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Antibiotic Sensitivity in *Pseudomonas aeruginosa* of Diabetic Patient's Foot Ulcer

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Abstract

Diabetes Mellitus (DM) patients are at risk to have the diabetic ulcer. The main reason for DM's patient with ulcer complication to be treated and healed in hospital is bacterial infection. One of many bacteria that infects diabetic ulcer is Pseudomonas aeruginosa. The effort to treat this infection is by using antibiotic. The use of antibiotic unfortunately, is often found inaccurate causing the microbe resistance to occur. To choose the right antibiotic, it needs to test the antibiotic's sensitivity towards Pseudomonas aeruginosa. The aim of this study is to determine the sensitivity of antibiotics against Pseudomonas aeruginosa. Sample used was taken from diabetic ulcers swab with grade III and IV Wagner. The identification of bacteria was managed using the biochemical test and Gram staining test. Antibiotic sensitivity was determined by Kirby Bauer method. Antibiotics that were found still sensitive towards Pseudomonas aeruginosa included ciprofloxacin, norfloxacin, imipenem, levofloxacin, meropenem, ceftriaxone, and cefotaxime, whereas cefadroxil and amikacin were resistant. Antibiotics that can be used for Pseudomonas aeruginosa in diabetic foot ulcer patients are ciprofloxacin, norfloxacin, imipenem, levofloxacin, meropenem, ceftriaxone, and cefotaxime.

Keywords : antibiotic, diabetic ulcer, Pseudomonas aeruginosa

BACKGROUND

Diabetes mellitus (DM) is a dangerous disease that is often called the silent killer. Diabetes mellitus is one of the degenerative diseases that require a careful handling. South-East Asia has a greatest rise in diabetes prevalence that is 8.6% (WHO, 2016). Indonesia is at the fourth position as the country with the highest number of people with 8.4 million people with the cases of diabetes mellitus. International Diabetes Federation predicts the number will increase in 2030 reached 21.3 million patients (Wid *et al*., 2004). Patients with diabetes are at risk for diabetic ulcers. DM patients are estimated to experiencing diabetic ulcers by 15% and 3-4% exposed to severe infections (Frykberg *et al*., 2006). The treatment of diabetic ulcers can be given by reducing the pressure on the skin. Surgery and the use of antibiotics are also important for the treatment of infected ulcers. Antibiotics are a class of drugs often used to treat the infection. However, the use of antibiotics is often imprecise leads to microbial resistance.

Diabetic ulcers are open sores on the skin surface. It is possible for complications in macroangiopati causing vascular insufficiency and neuropathy develop into an infection caused by aerobic and anaerobic bacteria (Tambunan, 2007). Diabetic patients with ulcers of Gram-negative bacteria are identified at most, which is 7 times more compared with gram-positive bacteria (Aulia, 2008).

Based on research from Sari and Apridamayanti (2015), it has been found that gram-negative bacterium, such as *Pseudomonas aeruginosa* is one of the bacteria that have the highest percentage in patients with diabetic ulcers. It is also reinforced in research of Manisha (2012) that the most Gram-negative bacterial pathogens in diabetic ulcers is *Pseudomonas aeruginosa* 48 (30.57)%, Klebsiella spp 35 (22.29), Escherichia coli 26 (16.56%) and Proteus sp 8 (4.37%).

As revealed in the research of Aulia (2008), *Pseudomonas aeruginosa* has the highest level of resistance to doxycycline, streptomycin, ampicillin, and erythromycin. On the other hand, there are few antibiotics that are still sensitive to *Pseudomonas aeruginosa* such as meropenem, cefotaxime, and amikacin.

Based on the above background this research was conducted to determine the sensitivity of antibiotics against *Pseudomonas aeruginosa* bacteria found in diabetic ulcers Wagner stage III and IV to help direct the administration of antibiotics in patients with diabetic ulcers.

