

DM Gangrene

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Identification of negative gram bacteria in diabetes mellitus gangrene in general area hospital dr. Soedomo Trenggalek

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ABSTRACT

Diabetes mellitus is a metabolic disease due to the increase of normal > blood glucose levels. Gangrene diabetes is tissue death due to obstruction of blood vessels that provides nutrients to obstructed tissue and is a form of complications from diabetes mellitus. The purpose of this research is to determine the presence of gram-negative bacteria on the wounds of Gangrene Diabetes Mellitus. This research is a descriptive Non-Analytic, conducted on April 15 to May 21, 2019, in the Microbiology Laboratory of STIKes Hutama Abdi Husada Tulungagung. The study sample was all patients with Gangrene Diabetes Mellitus wound in hospital dr. Soedomo Trenggalek, the sampling technique used was accidental sampling, obtained a sample of 4 Gangrene swabs. The study was conducted by the identification of gram-negative bacterial isolation. The results of the study were 4 samples, 2 were positive for *Pseudomonas aeruginosa* and 2 samples were positive for *Proteus* sp. Subsequent research results were analyzed descriptively. The conclusion of this research is that 100% positive gram-negative. The typical characteristics of the *Pseudomonas aeruginosa* bacteria are obtained on the MCH media (alkaline slope, alkaline base, negative gas, negative H₂S) and on the NAS produce greenish-yellow fluorescence, while the typical characteristics of *Proteus* sp are obtained on the MCH media (alkaline slope, alkaline slope, base negative) acid, positive gas, positive H₂S).

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INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by chronic hyperglycemia or an increase in blood glucose more than normal resulting from defects in insulin secretion, insulin action, or both. The importance of insulin as an anabolic hormone, and low insulin levels or insulin resistance of target tissues, especially skeletal muscle, tissue adipose, and to a lesser extent, the liver at the level of insulin receptors, signal transduction systems, and/or enzymes or effector genes responsible for metabolic disorders. Carbohydrate, lipid, and protein metabolism disorders result from the importance of insulin as an anabolic hormone. Some diabetic patients, especially type 2 diabetes in the early years of the disease have no symptoms, people with hyperglycemia and children with an absolute deficiency can suffer from polyuria, polydipsia, polyphagia, weight loss and blurred vision. the severity of symptoms is caused by the type and duration of diabetes. Uncontrolled diabetes can cause fainting, coma and if not treated due to death from ketoacidosis or rarely from the hyperosmolar nonketotic syndrome.¹



¹ Diabetes mellitus (DM) is a metabolic disease that causes hyperglycemia, due to insulin defects, insulin secretion, or both. A survey conducted by the world health organization (WHO) in 2004 there were 3.4 billion people in the world who have diabetes mellitus² and it is estimated that in 2030 will increase. The Riskesdas Report (2007) shows that in Indonesia there are 5.7% of people with Diabetes Mellitus.² In 2014, the estimated prevalence of diabetes³ in the world reached 9% at the age of 18 years. Diabetes is the cause of 1.5 million deaths in the world and more than 80% of deaths occur in low and middle-income countries. Based on the 2013 Riskesdas data, the prevalence of diabetes in Indonesia is 2.1% at the age of 15 years.³

The prevalence of diabetes in Indonesia is occupied by the province of East Java because diabetes is the top 10 most diseases. The number of DM patients according to Riskesdas increased from 2007 to 2013 by 330,512 patients.⁴ In the Trenggalek area itself, there are many patients who want to have their blood sugar checked. This is supported by data from the Medical Record Hospital Dr Soedomo in 2018 there were 151 patients who experienced Diabetes Mellitus with Gangrene, and in November there were 14 patients (inpatient registration).

