Antibiotic potency of \textit{Straptomyces drozdowiczii} on white \textit{Rattus norvegicus} which is infected with \textit{Acinobacter baumanii}

R Kawuri$^{1,3}$, I B G Darmayasa$^1$, C Gading$^2$

$^1$Biology Department Faculty Mathematic and Natural Sciences Udayana University, Bali, Indonesia.
$^2$Faculty of Medicine Udayana University, Bali, Indonesia.
$^3$Author to whom any correspondence should be addressed. E-mail: microbiologylaboratory@yahoo.com

Abstract. \textit{Acinobacter baumannii} multi drug resistant (MDR) bacteria are recognized as one of the aerobic bacteria resistant to some antibiotics. The aim of this study is to find appropriate doses of antibiotics used in white mice infected with MDR \textit{A baumannii}; toxicity liver organ and histopathology of treated white mice. Mice was infected with \textit{A baumanii} one day before treatment. Antibiotic \textit{Streptomyces drozdowiczii} dose treatment with concentration 7%, 6%, 5%, 4% and control (without treatment) and positive control with ciprofloxacin. Blood culture is taken after 5 days to determine total bacteria \textit{A baumanii} and liver function. Liver histopathology analysis is done by histological incisions with paraffin and staining methods. The results showed that \% of live animals were antibiotic concentrations of 4% 5%, 6%, 7%, positive controls and negative controls, ie sequentially 40%, 100%, 100%, 100%, 100% and 100%. All animals did not show liver damage from SGOT and SGPT analyzes. Histopathological analysis showed liver cells in animals tried positive control, negative control and administration of 7% antibiotic showed normal liver cells, Conclusion; 7% antibiotic is the best by not damaging the liver and \textit{A baumanii} bacteria is not present in the blood.

1. Introduction
Resistant \textit{A. baumannii} bacteria are often the cause of infectious and endemic outbreaks. This is supported by its ability to survive in various environmental conditions for a long time [1] and can even colonize in the hands of health workers [2]. These resistant \textit{A. baumannii} bacteria are known to have a broad clinical spectrum such as bacteremia, pneumonia, meningitis, urinary tract infections, skin and soft tissue infections, bloodstream infections, endocarditis, intra-abdominal abscesses, and surgical wound infections [3]. Resistance of \textit{A. baumannii} to several antibiotics is known to have begun to plague in 1992 at the Barcelona, Spain hospital. In 1997, \textit{A. baumannii} appeared resistant to carbapenem groups [4]. Acinobacter baumannii was a major concern because it had led to Multidrug Resistant \textit{A. baumannii} (MDR-A. baumannii) [5]. It is known that MDR-\textit{A baumannii} has been endemic in Asian and Middle Eastern countries [6]. To overcome bacterial resistance to antibiotics, it is necessary to find a new antibiotic. Filtrate Streptomyces sp. KCM2 can inhibit growth of MDR-\textit{A baumannii} with an inhibition zone of 23.44 mm with a Minimum Inhibitory Concentration (MIC) of 4\% (v / v) [7].

\textit{Streptomyces} sp. CMCM2 was identified as \textit{Streptomyces drozdowiczii} strain MDDM-11 16S ribosomal gene RNA, partial sequence (with 89\% similarity). The antibiotic filtrate produced by \textit{Streptomyces drozdowiczii}, which was analyzed using GCMS had 10 peaks, of which 3 peaks were the same. All 7 of them acted as antimicrobials and were identified as Cyclohexene (CAS) cyclohexene (10.53\%), 1Methoxy1buten3yne (14.60\%), Butanenitrite (CAS) nutyronitrite (10.44\%),
1Pentane3yne2methyl (12.85%), 3.3 .5Trimethylcyclohexylamine (13.37%), 9Borabicyclo (422) nonane-dimer (2.3%), 3Methyl2-oxo-2pyrane 6carboxylic acid (9.6%) [8].

The aim of the study was to find the right dose of antibiotics used in white rats infected with MDR A baumannii, to test the toxicity of the liver and to investigate the histopathology of the liver from treated white rats.

2. Method

Streptomyces drozdowiczii was grown for 5 days on YEMA media and incubated at 28 ± 2°C. Streptomyces drozdowiczii which was cultured in Petri dishes was taken using cork borer with 5 mm in diameter as many as 5 pieces and put in a glass bottle that had been filled with 100 mL of Yeast Extract Malt Broth media. Then incubated in a shaker with a speed of 80 rpm for 14 weeks. Then the centrifugation process was carried out at a speed of 10,000 rpm for 15 minutes so that the supernatant and pellet parts were obtained. The supernatant is filtered using a filter paper measuring 0.45 μm. The part taken is the filtrate while the residue is removed. The filtrate is then given the same volume of butanol (v / v) allowed to stand for 6 hours in the separating funnel. Antibiotic filtrate with n butanol solvent was evaporated with an evaporator and ready for use in experimental animals [9].

