

Sensitivity of *Escherichia coli* Bacteria Towards Antibiotics in Patient with Diabetic Foot Ulcer

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ABSTRACT

Chronic complication of diabetes mellitus is diabetic foot ulcers. Diabetic foot ulcer can be defined as an open wound in the feet which will become infected as the result of high blood sugar levels that develops and become place of bacteria. One of the bacteria in diabetic foot ulcer is *Escherichia coli*. Imprecise use of antibiotics in the treatment of diabetic foot ulcers can cause antibiotic resistance to bacteria. This study aims to determine antibiotic sensitivity of *Escherichia coli* bacteria in diabetic foot ulcer Wagner grade III and IV. Samples of diabetic foot ulcer swab's with Wagner grade III and IV were taken as *Consecutive sampling*. Identification of *Escherichia coli* bacteria is done by using a biochemical test and Gram stain test. Antibiotic sensitivity test is conducted by using Kirby Bauer's disc of diffusion method. *Escherichia coli* bacteria that were tested are sensitive to amikacine, gentamicine, ciprofloxacin, levofloxacin, ceftriaxone, cefotaxime, imipenem and meropenem antibiotics but showed resistant to cefadroxil.

Keywords: diabetic foot ulcers; *Escherichia coli*; antibiotic sensitivity

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INTRODUCTION

Diabetic ulcers are long-term complications of diabetes. In Indonesia particularly Pontianak, the incidence of diabetic ulcers is still very high. The highest prevalence of diabetes in Indonesia (2008) is located in West Kalimantan and North Maluku (respectively 11.1%) and Riau (10.4%) (Hasnawati *et al.*, 2009).

The number of patients with diabetic foot ulcer grade III and IV has a proportion of 27 (35%) patients with grade III and 23 (29.9%) patients with grade IV (Wahab *et al.*, 2015). In addition, 50% of cases of diabetic ulcer or gangrene will become infected as a result of high blood sugar level for the growth of pathogenic bacteria (Soejahjo & Poerwadi, 1991).

Diabetic gangrene identified that most of the Gram-negative of aerobic bacteria with a percentage of 88% and Gram positive bacteria with percentage of 12% (Aulia, 2008). *Escherichia coli* bacteria is a Gram-negative bacteria which has the highest percentage in patients with diabetic foot ulcer (Zubair *et al.*, 2010). Antibiotics are class of drugs most commonly used for bacterial infections. Appropriate antibiotic use will greatly assist the patient in the healing process in terms of both cost and time of healing (Decroli *et al.*, 2008). On

the other hand the irrational use of antibiotics can cause bacteria resistance problem to some antibiotics. Owing to the a forementioned background and the previous research the researchers are interested in conducting the research aiming at determining antibiotic sensitivity of the *Escherichia coli* bacteria found in diabetic ulcers Wagner grade III and IV.

METHODS

Tools

The tools used in this study, among other things are autoclave, petri dish (*Iwaki pyrex*), beaker (*Iwaki pyrex*), vernier caliper, ose needle, incubators, object glass, LAF, refrigerator, dissecting microscope (*Olympus CX51*), test tubes and erlenmeyer tube (*Iwaki pyrex*).

Materials

Materials used in this study, include the infusion of NaCl 0.9%, *Mc Farland*, transport media, clear plastic (puppet), Blood Agar, *Mac Conkey Agar*, *Salmonella Shigella Agar*, *crystal violet*, 96% alcohol, fuchsine, *Lugol's iodine*, biochemical reagents, and *Nutrient agar*. Meanwhile, the antibiotics used are class amino glycosides (amikacine and gentamicine), class of fluoroquinolones (ciprofloxacin and levofloxacin), class of cephalosporin (ceftriaxone, cefadroxil, and

cefotaxime), and the class of carbapenem (imipenem and meropenem).

Sampling

Samples were taken from patients with diabetic foot ulcer grade III and IV in Clinical Kitamura Pontianak in West Kalimantan. Swab samples are taken in the from of diabetic foot ulcer by using a sterile swab which are then stored in transport media (Sari & Apridamayanti, 2015).

Isolation and Identification of *Escherichia coli*.

Swabs taken from of diabetic foot ulcers are analyzed in Pontianak Health Laboratory Unit. Swab will be planted in three media: *Blood To Plate* (BAP), *Mac-Conkey Agar* (MCA), and *Salmonella shigela Agar* (SSA) media. Planting bacteria are carried on solid agar medium and incubated for 24 hours in the incubator at 37°C. *Escherichia coli* colonies then grow on *Nutrient Agar*. Afterwards the bacteria are identified biochemical tests and Gram stain test (Anggriawan et al., 2014 ; Widyastutik et al., 2013).

