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ANTIBIOTIC SENSITIVITY PROFILES AGAINST BACTERIA ISOLATES WHICH INFECTING PATIENTS WITH DIABETIC FOOT ULCERS LEVEL 3 AND 4 WAGNER IN PONTIANAK

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ABSTRACT
Diabetic foot ulcer is one of many complications of diabetes. This ulcer can lead to wound infection which happens when a patient has high level of blood glucose. Antibiotic is usually prescribed for diabetic foot ulcer patient to prevent the infection and accelerate the wound healing process. Hence, a potent antibiotic is needed. The aim of this research was to determine the profile of antibiotic sensitivity test against bacteria isolates from patients. The isolates were taken in Kitamura clinic in Pontianak in the year of 2015. Level of sensitivity test was determined from the inhibition zone of certain antibiotic against bacteria cultures from patients' isolates. The result of sensitivity test conducted on *Staphylococcus epidermidis* and *Pseudomonas putida* was 89%, *Micrococcus luteus* and *Enterobacter cloacae* was 55%, *Proteus mirabilis* was 42.8%, *Proteus vulgaris* was 33%, and *Proteus retgeri*, *Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus haemolyticus* and *Escherichia coli* was 11%. The result of the antibiotics sensitivity test was analyzed using One-way ANOVA followed by Tukey Post Hoc test. Result of statistical analysis shows From this statistic result that Ceftriaxon, Cefotaxim, Ciprofloxacin, Meropenem, Piperacillin-sulbaktam, Imipenem, and Amicasin were sensitive to patients' isolates, while Cefadroxil (72.70%) and ; Clindamycin (100%) resistance to patient isolates DFU.

KEYWORDS: antibiotic sensitivity, diabetic foot ulcer (DFU), resistance, Pontianak.

INTRODUCTION
Public health can be affected by food, level of stress, and regularity of exercise. Diabetes mellitus is a metabolic disease with hyperglycemia characteristics or elevated blood glucose levels above normal which is caused by the abnormalities of insulin secretion. It also can lead to impaired metabolism of carbohydrates, fats, and proteins. Indonesia is ranked fourth highest in the world for 8.4 million people having diabetes. International Diabetes Federation predicts there will be an increase in number of people with diabetes in Indonesia in 2030 to 21.3 million people.[1]

One of long-term diabetes mellitus complications is diabetic foot ulcer. Diabetic foot ulcer (DFU) is open wound on the foot where there is nerve damage in the leg because of the high blood glucose level. The research done by Wahab et al., 2015 shows that the number of people with diabetic foot ulcer stage III and IV have the high proportion that is 27 (35%) for patient stage III and 23 (29.9%) for patient stage IV.[3] In diabetic foot ulcer type III, it occurs in-abscess with or without osteomyelitis, while diabetic foot ulcer IV occurs in toe gangrene or distal foot with or without cellulitis. This condition is closely related to the delay in diagnosing the disease and taking care the wound as well as the extent of tissue damage.[4]

Diabetic foot ulcer (DFU) are common and serious problem in patient with diabetes, which is caused by anaerobic Positive-Gram bacilli and anaerobic Negative-Gram bacilli or anaerobe which spread rapidly in the foot causing the damage to tissue.[5] Antibiotic can be one of treatments in handling the infection. However, the irrational use of improper antibiotic and giving therapeutics of antibiotic can cause bacterial resistance to several antibiotic. It leads to other problems, such as rising cost of treatment, reduction in anti-bacterial activity, amputation or even causing the death. The repeated and improper use of antibiotics is the main cause of the increase in the number of bacteria that are resistant to antibiotic.[6] Based on the background and previous research, the researchers are interested in doing the research related to the antibiotic resistance pattern in isolated bacteria which infects the diabetic foot ulcer stage III and IV wagner. The aim of this research is determining antibiotic resistance profile occurring to diabetic foot ulcer stage III and IV Wagner arising due to

the use of antibiotic while wound care treatments, antibiotic used, culture isolate and sensitivity of antibiotic.

