MELYSA
Medical Laboratory Analysis and Sciences Journal
Volume 1 Issue 2, November 2019, page 70-76
ISSN: 2657-1471 (Online)
ISSN: 2656-937X (Print)
DOI: 1035584/melysa.v1i2.34

Identification of negative gram bacteria in diabetes mellitus gangrene in RSUD dr. Soedomo Trenggalek

Hariyanto[1a*], Nala Fidarotul Ulya[1b], Linda Popi Hartanti[1c]
[1]Diploma III in Medical Laboratory Technology, STIKes Hutama Abdi Husada, Jl. Iswahyudi Kedungwaru Tulungagung 66224, East Java, Indonesia
[a]hariyanto696@gmail.com*
[b] nalaulya88@gmail.com
[c]lindapopi40@gmail.com
*corresponding author

ARTICLE INFO

Article history:
Received Date: September 21th 2019
Revised Date: October 7th 2019
Accepted: October 22th 2019
Published: November 1st 2019

Keywords:
Diabetes Mellitus
Gangrene
Gram Negative Bacteria

ABSTRACT

Diabetes mellitus is a metabolic disease due to an increase in blood glucose levels normal. 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. *Pseudomonas aeruginosa* is one of the causes of gangrene infection nosocomial opportunistic infections in humans. The purpose of this study was to determine the presence of gram-negative bacteria in 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).

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Carbohydrate, lipid, and protein metabolism disorders result from the importance of insulin as an anabolic hormone. Low insulin levels achieve an adequate response and/or target tissue insulin resistance, especially skeletal muscle, tissue adipose, and to a lesser extent, liver, at the level of insulin receptors, signal transduction systems, and/or enzymes or effector genes responsible for this is a metabolic disorder. The severity of symptoms is caused by the type and duration of diabetes. Some diabetic patients show no symptoms especially those who suffer from type 2 diabetes during the early years of the disease, others with hyperglycemia and especially in children with absolute insulin deficiency can

suffer from polyuria, polydipsia, polyphagia, weight loss, and blurred vision. Uncontrolled diabetes can cause fainting, coma and if not treated because of death for ketoacidosis or rarely from a hyperosmolar nonketotic syndrome.[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 mellitus 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 diabetes 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).

Diabetes 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 patients 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, some studies show that about 40-80% of diabetic ulcers have an infection.[7] Approximately 15% of patients with DM develop foot ulcers, which eventually progress to osteomyelitis. Diabetic foot infections (DFIs) are common, complex and costly complications of DM.[8]

Patients with a diabetes mellitus diagnosis show vascular disease clinical signs, infection or neuropathy, in at least one of the lower limbs. Diabetic foot Wagner classification is the more commonly used parameter to quantify trophic lesions; in the case of the diabetic foot, this classification corresponds to Type I, evidenced with superficial ulcers without deep tissue involvement; Type II, where wounds reach tendons, joint capsule, and bones; Type III, where the abscess occurs (pus fluids accumulation), osteomyelitis (bone infection), osteoarthritis (joint wearing and tearing), and tendonitis (tendon inflammation); Type IV, evidenced with gangrene or localized gangrene incidence, usually in one part of the foot; Type V where gangrene is spread throughout the foot. Among those diabetes mellitus chronic complications, resulting from micro and macrovascular damages, diabetic foot is considered as one of the most-feared complications when patients are asked.[9] A common complication of these ulcers is an infection, which if left untreated, results in the need for distal limb amputation.[10]

Diabetic foot infections (DFI) cause significant health problems, reduce the patients' quality of life, cause a lower limb amputation, increase morbidity and increase the cost of

**Corresponding author: hariyanto696@gmail.com*

health services. DFI frequently require hospitalization of patients and could lead to death.[11] The development of a foot ulcer invariably involves the convergence of several pathological mechanisms.7 Diabetic peripheral sensorimotor neuropathy is a key factor in the majority of cases. As a result of damage to sensory nerves, minor trauma (from something as seemingly trivial as an ill-fitting pair of shoes or walking barefoot on unfamiliar terrain) can go unnoticed. Neuropathy can also deform the architecture of the foot to such a degree that joints and digits are placed in mechanically unfavorable positions, making them highly vulnerable to injury.[12]

The incidence of foot infections in persons with diabetes ranges from a lifetime risk of up to 25% in all persons with the diagnosis to 4% yearly in patients treated in a diabetic foot center. Diabetic foot infections (DFIs) occasionally present as cellulitis or post-traumatic (including postsurgical) infections, but are most commonly a consequence of ulcerations secondary to progressive peripheral polyneuropathy. This causes a loss of protective sensation, as well as foot deformities, gait disorders, anterior displacement of weight-bearing during walking, and reduced mobility. These neurological problems are commonly accompanied by arterial insufficiency and immunological disturbances.[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 sample of 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 sp.*[3]

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 37^0C for 24 hours then planted on Mac Conkey Agar (MCA) media at 37^0C for 24 hours. Further tests were carried out with Gram staining and biochemical reaction.The sampling technique in this study was accidental sampling.