Objectives

The aim of this study is to determine the sensitivy of antibiotic class of aminoglycoside (amikacin), class of cephalosporin (cefadroxil, ceftriaxone, and cefotaxime), class of carbapenem (imepenem and meropenem), and the class of quinolones (ciprofloxacin, levofloxacin and norfloxacin)

against *Pseudomonas aeruginosa* at the foot ulcers of diabetic degrees III and IV Wagner. It is also included the resources for the promotion of the prevention of antibiotic resistance on patients with diabetic ulcers degrees III and IV.

METHOD

Sampling

Samples of bacteria were taken from diabetic ulcer swab degrees III and IV Wagner in Clinic Kitamura Pontianak, West Kalimantan at 2015. Sample were taken using a sterile swab, then stored in a sterile transport medium and sealed. Sample was taken based on ethical clearence number 4270/ UN 22.9/ DT/ 2015. Bacteria contained in a sterile swab planted in the media blood agar and Mac-Conkey.

Isolation of *Pseudomonas aeruginosa*

The bacteria are grown on media blood agar and Mac-Conkey. Planting bacteria were performed directly on solid agar media and incubated for 24 hours in an incubator at a temperature of 32-40 °C (Aulia, 2008).

Identification of *Pseudomonas aeruginosa*

Identification has been performed with the Gram stain test and biochemical tests. Biochemical test was conducted on the test fermentation of sugars, fermentation of carbohydrates, motility, indole, H_2S production, urea, oxidase, and fermentative-oxidative (Forbes, 2002).

Antibiotic sensitivity testing

The test was performed using the Kirby-Bauer method used antibiotic disks . Media used was Mueller Hinton Agar (MHA) (Aulia, 2008). Meanwhile, the antibiotic disk used included ciprofloxacin, norfloxacin, levofloxacin, amikacin, cefadroxil, ceftriaxone, cefotaxime, imepenem and meropenem. The determination of antibiotic sensitivity was performed based upon the guidelines of Clinical Laboratories and Standards Institute (2014).

RESULT AND DISCUSSION

Isolation of *Pseudomonas aeruginosa*

The bacteria grown in two media included Mac-Conkey Agar (MCA) and Blood Agar Plate (BAP). This planting used the scratch method that has been selected for being more practical, economical and not time consuming as compared to the casting method requiring longer time and more materials. First of all, a sterile swab in amies media was lubricated on both media, and then looped round heated to glow aside some time after being etched in a zigzag pattern on both media. Planting bacteria were carried on solid agar medium and incubated for 24 hours in an incubator at a temperature of 32-40 ^{o}C. Once morphological observation was completed bacteria grew on both media.

Mac Conkey (MC) is a selective differential medium used to see the ability of bacteria to ferment glucose. *P.aeruginosa* colonies when grown will not be coloured because it is not

able to ferment lactose as shown in Figure 1. The blood agar media is a differential medium that can differentiate bacteria based on their ability to lyse red blood cells. *P.aeruginosa* colonies formed are round, convex, transparent and uneven edges. *P.aeruginosa* experience haemolysis Beta (β) or so-called haemolysis total, defined as the entire lysis of red blood cells. A clear zone, close to the colour and transparency of a basic media, surrounded the colony as seen in Figure 2.