Antibiotic Sensitivity Testing

Suspension of *E. coli* bacteria is conducted aseptically. Turbidity standard is equal with Mc Farland 0.5 standard that is equivalent to the amount of growth of 1×10^8 bacterial cells / ml and after similar then this suspension is used as test bacteria (ICMR, 2009). The suspension of *E. coli* is then etched into the whole *Mueller Hinton* (MH) media which were incubated using a sterile swab method -*Bauer Kirby*. Then, they were placed on the used disk of antibiotics consisting of : ciprofloxacin (5µg), cefadroxil (30µg), gentamicin (10µg), meropenem (10µg), imipenem (10µg), levofloxacin (5µg), amikacin (30µg), ceftriaxone (30µg) and cefotaxime (30µg). The determination of antibiotic sensitivity is based on the guidelines of *Clinical Laboratories and Standard Institute* (2014) (CLSI, 2014).

RESULTS AND DISCUSSION

The research carried out by Sari and Apridamayanti (2015) revealed the fact that there are 24 patients with diabetic foot ulcer which consisted of 15 patients grade III (62.5%) and 9 patients with grade IV (37.5%). The patient swabs was taken from diabetic foot ulcer which consisted of male as the majority of the patients with 14 patients (58.33%) and female patients of 10 patients (41.67%). The identified result shows the distribution of the highest number of patients in the range of 46-65 years age group (Sari & Apridamayanti, 2015). Diabetic ulcers often occur in those aged >50 years due to declining physiological body functions such as decreased secretion or insulin resistance so that the functional capability of the body to control high blood glucose become less than optimal. Sugar levels

Table 1. Characteristic of Diabetic Foot Ulcer Patients (Sari & Apridamayanti, 2015)

Characteristics of Patients	N = 24	
	Total Patient(n)	Percentage (%)
Age (year)		
< 45	3	12.5
46-65	17	70.83
66-85	4	16.67
Grade of ulcer		
Grade III	15	62.5
Grade IV	9	37.5
Sex		
Male	14	58.33
Female	10	41.67

which are not controlled will lead to long-term chronic complications of both macrovascular and microvascular one of which is diabetic foot ulcer (Frykberg, 2002).

Isolation and Identification of *Escherichia coli* in Diabetic Foot Ulcers.

Identification of *Escherichia coli* bacteria on *Blood Agar*, *Mac-Conkey* and *Salmonella shigela* are shown in Figure 1. Figure 1 shows the views from *Escherichia coli* bacteria colonies on media *Blood Agar*, *Mac-Conkey* and *shigela Salmonella Agar*. Identification of *Escherichia coli* on blood agar media showed gray colonies, smooth, convex, and lyses in the blood. *Escherichia coli* leads to α-hemolysis which partial lyses of red blood cells and hemoglobin. This results in a change of color around the colonies which become gray.

Identification of *Escherichia coli* bacteria on *Mac Conkey* media showed moderate colony, red brick, smooth and convex. The ability of *Escherichia coli* ferment lactose led to decrease of pH then facilitated *neutral red* absorption, making it easier to turn the colony into a red brick. This is conducted in order to make the media of *Salmonella Shigella*, *Escherichia coli* be seen as a small colony of pink.

Identification of *Escherichia coli* using Gram staining and biochemical tests is carried out to ensure that the *Escherichia coli* bacteria isolated. The results of biochemical test that can be seen in Table 2 explains that the test of sugar consist of glucose, lactose, mannitol, maltose and sucrose. This showed positive results as marked by a change of media sugar from red to yellow which means that the *Escherichia coli* ferment sugar. Indole test results showed positive results which is characterized by the formation of a layer of red ring at