Diabetes⁵ mellitus can cause various complications both macrovascular and microvascular. The impact of Diabetes Mellitus on the quality of human resources and the increase in health costs is quite large so that a type two DM control program is needed. Type two Diabetes Mellitus can be prevented by knowing the risk factors. DM risk factors are divided into risk factors but can be changed by humans, in this case, can be in the form of eating patterns, patterns of daily habits such as eating, resting patterns, activity patterns and stress management. The second factor is risk factors but cannot be changed such as age, gender and factors of patients⁶ with a family background with Diabetes.⁵

Manifestations of angiopathy in people with diabetes mellitus in the form of narrowing and blockage of peripheral arteries which mainly occur in the lower extremities. The earliest blood vessels that experience angiopathy is the tibial arteries. Arterial abnormalities due to diabetes mellitus also often affect the distal part of the deep femoral artery, popliteal arteries, tibial arteries and digitalis pedis arteries, as a result of the distal tissue perfusion from the lower extremity is reduced and ulcers arise which can then develop into necrosis/gangrene which is not infrequently ended with amputation.⁶ The presence of open sores on the skin will facilitate the invasion of bacteria,⁷ some studies show that about 40-80% of diabetic ulcers have an infection.⁷ Diabetic foot infection (DFI) is a common, complex and expensive complication in people with diabetes. About 15% of DM patients⁸ have foot ulcers which eventually become osteomyelitis.⁸

Patients with a diagnosis of diabetes mellitus show clinical signs of vascular disease, infection or neuropathy, in at least one of the lower limbs. Wagner's classification of diabetic foot is a more commonly used parameter for measuring trophic lesions; in the case of the diabetic foot, this classification according to Type I is proven by superficial ulcers without deep tissue involvement. Type II, where the wound reaches the tendons, joint capsules, and bones. Type III where abscesses (pus accumulated fluid), osteomyelitis (bone infection), osteoarthritis (joint use and tearing), and tendonitis (tendon inflammation). Type IV is evidenced by gangrene or a local gangrene incident, usually in one leg. Type V where gangrene spreads throughout the leg. Among those chronic complications of diabetes mellitus, resulting from micro and macrovascular damage, when asked about diabetic ulcers is one of the complications that patients fear.⁹ Complications of these ulcers are infections which if left untreated will cause amputations of distal limbs.¹⁰

Patients with diabetic foot infections (DFI) are often hospitalized because they can cause significant health problems leading to death.¹¹ Peripheral diabetes sensorimotor neuropathy is a key factor in the majority of cases. Lack of patient awareness such as not wearing shoes or footwear when walking so that they are vulnerable to injury and can damage sensory nerves.¹²



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Foot infections in diabetics with a lifetime risk of around 25% in people with a diagnosis, patients treated at the diabetic foot center reach 4% per year. DFI sometimes appears after trauma infection but most often the consequences of secondary ulceration to progressive peripheral polyneuropathy can cause disruption of gait and loss of sensation due to foot deformity. These neurological problems are usually accompanied by arterial insufficiency and immunological disorders.¹³

One complication of diabetes mellitus is an ulcer where superficial infections occur in the skin of the patient. The risk of ulcers in diabetics is 29 times greater. The entry of bacteria becomes the beginning of ulcers and high glucose levels become a strategic place for bacterial development. Bacteria found in diabetic ulcers are a combination of aerobic and anaerobic bacteria. Research on the profile of diabetic ulcers in Banda Aceh City aims to identify bacteria and antibiotic sensitivity to bacterial ulcers. The results showed that the total bacteria found from the sample¹⁰ 57 diabetic ulcer pus was 207 bacteria. The most common bacteria found in a row. *Staphylococcus sp.*, *Klebsiella sp.*, *Proteus sp.*, *Shigella sp.*, *Escherichia coli*, and *Pseudomonas s*².