MATERIAL AND METHODS

Material

Materials used are petri dish (Iwaki pyrex), Ose needle, object glass, test tube (Iwaky pyrex), tweezers, digital camera (Canon), hot plate, erlemeyer (Iwaki pyrex), beaker glass (Iwaki pyrex), autoclave, microscope (Olympus CX51), incubator, Laminar Air Flow, bunsen, fredge, and calipers. NaCl 0,9%, Ames transport, Mc Farland (oxoid), blood agar plate, Mac Conkey Agar (oxoid), Salmonella Shigella Agar, crystal violet (merck), alkohol 96%, fukhsin (merck), lugol's iodin, Glucose (merck), Sacarose (merck), Maltose (merck), Manitol (merck), Sucrose (merck), Triple Sugar Iron Agar (TSIA) (oxoid), Simmon Citrate agar (oxoid), Sulfur Indole Motility Media (SIM) (oxoid), Urea Agar Base (oxoid), Glucose Of-Gelatin (oxoid), Nutrient Agar (merck) and Antibiotic discs were used in this research are Aztreoname (30µg), Clindamycin (10µg), Piperacilim-tazobactam (110µg), Ciprofloxacin (5µg), Cefadroxil (30µg), Gentamicin (10µg), Meropenem (10µg), Imipenem (10µg), Levofloxacin (5µg), Amikacin (30µg), Ceftriaxone (30µg) and Cefotaxime (30µg).

Sample

In this research the population is drawn from patient who have been diagnosed with diabetic foot ulcer stage III and IV Wagner by doctor at Kittamura Clinic based on medical record in october to november 2015. This research has been approved by ethics committee from Faculty of medicine University of Tanjungpura ethics commission no 4870/UN22.9/DT/2015. The sample were grown and identified in Health Laboratories Unit Pontianak. The samples were collected by Purposive Sampling methode. The sample size that was used to monitor the wound condition was determined by Slovin formula.

Isolation of Bacteria

Ames transport were incubated for 18-24 hours at 37°C. Bacteria was isolated by scraping upward growth blood agar media, Mac Conkey and Salmonella-Shigella agar and replicated in BHI broth. Bacteria was isolated directly on solid agar media and incubated for 12 hours at 32-40°C.[7]

Sensitivity test of bacteria

Method for determining the diameter of inhibition zone is the agar diffusion disc method. The bacteria species maintained in BHI at -20°C; 300 mL of each stock-culture was added to 3 mL of BHI broth. After overnight incubation, bacterial suspension (inoculum) was diluted with the sterile physiological solution then put the papper dish containing 20 µL of antibiotic were placed on the surface of the agar and then incubated at 37°C for 18-24 hours. Inhibition diameter is measured using calipers. Diameter of inhibition adjusted with CLSI table for each antibiotic.[8]

The antibacterial effectiveness is seen from the diameter of the inhibitory zone which appears as a clear area surrounding the dish of bacteria because of diffused antibiotic. The drag zone diameter is measured from the edge of the dish to the edge of the clear drag zone.

Data Analysis

The data was obtained in the form of the inhibitory zone diameter from each test, then the inhibit zone data was compare to the CLSI table to determine the antibiotic it was sensitive, intermediate or resistant. The inhibit zone data was analyzed using SPSS 18.0 to assess if there were any significant differences in the inhibit zones on the test results in order to make a conclusion which antibiotic had the best sensitivity.

Patient medical record data obtained from the Kittamura clinic in Pontianak was used to observe the antibiotic suitability which is given to the patient, then the data was presented in the diagrams and narratives related to antibiotic suitability with infection.

RESULT AND DISCUSSION

Characteristics of the Test Subject

The study was conducted in October-December 2015 with 24 people who had diabetic ulcers stage III and IV Wagner. Medical record data was obtained from Kittamura Clinic of Pontianak in October until November 2015. Based on medical record data, the description related to gender, age, degree of wound and blood glucose level average was obtained from all tests subjects which is shown in table 1.