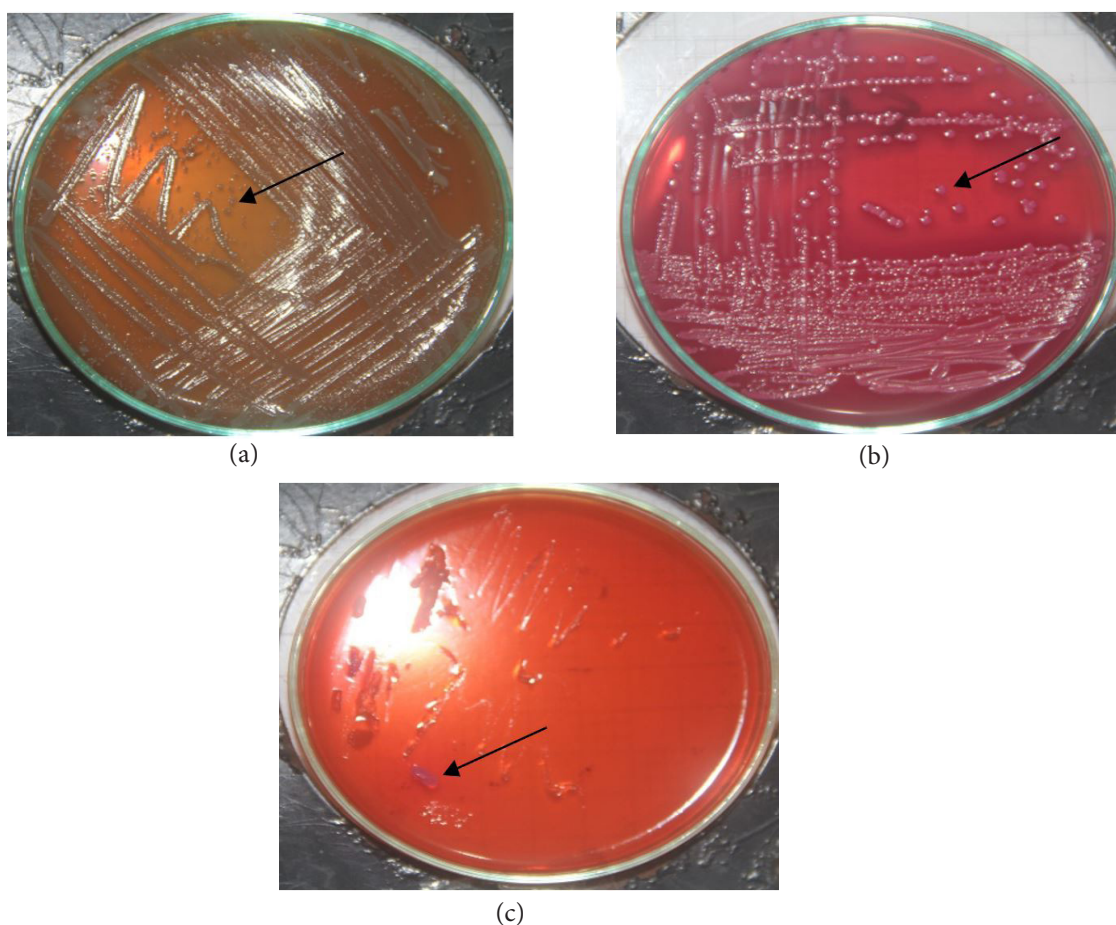


Figure 1. Colonies of *Escherichia coli* on Blood Agar (a), Mac-Conkey (b), and *Salmonella shigella* Agar (c)

the surface of the culture. It means that these bacteria have triptophanase enzymes. Motility test results indicated positively in that it showed that this bacterium has flagella.

In the test using TSIA media, *Escherichia coli* gave a positive result on the slopes acid (lactose and sucrose) and the bottom of the tube (glucose) when the media becomes yellow. This can occur because the bacteria *Escherichia coli* can ferment all the carbohydrates glucose, lactose and sucrose in TSIA media.

Escherichia coli shows fermentative test results on oxidative/fermentative as marked by a color change of the second tube from green to yellow. The obtained results showed that the bacteria were identified as *Escherichia coli*. These results are supported by the research carried out by Widiyastutik *et al* (2013) that *Escherichia coli* showed positive results in a test of glucose, maltose, mannitol, lactose, sucrose, indole, motility, and TSIA while Simmon citrate and urea showed negative results (Widiyastutik *et al.*, 2013).

Cell *E. coli* shows red bacilli shape as seen in Figure 2. This is because the cell wall of Gram-negative bacteria has a thick lipid content and when added by crystal violet staining the cell wall of Gram-negative bacteria

Table 2. Biochemical Test Result of *Escherichia coli*

Biochemical Test	Result
Glucose	+, Gas (+)
Lactose	+
Mannitol	+
Maltose	+
Sucrose	+
Indole	+
Motility	+
TSIA (<i>Triple Sugar Iron Agar</i>)	+, H ₂ S (-)
Simmon Citrat	-
Urea	-
Oxides	-
Oxidative/Fermentative	+



Figure 2. Gram-staining Result with 1000x Magnification

it will absorb the dye. However when it is given alcohol, the crystal violet in the Gram negative faded since the structure of their cell walls are largely composed of lipids. When the second dye that is water fuchsin is added, the cell wall of Gram-negative bacteria will reabsorb dye red Gram-negative bacteria (Radji, 2012).