The dominance of gram stem family *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, and *Proteus mirabilis*) has recently been reported as the largest aerobic group. For example, an average of 1.8 pathogenic bacteria per diabetes wound. Samples were reported in one study, were, 51.2% were Gram-negative bacteria which were quite high.¹⁴

The purpose of this research is because of the magnitude of foot problems in diabetics, especially if there is Gangrene, researchers are interested in researching with the title "Identification of gram-negative bacteria in Wound Gangrene Diabetes Mellitus in Dr. Soedomo Trenggalek Regional Hospital in 2019"

MATERIALS AND METHODS

This research was conducted on April 15-May 21 2019. Sampling was carried out at dr. Soedomo Trenggalek and the examination and research were conducted at the Microbiology Laboratory of STIKes Hutama Abdi Husada Tulungagung. The sample used was all the samples in the study of all Diabetes patients who experienced Gangrene who were hospitalized at RSUD dr. Soedomo Trenggalek on 15 April-21 May 2019. Gangrene swab samples were obtained from diabetics at RSUD dr. Soedomo Trenggalek by swabbing on gangrene uses a sterile swab. The swab is put into a test tube containing Pz sterile and has been labeled with the code or initials of the respondent's name. Samples were taken to the laboratory using a cool box. The purpose of using the cool box is to avoid contamination from the outside and can inhibit the growth of pathogenic bacteria during the process of bringing the sample to the laboratory so it is expected that the results obtained from the microbiological examination can really describe the bacteria in the sample. Then carried out identification. Identification is done by bacterial isolation. Isolation was carried out by means of Gangrene swab samples planted in Nutrient Broth / Bouillon media incubated 37°C for 24 hours then planted on Mac Conkey Agar (MCA) media at 37°C for 24 hours. Further tests were carried out with Gram staining and biochemical reaction. The sampling technique in this study was accidental sampling. Accidental sampling technique, which is the technique of determining samples accidentally by taking samples that happened to be encountered at that time. This type of research is to use Non-Analytical Descriptive research which is a study conducted with one variable and does not require a hypothesis.

RESULTS AND DISCUSSION

The results of the study of four Gangrene Diabetes Mellitus wound samples were carried out by means of identification. Identification was carried out by means of gangrene Diabetes Mellitus wound swab culture. The following results are obtained :



Table 1 Culture results of Gangrene Diabetes Mellitus wound swab samples on the media

No	Media	Sample Code			
		Sample A	Sample B	Sample C	Sample D
1.	MCA	Laktose +	Laktose +	Laktose -	Laktose -
2.	Gram Staining	Gram -	Gram -	Gram -	Gram -
3.	KIA	L = Alkali	L= Alkali	L= Alkali	L= Alkali
		D = Acid	D = Acid	D= Alkali	D= Alkali
		H ₂ S = +	H ₂ S = +	H ₂ S = -	H ₂ S = -
		Gas = +	Gas = +	Gas = -	Gas = -
4.	NAS	Non Fluorescent	Non Fluorescent	Fluorescent	Fluorescent
5.	Biokimia Reactions				
	a. Glucose	+	+	-	-
	b. Laktosa	-	-	-	-
	c. Sukrosa	-	-	-	-
	d. Maltosa	-	-	-	-
	e. Mannosa	-	-	-	-
	f. VP/MR	+	+	-	-
	g. Citrat	+	+	+	+
	h. Urea	+	+	-	-
	i. Motil	+	+	+	+
	j. Lysin	-	-	-	-
	k. Indol	+	+	-	-

In this discussion report the condition of the Gangrene population in RSUD dr. Soedomo Trenggalek in mid-April to mid-May has decreased, which previously every month there were Gangrene cases an average of 12 patients. Difficulties in this study, the number of samples in this study is limited. Based on data from the Hospital Medical Record dr. Soedomo, there were several Gangrene patients who were declared cured 8 and died 4, so that in the month of this study there were only 4 respondents (new patients). This study presents 4 samples from the existing Gangrene population. The result is a low sample variation.