**Corresponding author: hariyanto696@gmail.com*

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 D = Acid H_2S = + Gas = +	L= Alkali D = Acid H_2S = + Gas = +	L= Alkali D= Alkali H_2S = - Gas = -	L= Alkali D= Alkali H_2S = - Gas = -
4.	NAS	Non Fluo-rescent	Non Fluo-rescent	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. Gram-negative motile and frequently swarming bacteria of the genus *Proteus* and the family *Enterobacteriaceae* are opportunistic human pathogens.[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.*

**Corresponding author: hariyanto696@gmail.com*

Proteus spp is gram-negative, facultatively anaerobic, rod-shaped. It has clustered motility, urease activity usually does not ferment lactose. Because it belongs to the family Enterobacteriaceae, the general behaviour is applied to this genus: Active genus, not forming spores, non-capsule negative oxidase, but positive catalase and nitrate. To identify Proteus spp, specific tests include a positive urease test and phenylalanine deaminase.[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 C and sample D contain characteristics of the bacterium Pseudomonas aeruginosa. Pseudomonas aeruginosa is emerging 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), piorubin (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]

Pyogenic wound infections were found prevalent in the tertiary care hospital and E. coli isolates showed the highest incidence followed by *S. aureus, P. aeruginosa, K. pneumoniae, A. baumannii, Citrobacter, P. mirabilis, and streptococcus sp*. Bacterial isolates exhibited high to moderate levels of resistance against different classes of antibiotics. The susceptibility data from this report may be worth consideration while implementing empiric treatment strategies for pyogenic infections.[22]

The microorganisms involved in DFI show various epidemiology depending on the characteristics of the patient, the clinical risk factors, the wounds (extension and depth) and the microenvironment. The epidemiology of osteomyelitis reflects the one found in soft tissue infections, rarely mono-microbial and more often poly-microbial. S. aureus (up to 50% of cases), S. epidermidis (about 25%), Streptococci (about 30%) and Enterobacteriaceae (up to 40%) are the most commonly detected bacteria in DFO. Among the Gram-negative, Escherichia coli, Klebsiella pneumonia and Proteus, are the most common microorganism followed by Pseudomonas aeruginosa.[23]

CONCLUSIONS

**Corresponding author: hariyanto696@gmail.com*

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 H_2S) 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 H_2S)

ACKNOWLEDGEMENT

The preparation and writing of this journal cannot be separated from the help, guidance and support of various parties. Therefore, on this occasion, the author would like to thank all the staff and employees of the Soedomo Trenggalek Hospital Laboratory who have helped a lot during the research to completion so that the preparation of this journal can run smoothly.