According to table 1, it can be seen that the percentage of male having Diabetic Foot Ulcer (DFU) level III and IV Wagner is more than female, that is 58.33%. This research is in line with the research done by Radji et, al (2014) which resulted higher number of DFU prevalence in man than woman. It is because the physical activity level of men is higher than women, thus men become susceptible subject in suffering from diabetic ulcer.[10] Based on Ahmad, et al., 2017 reaserch male predominance due to greather exposure to external environment and trauma. Barefoot walking is one of another common factor of DFU this leads to burns, especially on the feet of diabetic patient and resulting of chronic ulcers. The high number of diabetes is in 46 – 60 years old. It is the productive age group that the adult patients are still actively working and foot ulcer treatment is less intensive. Wild, et al., 2004 stated that there is high number of people with diabetes in age 45–64 years old, thus there will be the high risk of diabetes for that people in those ages. The average blood glucose in this research is 267.27 mg/dl which is categorized as hyperglycemia condition.

Profiles Antibiotic Sensitivity

According to antibiotic sensitivity test result to each bacterial isolate, it can be determined the resistance percentage from each bacterial sensitivity. The tested antibiotic in this research are amikacin, gentamicin, ciprofloxacin, levofloxacin, ceftriaxon, cefadroxil, cefotaxim, imipenem and meropenem. The antibiotic sensitivity test followed the guidelines issued by *Clinical Laboratories Standart Institute* (CLSI). The sensitivity test result can be seen in figure 1.

The results of the test sensitivity can be seen in Figure 2. According to Figure 2 the highest resistance in Positive-Gram bacteria is *Staphylococcus epidermidis* by 89%. In this research, *Staphylococcus epidermidis* is found 4 isolates from patient DFU and antibiotic sensitivity test result show that *Staphylococcus epidermidis* has resistance against Amikacin, ceftriaxone, cefotaxime, piperacillin-sulbactam, cefadroxil, ciprofloxacin, levofloxacin and imipenem. In line with the reaserch Roberto, et al 2013 of resistance patterns of *Staphylococcus epidermidis* with antimicrobial agents cefotaxim, levofloxacin, ciprofloxacin and imipenem are respectively 41%, 42%, 81% and 93% biofilm producer. The resistance in *Staphylococcus epidermidis* is caused by the ability of producing enzymes to break down the β-lactamase enzyme, and the formation of biofilm layer.[12,13] Based on Roberto, et al 2013 *Staphylococcus epidermidis* resistance to antibiotics were assessed by phenotypic as *mec*A gene as well as correlation with biofilm production and the relationship with infection site.

The result of test on gram negative bacteria can be seen in Figure 3. According to Figure 3, the highest resistance in case of gram negative bacteria was observed in *Pseudomonas putida* accounting to approximately 89%. The resistance of antibiotic are Ceftriaxon, Cefotaxim, Piperacillin-Tazobactam, cefadroxil, ciprofloxasin, Levofloxasin, Imiphenem, and Merophenem. According to research by Aneta et al., 2015 *Pseudomonas putida* confers resistance on ticarcillin-clavulanic acid, ciprofloxacin, imipenem, meropenem. In this research, *Pseudomonas putida* is found one isolate from patient DFU and antibiotic sensitivity test result show that *Pseudomonas putida* has resistance against ceftriaxone, cefotaxim, pipperacillin-sulbactam, cefadroxyl ciprofloxacin, levofloxacin, imipenem and merophenem. Ceftriaxone, cefotaxim and cefadroxil are cephalosphorins antibiotics grup; imipenem and merophenem are carbaphenem antibiotics group. Both of them cephalosphorins and carbaphenem group antibiotics also have a beta-lactam ring inside their structures, the resitence occurring because of the ability of bacteria in producing β-lactamase enzyme, that hydrolyzes the main beta-lactam ring[12] but receptors. Ciprofloxacin, and levofloxacin are the fluoroquinolone antibiotics. The mechanism of action from these antibiotics is by inhibition of gyrase and topoisomerase IV enzyme activity in DNA formation. The resistance mechanism in *Pseudomonas putida* occurs through the TtgABC code wich is capable of binding different antibiotic structurally and induces multidrug efflux pumps in responding resistance mechanisms[13]. According to Zgurskaya, et al.,2000, it is known that negative-Gram bacteria have an efflux pump system that RND (Resistance Nodulation Division) – OMF (Outer Membrane Factor) is capable of removing antibiotic inside the periplasm microbes out of cells, it explains one of the mechanisms of resistance that occur in gram negative bacteria, that one of them is *Pseudomonas putida*. The most common isolates found in the patients is *Proteus mirabilis* bacteria of 14 isolates in which *Proteus mirabilis* bacteria has antiviral resitance of 42.8%, i.e cefadroxil, clindamycin, and aztreonam. Pitout et al., 1997 research shows that *Proteus mirabilis* has TEM-1, SHV-1 gene, so it can be concluded that the occurrence of cefadroxil resisting to *Proteus mirabilis* bacteria is due to the presence of TEM-1, SHV-1 in bacteria plasmids. Resistance to aztreonam is associated with the formation of betalactamase enzymes by bacteria. Betalactamases that can hydrolyze the betalactam ring in aztreonam are coded by TEM-3 and SHV-2.