2.1. Toxicity tests on liver

Toxicity tests on liver and kidney white rats (Rattus norvegicus) were carried out by providing 30 male rats that had been acclimatized weighing about 30g. Antibiotic dosage treatments were concentrations of 7%, 6%, 5%, 4% and Control (without treatment) and positive control using ciprofloxacin antibiotics. Each treatment was repeated 5 times to obtain a total sample of 30 white rats. A total of 20 mice were given 1 mL of filtrate with different concentrations every day for 5 days by giving orally. Whereas the next 5 mice were only given aquadest and 5 mice were given ciprofloxacin antibiotics at the same time.

After the end of the study the rat was anesthetized with ether. Rat blood collection is done through the eyes with a capillary pipette and collected in a test tube and then placed at room temperature in a sloping position. The frozen blood is then centrifuged at 3000 rpm for 20 minutes and the serum separated. SGOT levels, SGPT can be determined using a Medical System Analyzer with a fluitest crea kinetic reagent, and cool reagent. Calculation of total bacteria from serum is done by dilution method.

2.2. Histopathology of the liver

The difference in liver cells from rats used as controls with treated mice can be seen by making a heart incision. Rats that have been anesthetized and their blood has been taken are then dissected and their organs are taken and put in a container that contains a formalin buffer in order to prevent damage to the cells and the tissues that make up the organ. Then the organ was inserted into a small flakon which contained Bouins solution and fixed for 24 hours.

Then dehydration with multilevel alcohols from 70%, 80%, 90% and 100% (carried out twice) for 15 minutes each. After that, it is de-alcoholized with pure xylol for 24 hours. The next process, the organ was infiltrated with paraffin consisting of 3 stages: paraffin I for 30 minutes, paraffin II for 60 minutes and paraffin III for 90 minutes, all of these stages were incubated at the same temperature of 80°C. Then the liver and kidney organs are placed in paraffin blocks and then left to freeze and then shaped like a trapezium. This shape is attached to the microtum holder. Blocking is done with 8μm thickness until a long slice band is formed. Paraffin tape is placed on a glass object that has been smeared with Meyers albumin and dripped with water then placed on a hot plate until it is dry. Then continued with dewox namely preparations put into xylol then dehydrated with 100% level alcohol (done 2 times), 96%, 80%, 70% for 5 minutes each. Preparations were then stained with hematoxylin erlich 15 minutes then rinsed with 70% water and alcohol and re-stained with eosin. Preparations are re-dehydrated with alcohols of 70%, 80%, 96% and 100% (carried out twice) for 5 minutes each, then the purification process is carried out using xylol and given DPX adhesive media and covered with a glass cover and dried over hot plate. Preparations were observed under a microscope with 10 or 40 times enlargement and documented [10].
3. Results and Discussion
The percentage of live rats data after being given antibiotic dose treatment for 5 days after being infected with pathogen *A baumanii* showed negative control and doses of 5%, 6% and 7% of animals tried to live 100%, while positive control with antibiotic Ciprofloxasin% lived experimental animals by 80% and antibiotics 4% by 40% (Table 1).

**Table 1.** Percentage of life of rats after being given antibiotic dose treatment for 5 days after infection with *A. baumanii* pathogen.

| No | Treatment | Dead rats | % live |
|----|-----------|-----------|--------|
| 1  | Control + | 1         | 80 %   |
| 2  | Control - | 0         | 100 %  |
| 3  | Antibiotic 4 % | 3  | 40 %   |
| 4  | Antibiotic 5 % | 0  | 100 %  |
| 5  | Antibiotic 6 % | 0  | 100 %  |
| 6  | Antibiotic 7 % | 0  | 100 %  |

The total bacteria in animal blood trying to show the number of *A baumanii* bacteria varied as shown in Table 2.