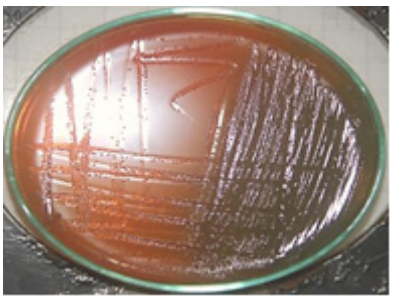

Figure 1. Colonies of Pseudomonas aeruginosa on Mac-Conkey agar

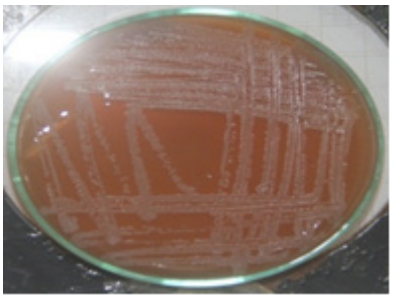

Figure 2. Colonies of Pseudomonas aeruginosa on blood agar

Identification of *Pseudomonas aeruginosa*

The identification of *Pseudomonas aeruginosa* was performed using Gram staining and biochemical tests. Biochemical tests included the fermentation of sugars, fermentation of carbohydrates, motility, indole, H_2S production, urea, oxidase, and fermentative-oxidative.

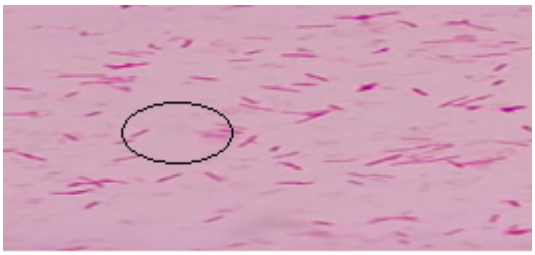

Figure 3. Gram staining Pseudomonas aeruginosa in microscopy
Description: The bacteria Pseudomonas aeruginosa is a gram-negative bacteria and shaped bacillus

As seen in figure 3, Gram staining test results showed that the bacterium *P.aeruginosa* was a Gram-negative bacterium for showing some results in red. The results were obtained in accordance with the description Mayasari (2006) stating that *P.aeruginosa* has been shaped gram-negative bacterium bacillus, *Pseudomonas aeruginosa* is a Gram-negative bacteria because bacteria are in red cells. The red colour as seen in bacterial cells was due to the release of the dye crystal violet for leaching using alcohol. The cell wall of Gram-negative bacteria that have a peptidoglycan layer is thinner than Gram-positive bacteria. Gram-negative bacteria with a thin peptidoglycan layer would be more easily dislodged and replaced by safranin red. Safranin got into the cells of bacteria and replaced crystal violet so that the colours seen are red. Bacterial biochemical test is a method or treatment performed to identify and determine a pure culture of the isolated bacteria through the properties of physiology. Table 1

Table 1. Test results biochemistry *Pseudomonas aeruginosa*

Type of Test	Result	Information
Glucose	-	Not forming yellow
Lactose	-	Not forming yellow
Mannitol	-	Not forming yellow
Sucrose	-	Not forming yellow
Maltose	-	Not forming yellow
Citric	+	Forming in blue
Motility	+	Spreading white
Indole	-	Not forming a layer of red ring
Urea	-	Not forming pink
Oxidase	+	Forming in blue
H_2S	-	Not forming a black precipitate
Fermentative/ Oxidative	Oxidative	The colour changes to yellow on one of the tubes

shows that the bacteria *Pseudomonas aeruginosa* in motility test was positive in this case as shown by the spread of white as the roots around the inoculation. This showed the movement of bacteria inoculated, indicating that these bacteria had flagella. The bacteria were also positive in the test Simmon citrate as indicated by a colour change from green to blue.It shows that these bacteria utilize citrate as a carbon source.

Oxidase test is a biochemical reaction carried out to see their cytochrome oxidase, an enzyme that is usually called indophenol oxidase. Test oxidative/fermentative was conducted with an aim to know the nature of oxidation or fermentation of bacteria to glucose by using two tubes, one of which was as media to use paraffin. *Pseudomonas aeruginosa* results of discoloration on one of the tubes from green to yellow indicate the bacteria from oxidative.

The results showed that the bacterium was identified as the bacterium *Pseudomonas aeruginosa.* These results are consistent with Sulistiyaningsih (2010) showing that the bacteria *Pseudomonas aeruginosa* in biochemical tests of glucose, lactose, mannitol, maltose, sucrose, indole, urea, H_2S showed a negative result on the test, while motile, oxidase and Simmons citrate showed positive ones.