According to the results of sensitivity test on bacteria in isolate patients, it can be seen the antibiotic that have resistance to bacteria. The resistance profile occurring in antibiotic is shown in Figure 4. In Figure 4, the highest antibiotic resistance is in clindamycin antibiotic by 100% and cefadroxil by 72.70%. Clindamycin has the highest resistance on *Staphylococcus aureus* and *Proteus mirabilis* as observed from the result of the sensitivity test performed resistance in clindamycin. It occurs because of resistance to lincosamide wich can occur due to the methylation in ribosome causing obstruction of binding site protein, target mutation, and increased bacterial efflux pump in this case clindamycin resistance occurs due to increased activity of efflux pump.

This study in line with Haritha et al., 2014 reaserch, that the first generation of chepalosphorins drugs such cefadroxyl has show 80% resistance. Cefadroxyl resistance can occur due to structural changes of bacteria, in which bacteria can modify themselves and are capable of producing enzymes that can inactivate antibiotic[16] and according to[6], giving inappropriate antibiotic and unnecessary use of antibiotic may increase resistance.

The sensitivity test results obtained is tested statistically by using SPSS 18.0 to determine the antibiotic which have good activity against test bacteria. These results were then analyzed using One-way ANOVA followed by Tukey Post Hoc test. From this statistic result, it is known that Ceftriaxon, Cefotaxim, Ciprofloxacin, Meropenem, Piperacillin-sulbaktam, Imipenem, and Amicasin were sensitive to patients' isolates.

The Appropriate use of antibiotic

According to Lipsky, et al., 2012, the use of empiric antibiotic is based on patient clinical data and epidemiological data, but the use of definitive antibiotic based on frequent infections in the DFU. Antibiotic use was considered to be partially proper when if it is contained the standart treatment regiment and duration that was indicated foer the patient's clinical infection or prophylaxis. Antibioitic use improper when the clinical condition of the patient did not justify use of antibiotics for either treatment or prophylaxis (incorrect decision and data sufficient).[17] The use of antibiotic is based on bacterial culture test and bacterial sensitivity test.

According to the researches, the analysis to the compatibility of prescribing antibiotic definitive in DFU infection at Kittamura Clinic Pontianak from October-November 2015 can be seen in Figure 5. Figure 5 shows the number of prescribers that are not compatible for the use of a definitive antibiotic with a bacterial culture test inappropriate use antibiotics by 75%, appropriate use antibiotics by 20.83% and untested antibiotic by 4.2%. Antibiotic which is not tested and used as a definitive antibiotic therapy is metronidazole. The use of metronidazole in definitive treatment of DFU patients in this reaserch is not appropriate because there is no anaerobic bacteria found in the patient isolates. According to Brook et al., 2013, the use of antibiotic combination for anaerobic bacteria is based on the condition level of infected bacteria and it can be combined with aerobic bacteria. From this research, it is known that the inaccuracy of drug used in antibiotic with the type of bacteria that infect is 38.9% and excessive antibiotic is 61.1%. According to Krushna, et al., 2010 research there was lack of good laboratories for culturing and sensitivity testing, wich cause improper diagnosis and hence irrational antibiotic prescribing in human. An inappropriate use of antibiotics is one of the causes of antibiotic resistance.