**Table 2.** The total number of *A. baumanii* bacteria in the blood of the rat after treatment.

| No | Treatment   | Total bacteria, cfu/ml |
|----|-------------|------------------------|
| 1  | Control +   | 340                    |
| 2  | Control -   | 0                      |
| 3  | Antibiotic 4% | 240                    |
| 4  | Antibiotic 5% | 160                    |
| 5  | Antibiotic 6% | 120                    |
| 6  | Antibiotic 7% | 0                      |

3.1. SGPT SGOP Analysis
Analysis of SGOT and SGPT was carried out in the Regional Laboratory of Bali Province. The results showed that all antibiotic doses had no effect on liver function (Table 3). The normal concentration of SGOT in rats was 76 - 208 U/L, while SGPT ranged between 30 - 314 U/L [11]

**Table 3.** Results of analysis of liver SGOT and SGPT

| No | Code Sample | SGOT       | SGPT       |
|----|-------------|------------|------------|
| 1  | Control +   | 200 U/L    | 60 U/L     |
| 2  | Control -   | 310 U/L    | 110 U/L    |
| 3  | Antibiotic 4% | 320 U/L    | 100 U/L    |
| 4  | Antibiotic 5% | 210 U/L    | 150 U/L    |
| 5  | Antibiotic 6% | 150 U/L    | 100 U/L    |
| 6  | Antibiotic 7% | 160 U/L    | 80 U/L     |

3.2. Histopathology Analysis
The results of liver histopathology analysis showed that positive control, negative control and 7% antibiotic dose treatment were normal liver cells (Figure 1), while the treatment of antibiotic doses of 4% 5% and 6% liver cells experienced showed mild liver damage and could return to normal when treatment was stopped (liver cells experiencing hydrophic degenerative) (Figure 2).
Figure 1. Rats liver histopathology. Control positive (a); Control Negative (b) and Dose 7% of antibiotic (c) shows normal liver cells (Arrows)

Figure 2. Rats liver histopathology Dose 4% (a); Dose 5% (b) and Dose 6% of antibiotic (c) shows hydrophic degenerative liver cell (Arrows)

The results of the SGOT and SGPT blood serum analysis of rats showed that it was still categorized as a normal range of positive control, negative control and all treatments for antibiotic dosing. The results of histopathological analysis of the liver showed positive control treatment, negative control and antibiotic dosing treatment of 7% of liver cells showed normal conditions while treatment of 4%, 5% and 6% antibiotic doses of liver cells experienced hydrophic degeneration.

Fluoroquinolones, including ciprofloxacin, are metabolized in the liver and excreted by the kidneys. Side effects of fluoroquinolones in the liver include transient increases in enzyme levels of liver function, cholestatic jaundice, hepatitis, and liver failure. Liver enzyme abnormalities have been recorded in 2%-3% of all recipients of fluoroquinolone and 1%-3% of recipients of ciprofloxacin [12].

The highest levels of serum transaminases and alkaline phosphatase occur. There is usually mild elevation that is not associated with clear and reversible clinical findings with discontinuation of therapy. Elevated serum aspartate aminotransferase levels, alanine aminotransferase, or alkaline phosphatase occurred in 24.9, 24.0, and 17.8 patients per 1,000 treated with ciprofloxacin, ofloxacin, and pefloxacin, respectively [13].

Patients with Drug Induced Liver Injury (679), 12 had hepatotoxicity from fluoroquinolones (6 ciprofloxacin, 4 moxifloxacin, 1 levofloxacin, and 1 gatifloxacin). There were 4 cases of hepatocellular injury (increased levels of alanine aminotransferase, 4 cases of cholestatic injury (elevated alkaline phosphatase levels) and 4 mixed cases [14].

More severe liver injuries associated with fluoroquinolones remain rare. The incidence of hepatotoxicity due to ciprofloxacin is considered very low, although two fatal cases of acute liver failure in elderly men have been documented [15]. Ciprofloxacin sometimes causes acute liver injury within two days to two weeks after the start of antibiotic treatment. Hepatotoxicity can occur as a result of direct chemical effects because many drugs are concentrated in the liver; hepatotoxicity can also occur due to drug allergies or hyper sensitivity reactions [16]. Fluoroquinolone liver injury can be very severe, causing prolonged jaundice, acute and chronic liver morbidity and failure resulting in death or transplantation.
This shows that the 7% antibiotic dose is the best because the liver cells are normal and supported by the total number of bacteria in the blood negative compared to the treatment of other antibiotic doses. Positive control where the experimental animals were given ciprofloxacin antibiotics 1.3 mg of body weight of rats in their blood there were still *A baumannii* bacteria of 340 cfu / ml. The percentage of rat deaths also showed an antibiotic dose of 7% of all live animals (100%). Practically, the results of this study can be used as a reference to develop treatment strategies using new antibiotics produced by Streptomyces drozdowiczii to control diseases caused by Multi Drug Resistant *Acinobacter baumanii* (MDRA-baumanii).

4. Conclusion
Antibiotic dose of 7% is the best because it does not damage the liver and *A baumannii* bacteria is not present in the blood.

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