REFERENCES

1. Akram T Kharroubi, Hisham M Darwish. Diabetes mellitus: The epidemic of the century. *World J Diabetes*. 2015; 25; 6(6): 850-867. doi:10.4239/wjd.v6.i6.850
2. Yohanna Eclesia Lumban Gaol, Erly, Elmatris Sy. Pola Resistensi Bakteri Aerob pada ulkus diabetic terhadap beberapa antibiotic di Laboratorium Mikrobiologi RSUP Dr. M. Djamil Padang Tahun 2011-2013. *J Kesehatan Andalas.* 2017;6(1):164. doi:10.25077/jka.v6i1.664
3. Nur A, Marissa N. Gambaran Bakteri Ulkus Diabetikum di Rumah Sakit Zainal Abidin dan Meuraxa Tahun 2015. *Buletin Penelitian Kesehatan*. 2016; 44 (3):187 – 196. doi:10.22435/bpk.v44i3.5048.187-196
4. Kementerian Kesehatan RI. *Profil Kesehatan Indonesia*. Jakarta: Depkes RI; 2014
5. Isnaini N, Ratnasari R. Faktor risiko mempengaruhi kejadian Diabetes mellitus tipe dua. *J Kebidanan dan Keperawatan Aisyiyah.* 2018;14(1):59-68. doi:10.31101/jkk.550.
6. Wijoseno G. Jantung, pembuluh darah arteri, vena dan limfe. Dalam: de Jong W, editor. Buku ajar ilmu bedah. Edisi ke-4. Jakarta: EGC; 2017
7. Richard, J. L., Sotto, A. & Lavigne, J., New insights in diabetic foot infection. *World J Diabetes*. 2011;2(2):24-32. doi:10.4239/wjd.v2.i2.24
8. Benwan Khalifa Al, Mulla Ahmed Al, Rotimi Vincent O. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. *Journal of Infection and Public Health.* 2012; 5(1):1-8. doi:10.1016/j.jiph.2011.07.004
9. Vivanco, A.L., Espinoza, F.M., Mayorga, F.C., Luna, J.S., Tandazo, M.C., Rueda, E.Y.R. and Davila, K.D. Bacteriological Profile in Diabetic Foot Patients. *Journal of Diabetes Mellitus*. 2017; 7:265-274. doi:10.4236/jdm.2017.74021
10. Banu A, Noorul Hassan MM, Rajkumar J, Srinivasa S. Spectrum of bacteria associated with diabetic foot ulcer and biofilm formation: A prospective study. *AMJ.* 2015;8(9): 280-285. doi:10.4066/AMJ.2015.2422
11. Nese Saltoglu, Onder Ergonul, Necla Tulek. Influence of multidrug resistant organisms on the outcome of diabetic foot infection. *International Journal of Infectious Diseases.*2018;70:10-14. doi:10.1016/j.ijid.2018.02.013
12. Powlson Andrew S Anthon, y P. Coll. The treatment of diabetic foot infections. *J Antimicrob Chemothe.* 2010; 65(3):3–9. doi:10.1093/jac/dkq299
13. Uckay Ilker, Javier Arago´n-Sa´nchez , Daniel Lew, Benjamin A. Lipsky. Diabetic foot infections: what have we learned in the last 30 years? *International Journal of Infectious Diseases*.2015;40: 81-91. doi:10.1016/j.ijid.2015.09.023

**Corresponding author: hariyanto696@gmail.com*

14. Heravi FS, Zakrzewski M, Vickery K, Armstrong DG, Hu H. Bacterial Diversity of Diabetic Foot Ulcers: Current Status and Future Prospectives. *Journal of Clinical Medicine*. 2019; *8*. doi:10.3390/jcm8111935
15. Eleonora Aquilini, Joana Azevedo, Natalia Jimenez, Lamiaa Bouamama, Juan M. Toma´s, and Miguel Regue. Functional Identification of the Proteus mirabilis Core Lipopolysaccharide Biosynthesis Genes. *Journal of Bacteriology*. 2010;192(17): 4413–4424. doi:10.1128/JB.00494-10
16. Banaz M. Latif, Salah. S. Zayn Aleabdyn and Ibraheem S. Ahmed. A Comparative Bacteriological and Molecular Study on Some Virulence Factor of Proteus spp Isolated from Clinical and Environment Specimens. *Int.J.Curr.Res.Aca.Rev.* 2017; 5(5): 26-33. doi:10.20546/ijcrar.2017.505.005
17. Janak, Kishore. Isolation, Identification & Characterization of Proteus penneri - a missed rare pathogen. *Indian J Med Res.* 2012; 135(3): 341–345.
18. Amrullah Shidiki, Bijay Raj Pandit and Ashish Vyas. Characterization and Antibiotic profile of Pseudomonas aeruginosa isolated from patients visiting National Medical college and Teaching Hospital, Nepal. *Acta Scientific Pharmaceutical Sciences. 2019*;3(7): 02-06. doi:10.31080/ASPS.2019.03.0296
19. Jawetz, Melnick. et.al. Mikrobiologi Kedokteran, Alih Bahasa Aryandhito Widhi Nugroho et.al., editor edisi Bahasa Indonesia Adisti Adityaputri Edisi 25, Jakarta: EGC; 2012
20. Harti, a.s. *Mikrobiologi Kesehatan: Peran Mikrobiologi Dalam Bidang Kesehatan.* Yogyakarta: Andi Offset; 2015
21. Carrol, K, C., Stephen A Morse., Timothy Mietzner., Steve Miller. Jawetz, Melnick. & Adelberg. *Mikrobiologi Kedokteran* (ed 27). Jakarta: ECG; 2017
22. Trojan R, Razdan L, Singh N. Antibiotic Susceptibility Patterns of Bacterial Isolates from Pus Samples in a Tertiary Care Hospital of Punjab, India. *International Journal of Microbiology*. 2016;2016:2-4. doi:10.1155/2016/9302692
23. Giurato L, Meloni M, Izzo V, Uccioli L. Osteomyelitis in diabetic foot: A comprehensive overview. *World J Diabetes*. 2017;8(4): 135-142. doi: 10.4239/wjd.v8.i4.135

**Corresponding author: hariyanto696@gmail.com*