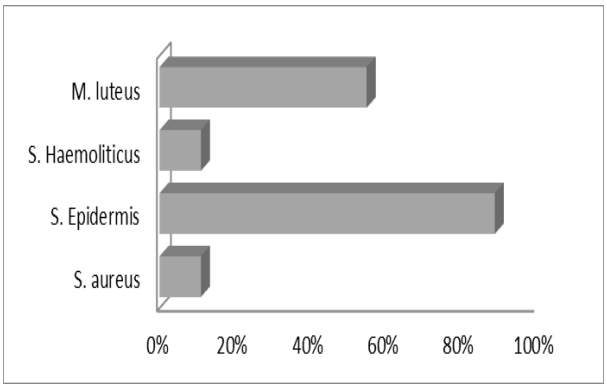

Figure 1: Antibiotic Resistance Pofile Negative-Gram Which Infected Patient Diabetic Foot Ulcer Level III And IV Wagner.

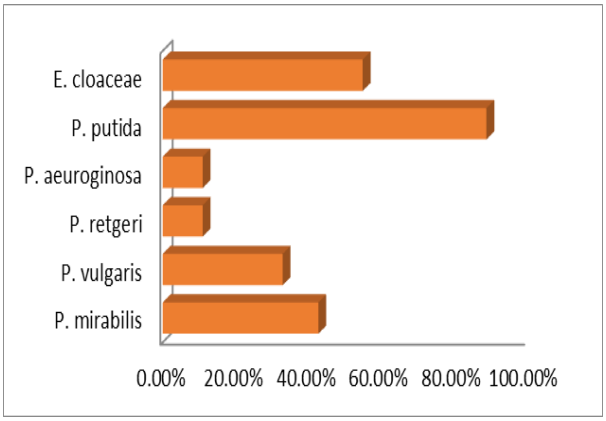

Figure 2: Antibiotic Resistance Pofile Positive-Gram Which Infected Patient Diabetic Foot Ulcer Level III And IV Wagner.

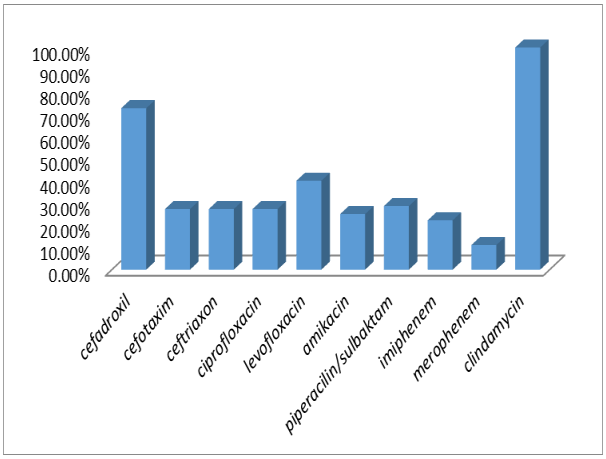

Figure 3: Antibiotic Resistance Profiles.

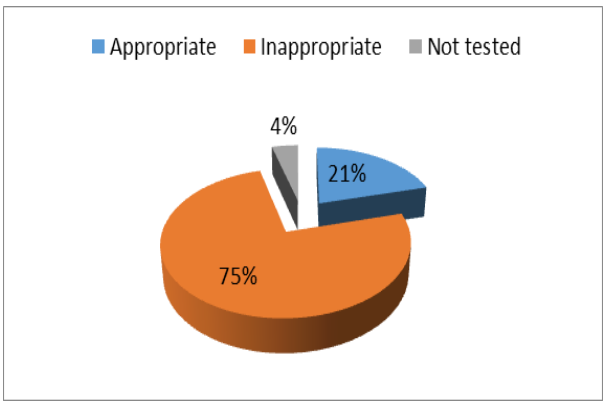

Figure 4: Percentage appropriate use of antibiotic in patient with diabetics foot ulcer (DFU) in Kittamura Clinic Pontianak October-November 2015 periode.