Antibiotic sensitivity test

Antibiotic sensitivity testing was carried out on the positive samples *Pseudomonas aeruginosa* and was selected randomly. Media test using Mueller Hinton Agar (MHA). The disk shaped antibiotics were used so that unnecessary antibiotic suspensions manufacture antibiotics. Antibiotics were testedi.e.cefotaxime,ceftriaxone,norfloxacin, imepenem, meropenem, levofloxacin, amikacin, cefadroxil, and ciprofloxacin.

Based on the test results of sensitivity as seen in Table 2, the sensitive antibiotics included ciprofloxacin, norfloxacin, imipenem, meropenem, levofloxacin, ceftriaxone and cefotaxime while cefadroxil and amikacin were resistant toward *P. aeruginosa.*

Table 2. Antibiotic sensitivity test result

Antibiotic	Dose (µg)	Zone of inhibition (mm)	information
Ciprofloxacin	5	35.3	Sensitive
Cefadroxil	30	0	Resistant
Norfloxacin	10	30.67	Sensitive
Imipenem	10	24.67	Sensitive
Meropenem	10	32	Sensitive
Levofloxacin	5	30	Sensitive
Amikacin	30	14.67	Resistant
Cefotaxime	30	34	Sensitive
Ceftriaxone	30	32.67	Sensitive

Ciprofloxacin, norfloxacin and levofloxacin are the quinolone class of antibiotics. Imipenem and meropenem are a class of carbapenem antibiotic as the large class of beta-lactamase. Cefotaxime and ceftriaxone are third generation cephalosporin class of antibiotics (Setiabudy, 2011).

Amikacin is an aminoglycoside class of antibiotics. Resistance of amikacin causes of a genetic mutation that results in the disruption of protein synthesis. In this case, the wrong types of amino acids in a polypeptide chain spliced to form a type of protein that is wrong. While the decline in the antimicrobial activity of amikacin was caused by the modification of enzymes, efflux pumps and increased activity as occurred 16S rRNA methylation. Modification enzymes that occur can be acetylated by the enzyme acetyltransferase, adenylation by nukleotidiltranferase and phosphorylation by fosforiltranferase. Increased efflux pumps may occur because of the XY gene Mex - OPR M which encodes the activation of efflux pump. Methylation 16S rRNA gene can occur because RmtA, RmtB, ARMA and RmtD which encodes bacteria (Meletis and Bagkeri, 2013).

Cefadroxil is a first generation cephalosporin class of antibiotics. This class of antibiotics is more effective for Gram-positive bacteria. Cefadroxil resistance is caused by the formation of beta-lactamase enzymes. This enzyme is produced from gene TEM1, TEM2, and SHV1 and can inhibit the action of cefadroxil namely by hydrolysing the beta-lactam ring contained in cefadroxil so that this drug can not bind to its receptor (Meletis and Bagkeri, 2013).

In previous study from Akbar *et al* (2014), it is stated that meropenem is good choice for diabetes patients with foot ulcer. The same results in this study were obtained by Sulistiyaningsih (2010). However Wahab *et al* (2013) reported that *Pseudomonas aeruginosa* may be sensitive to amikacin. There are different results with this study with Manisha (2012) research which stated that there is resistance in the levofloxacin and ciprofloxacin against *Pseudomonas aeruginosa*. This can occur because of differences in strains of *Pseudomonas aeruginosa* isolates which obtained. The different strains of *Pseudomonas aeruginosa* will affect the sensitivity test results because of the type of protein produced is different (Meletis and Bagkeri, 2013).

CONCLUSION

Pseudomonas aeruginosa isolates in this study were sensitive to levofloxacin, norfloxacin, cefotaxime, ceftriaxone, ciprofloxacin, imipenem, and meropenem, but resistant to cefadroxil and amikacin.

Acknowledgments

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