

Korn FW and Jurgen HK, 2002. *The Family Streptomycetaceae*. www. biology. eku. edu/piece/streptomyces.

Lestari, 2009. *Fecal Carriage of ESBL Producing Enterobacteriaceae Among Patient and Healhty Persons From Java Indonesia*. Rotterdam: Departemen of

Medical Microbiology and Infectious Diseases, Erasmus Universiteit.

Madigan TM, Martiuko JM, Parker J, 2002. *Biology of Microorganisms*, 9 th edition. Prentice Hall International. London.

Noor YR, Khazal M, Suryadiputra INN, 1999. *Panduan Pengenalan Mangrove di Indonesia*. PKA/WI-IPB.

Paterson DL and Bonomo RA, 2005. Extended Spectrum β-Lactamases: a Clinical Update. *Clinical microbiology reviews*. Vol. 18 (4): 657-686.

Pfaller MA and Segreti J, 2006. Overview of the Epidemiological Profile and Laboratory Detection of ESBL. *Clinical Infectious Diseases*. Vol. 42.

Pitout JDD and Laupland KB, 2008. Extended Spectrum β-Lactamase-producing *Enterobacteriaceae*: an Emerging Public-health Concern. *Lancet Infect Dis*. Vol. 8. 159-166.

Podschun R and Ullman U, 1998. *Klebsiella* spp as Nosocomial Pathogens: Epidemiology, Taxonomy, Typing Methods, and Pathogenicity Factors. *Clinical Microbiology Reviews*. Vol. 11 (4): 589-603.

Prescott LM, John PH, Donald AK, 2003. *Microbiology*. McGraw Hill Higher Education. Singapura. 546-547.

Rang HP, Dale MM, Ritter JM, Flower RJ, 2007. *Pharmacology*. Churchill Livingstone Elsevier.

Ramphal R and Ambrose P, 2006. Extended Spectrum β-laktamases and Clinical Outcomes: Current Data. *Clinical Infectious Diseases*. Vol. 42.

Retnowati W, 2008. *Diversitas Genotipe 16S rRNA Streptomyces sp. Isolat Tanah Ekosistem Mangrove Pantai Timur Surabaya*. Disertasi. Universitas Airlangga. Surabaya.

Sahly H, Aucken H, Benedi V.J., Forestier C, Fussing V, Hansen D.S.,Ofek I, Podschun R, Sirot D, Sandvang D, Tomas J.M., Ullmann U, 2003. Impairment of Respiratory Burst in Polymorphonuclear Leukocytes by Extended- Spectrum Beta-Lactamase-Producing Penghasils of *Klebsiella pneumonia*. *Eur J Clin Microbiol Infect Dis*. Vol. 26. 20-26.

Sharma, 2010. Plasmid Profil of ESBL Producing Gram-Negative Bacteria and Correlation with Susceptibility to β-Lactam Drugs. *Indian Journal of Pathology and Microbiology*; 53(1), January-March 2010. http://www.ijpmonline.org. Akses: 17 Maret 2010.

Vakulenko SB and Mobashery S, 2003. Versatility of Aminoglycosides and Prospect for Their Future. *Clinical Microbiology Reviews*. Vol. 16 (3): 430-450.

Wan Ho M, 2008. *Rekayasa Genetika*. Edisi Bahasa Indonesia. Yogyakarta: InsistPress.

Wibowo J, 1990. *Teknologi Fermentasi*. Proyek Pengembangan Pusat Fasilitas Bersama Antar Universitas (Bank Dunia XVII) PAU Pangan dan Gizi. Universitas Gajah Mada. Yogyakarta.

Wong A and Beringer, 2001. Therapeutic Challenges Associated with Extended-Spectrum Beta-Lactamase- Producing E. Coli and K. pneumonia. *J Pharmacotherapy*; 21 (5): 583-59.