Table 1: Demographic data of test subject.

Variable	Frekuensi (n)	Percentase (%)
Gender		
Man	14	58.33 %
Women	10	41.67%
Age		
< 50 years	7	29.17%
50-59 years	11	45.83%
> 60 years	6	25%
Wound degree		
Level III	15	62.5%
Level IV	9	37.5%
Mean of blood glucose	267.27 mg/dl	

Table 2. Sensitivity Test Towards Some Antibiotics agaist bacteria.

Antibiotics agent	Inhibition Zone (mm)			
	Enterobacter cloacae	***Escherichia coli***	***Pseudomonas aeruginosa***	***Pseudomonas putida***
Cefotaxime	-	40±0.0	34±2.0	-
Ceftriaxone	-	39.3±1.1	32.6±2.3	-
Aztreonam	-*	-*	-*	-*
Clindamycin	-*	-*	-*	-*
Gentamycin	-*	19.6±0.5	-*	-*
Cefadroxil	28±2.0	14.6±1.1	-	-
Ciprofloxacin	-	36.6±1.1	35. ±1.1	-
Amikacin	-	21.3±2.3	34±2.0	20±0.0
Piperacillin–tazobactam	29.3±1.1	-*	-*	-
Levofloxacin	-	35.3±2.3	30±2.0	-
Imipenem	28.6±2.3	28.6±2.3	24.6±1.1	-
Meropenem	31.3±2.3	30±0.0	32±2.0	-

NB. * Tests not Performed

Table 3: The determination of antibiotic sensitivity from isolated patient DFU based on the guidelines of Clinical Laboratories and Standards Institute (CLSI, 2014).

Bacteria	LVP	CFT	CRO	CLD	GTC	CFD	CIP	IMP	MRP	PPC/TZB	AMC
P. mirabilis	S	S	S	R	I	R	S	-	-	-	-
E. cloaceae	R	R	R	-	-	S	R	S	S	S	R
S. epidermidis	R	R	R	-	-	R	R	R	S	R	R
P. rettgeri	S	S	S	R	-	R	S	S	S	S	S
P. aeuroginosa	S	S	S	R	-	R	S	S	S	-	-
E. coli	S	-	S	-	S	R	S	S	S	-	S
S. aeureus	S	S	S	R	S	-	S	-	-	-	-
M. Luteus	R	-	R	-	-	S	R	S	S	S	R
P. putida	R	R	R	-	-	R	R	R	R	R	S
S. haemoliticus	R	S	S	-	-	R	S	S	S	S	S
P. vulgaris	-	-	R	-	-	S	S	S	S	S	S

Nb. (-): not tested
S : Sensitive antibiotic
R : Resistance antibiotic
I : Intermediate antibiotic
LVP : Levofloxacin
CFT : Cefotaxime
CRO : Ceftriaxone
CLD : Clindamicin
GTC : Gentamicin
CFD : Cefadroxyl
CIP : Ciprofloxacin

IMP : Imiphenem
MRP : Merophenem
PPC/TZB: Pipperacillin-Tazobactam
AMC: Amikacin

CONCLUSION

From this research it can be concluded that Ceftriaxon, Cefotaxim, Ciprofloxacin, Meropenem, Piperacillin-sulbaktam, Imipenem, and Amicasin were sensitive to patients' isolates, while Cefadroxil 72.70% and; Clindamycin 100% resistance to patient isolates DFU. The highest resistance bacteria is *Staphylococcus epidermidis* and *Pseudomonas putida* by 89% agaist antimicrobial agent.

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