Based on Table 1 it can be seen that 2 samples namely sample A and sample B show the characteristics of the *Proteus sp* bacteria that show Gram (-) negative. *Proteus sp* bacteria are a family of Enterobacteriaceae which are gram-negative and have motile swarming. *Proteus sp* including pathogenic germs.¹⁵ Judging from the results of Gram staining and planting in the MCH media which shows the results of the alkaline slope, acid-base, producing H₂S and gas. Planting on NAS media and biochemical reactions also showed characteristics of the *Proteus sp*.¹⁵

Proteus sp bacteria are gram-negative rod-shaped bacteria, have motile swarming, positive urease, do not ferment lactose, are not sporous, have no capsules, catalase and nitrate are positive. The specific test to identify proteus sp is a positive urease and phenylalanine test.¹⁶

Another Korean study focused on bacteremia because the Proteaceae isolated 132 strains, but only two were *P. penneri*. In this study, *P. penneri* was isolated from the patient's urine, drainage fluid or pus from the patient and which had several underlying diseases besides diabetic foot ulcers, which were found as a new presentation.¹⁷



Sample 19 and sample D contain characteristics of the bacterium *Pseudomonas aeruginosa*. *Pseudomonas aeruginosa* is found as a major nosocomial pathogen.¹⁸ this can be seen from Gram staining and planting on MCH media which shows the results of the alkali slope, alkaline base, and does not produce H₂S as well as gas, which then begins to dig bacteria into NAS media and Biochemical Reactions. The results of the above research The previous research conducted is that the characteristics of *Pseudomonas aeruginosa* are classified as Gram-negative bacteria where at the time of Gram's staining will produce a red colour, because it is needed iodine-coloured chemicals and become translucent, these bacteria can be coloured again with safranin.¹⁹ Test results on MCH media on the slope and bottom of the media are red and no black and gas forms. *Pseudomonas aeruginosa* bacteria do not form acids because the bacteria cannot ferment glucose and lactose.²⁰ *Pseudomonas aeruginosa* does not ferment the media of glucose, mannitol, saccharose, maltose and lactose, this can be seen from the media which does not turn yellow. These bacteria produce negative results on the indole test, the colour turns yellow. These bacteria produce negative results on the indole, Methyl Red, and Voges-Proskauer tests. Positive motility is characterized by the growth and spread of bacterial turbidity in all media. These bacteria can use citrate as a carbon source. The colonies formed appear smooth, round with a greenish fluorescence colour. *P. aeruginosa* strains produce fluorescent pigments including pyoverdine (green), piourubin (dark red), piomelanin (black).

Infection in ulcers and diabetic gangrene is generally caused by polymicrobial. From several studies showing aerobic bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*) and anaerobic bacteria (*Peptostreptococcus sp.*) Are the main causes of infection in ulcers/gangrene and slow healing. *Pseudomonas sp.* has characteristics such as Gram-negative, rods/coccus, obligate aerobes, motiles have a polar flagellum. This bacterium also causes disease in humans. Can cause infections in wounds and burns, causing bluish-green pus also cause invasive external (malignant) in diabetics.²¹

E. coli bacteria showed the highest incidence followed by *S. aureus*, *P. aeruginosa*, *K. pneumoniae*, *A. baumannii*, *Citrobacter*, *P. mirabilis*, and *Streptococcus spp* for pyogenic wound infections found in hospitals. After bacterial isolation, it showed a high to moderate level of resistance to various antibiotic classes. Reported vulnerability data can be considered for implementing treatment strategies.²²

Microorganisms contained in DFI usually depend on the characteristics of the patient, clinical risk factors, injuries suffered. Epidemiology osteomyelitis found polymicrobial infections in soft tissue infections more often. *S. aureus* (up to 50% of cases), *S. epidermidis* (around 25%), *Streptococci* (about 30%) and *terobacteriaceae* family (up to 40%) are most commonly detected in DFO, namely gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae* and *Proteus, Pseudomonas aeruginosa*.²³

CONCLUSIONS

Based on the results of studies that have been carried out on four samples of Gangrene Diabetes Mellitus wounds in RSUD dr. Soedomo Trenggalek research can be concluded that from the four samples obtained two positive samples of *Pseudomonas aeruginosa* (50%), and two positive samples of *Proteus sp.* As well as the typical characteristics of the *Pseudomonas aeruginosa* bacteria are obtained on the MCH media (alkaline slope, alkaline base, negative gas, negative H₂S) and on the NAS produce greenish-yellow fluorescence, while the typical characteristics of *Proteus sp* are obtained on the MCH media (alkaline slope, acid-base, positive gas, positive H₂S)

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