Antibiotic Sensitivity of *Escherichia coli*

Escherichia coli bacteria are found in diabetic foot ulcers Wagner stage III and IV as much as 8.33% (Sari & Apridamayanti, 2013). Antibiotic sensitivity results in Table 3 namely amikacine and gentamicine are sensitive to *Escherichia coli*. Amikacine and gentamicine belong to the class of aminoglycoside antibiotics. Antibiotic sensitivity due to the amikacin and gentamicin antibiotic will invade the cell and then bound to the 30S ribosome and ultimately inhibit protein synthesis (Setiabudy & Gunawan, 2007). This antibiotic binds component of the 30S ribosomal 16S subunit (Kohanski *et al.*, 2010). This binding causes a mismatch of codone and anticodone causing mistranslation (Lambert, 2012). The reading of the wrong amino acid into a polypeptide chain form a type of wrong protein (Setiabudy & Gunawan, 2007). Table 3 shows that ciprofloxacin and levofloxacin are sensitive to *Escherichia coli*. Ciprofloxacin and levofloxacin antibiotics are included in the class

of fluoroquinolone antibiotics. The mechanism of fluoroquinolone antibiotics work by inhibiting the DNA gyrase enzyme in bacteria that cause relaxation function in DNA *supercoiling positive* experienced at the time of transcription in the process of DNA replication (Setiabudy & Gunawan, 2007). This leads to disrupted process of DNA synthesis which cause cell's death. Ceftriaxone and cefotaxime are sensitive to *Escherichia coli* but cefadroxil is resistant to *Escherichia coli*. Ceftriaxone, cefadroxil, and cefotaxime belong to the cephalosporin class of antibiotics. Table 3 also shows that the imipenem and meropenem antibiotics are sensitive to *Escherichia coli*. Imipenem and meropenem belong to the class of carbapenem antibiotics.

Cephalosporin and carbapenem antibiotics are included into *beta-lactamase* antibiotics. The action mechanism of action of antibiotic sensitivity is by inhibiting the cell wall synthesis (Setiabudy & Gunawan, 2007). Bacterial cell walls are formed of peptidoglycan which are composed of polysaccharides and polypeptides. The workplace of antibiotics on bacterial cell wall is in peptidoglycan layer. The barriers of cell wall synthesis occurs because beta-lactam antibiotics bind to the enzyme transpeptidase that the enzyme known as PBP (*penicillin-binding-protein*). This causes a chain reaction between the disturbed peptidoglycan transpeptidation that ultimately lead to the synthesis of peptidoglycan which will be inhibited which and thus causes a loss of strength of the cell wall and subsequent cells's death (Setiabudy & Gunawan, 2007).

Cefadroxil is an antibiotic that showed resistance to *Escherichia coli*. Antibiotic resistance can be caused by bacteria's ability to produce *β-lactamase* enzyme that will hydrolyze bonds in *β-lactam* ring and lead to inactivation of antimicrobials. Plasmid of extra-chromosomal genetic material can carry the gene

Table 3. Antibiotics Sensitivity Result

Antibiotics	Dose	Inhibition Zone Diameter (mm)	CLSI Diameter Guideline (mm)			R/I/S
			R	I	S	
Ciprofloxacin	5 µg	36.67 ± 1.15	≤15	16-20	≥21	Sensitive
Gentamisin	10 µg	19.67 ± 0.578	≤12	13-14	≥15	Sensitive
Cefadroxil	30 µg	14.67 ± 1.15	≤15	16-20	≥21	Resistant
Levofloxacin	5 µg	35.33 ± 2.3	≤13	14-16	≥17	Sensitive
Imipenem	10 µg	28.67 ± 2.3	≤19	20-22	≥23	Sensitive
Meropenem	10 µg	30 ± 0	≤19	20-22	≥23	Sensitive
Cefotaxime	30 µg	40 ± 0	≤22	23-25	≥26	Sensitive
Ceftriaxone	30 µg	39.33 ± 1.15	≤19	20-22	≥23	Sensitive
Amikacine	30 µg	21.33 ± 2.3	≤14	15-16	≥17	Sensitive

coding for antibiotic resistance. Plasmids in *Escherichia coli* can produce the enzyme β -lactamase gene coding for ESBL (Rice & Bonomo, 1996). Resistance occurs because the gene encoding of β -lactamase enzyme is TEM-1, TEM-2 or SHV-1 (Paterson & Yu, 2005 ; Rice, 1999).

Antibiotic treatment should be based on the results of bacterial culture. On the other hand if not yet examined, leads to culture and sensitivity of the bacteria which is the reason why antibiotics should empirically be given to diabetic foot ulcer (Cahyono, 2007). This is conducted in order to prevent the occurrence of antibiotic resistance to *Escherichia coli*.

CONCLUSIONS

Escherichia coli which was found in diabetic foot ulcers Wagner grade III and IV are sensitive to the antibiotic such as amikacine, gentamicine, ciprofloxacin, levofloxacin, ceftriaxone, cefotaxime, imipenem, and meropenem. However *Escherichia coli* is resistant to the first generation of cephalosporin antibiotic that is cefadroxil.

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