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ANALYSIS OF DIFFERENCES IN GERM PATTERNS IN PUS CULTURE AND TISSUE CULTURE EXAMINATION IN PATIENTS WITH DIABETIC FOOT ULCERS

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ABSTRACT

Background: Diabetic foot ulcer (DFU) is one of the chronic complications of diabetes mellitus that can increase the risk of amputation and lead to high treatment costs if the infection is not properly managed. Identifying the etiology of the infection is crucial for determining effective treatment. This study aims to analyze the differences in infection patterns obtained through pus culture and tissue culture in patients with diabetic foot ulcers.
Methods: This prospective descriptive study involved 41 patients treated at Adam Malik Hospital, Medan. Ulcer samples were collected using medical record data: sterile swabs for pus culture and tissue samples for tissue culture. The bacterial patterns identified from both culture methods were compared using the Kruskal-Wallis statistical test.
Results: Gram-negative bacteria dominated both culture methods, with Pseudomonas spp being the most common in pus cultures and Escherichia coli in tissue cultures. A significant difference was found between the two methods in bacterial identification (p=0.002).
Conclusion: There is a significant difference between tissue culture and pus culture in DFU, particularly in the number and types of bacterial isolates. This difference is not coincidental, supporting IDSA's recommendation to prioritize tissue culture over swab culture. These findings are consistent with other studies in the field.

***Keywords**: DFU, Pus culture, Tissue culture, Bacterial patterns.*

ABSTRAK

***Latar Belakang**: Ulkus kaki diabetik (DFU) merupakan salah satu komplikasi kronis diabetes melitus yang dapat meningkatkan risiko amputasi dan menyebabkan biaya pengobatan yang tinggi jika infeksi tidak dikelola dengan baik. Mengidentifikasi etiologi infeksi sangat penting untuk menentukan pengobatan yang efektif. Penelitian ini bertujuan untuk menganalisis perbedaan pola infeksi yang diperoleh melalui kultur nanah dan kultur jaringan pada pasien dengan ulkus kaki diabetes.*

***Metode**: Studi deskriptif prospektif ini melibatkan 41 pasien yang dirawat di RS Adam Malik, Medan. Sampel ulkus dikumpulkan menggunakan data rekam medis: swab steril untuk kultur nanah dan sampel jaringan untuk kultur jaringan. Pola bakteri yang diidentifikasi dari kedua metode kultur dibandingkan menggunakan uji statistik Kruskal-Wallis.*

***Hasil**: Bakteri gram-negatif mendominasi kedua metode kultur, dengan Pseudomonas spp menjadi yang paling umum dalam kultur nanah dan Escherichia dalam kultur jaringan. Perbedaan yang signifikan ditemukan antara kedua metode dalam identifikasi bakteri (p = 0,002).*

***Kesimpulan**: Ada perbedaan yang signifikan antara kultur jaringan dan kultur nanah dari DFU, terutama dalam jumlah dan jenis isolat bakteri. Perbedaan ini bukan kebetulan, mendukung rekomendasi IDSA untuk memprioritaskan kultur jaringan daripada kultur swab. Temuan ini konsisten dengan penelitian lain di lapangan.*

***Kata kunci**: DFU, Kultur Pus, Kultur jaringan, Pola bakteri*

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1. Introduction

Diabetes Mellitus (DM) is a condition that can involve complications throughout the body, including wounds on the feet of patients with DM. DM is one of the global health issues whose prevalence continues to increase each year. It is estimated that the number of diabetes sufferers worldwide will reach 643 million by 2030 and 784 million by 2045, with the majority of patients living in low- and middle-income countries. In Indonesia, the prevalence of DM also shows a significantly high rate, especially in North Sumatra, where the prevalence exceeds 1.8%, and in some districts/cities, it reaches 2.9%.[1]. The pathophysiology of DFUs involves metabolic dysfunction, diabetic immunopathy, diabetic neuropathy, and angiopathy. The processes by which hyperglycemia causes peripheral nerve damage are related to adenosine triphosphate deficiency, the polyol pathway, oxidative stress, protein kinase C activity, and proinflammatory processes. In the context of hyperglycemia, the suppression of endothelial nitric oxide production leads to microcirculation atherosclerosis, heightened inflammation, and abnormal intimal growth. Diabetic neuropathy involves sensory, motor, and autonomic neuropathies. The interaction between these neuropathies forms a callus that leads to subcutaneous hemorrhage and skin ulcers. Hyperglycemia causes peripheral vascular changes that result in endothelial cell dysfunction and decreased vasodilator secretion, leading to ischemia. The interplay among these four preceding pathophysiological factors fosters the development and progression of infections in individuals with diabetes. Charcot neuroarthropathy is a chronic and progressive degenerative arthropathy characterized by heightened blood flow, increased calcium dissolution, and repeated minor trauma to insensate joints [2]. One of the most common chronic complications of DM is diabetic foot ulcers (DFU). Diabetic foot ulcers

are one of the main causes of morbidity in diabetes patients and are a significant factor that can lead to lower extremity amputations, resulting in increased healthcare costs. These ulcers occur due to peripheral neuropathy, peripheral vascular disease, and poor wound healing in diabetic patients. Infected ulcers can progress to severe infections that increase the risk of amputation by up to 80% if not managed properly.[2]. Bacterial identification is crucial in selecting the appropriate antibiotics to improve healing outcomes and reduce the risk of antibiotic resistance. The Infectious Diseases Society of America (IDSA) recommends obtaining specimens from deep tissue through biopsy or curettage, rather than using swab methods. The variation in bacterial profiles of diabetic ulcers is influenced by geographic, climatic, and cultural factors, making international guidelines for antibiotic selection difficult to apply in developing countries. In Indonesia, research on identifying diabetic foot infections based on tissue culture is still limited, and many healthcare centres continue to use swab techniques.[2]. IDSA stated that the benefits obtained from tissue culture are that the microbial representation is more varied and dominates compared to swab culture. This study aims to compare two specimen collection methods, tissue culture and pus culture, for identifying organisms in diabetic foot infections to guide appropriate antibiotic therapy. [3].

2. Methods

This research is a descriptive study with a prospective design aimed at analyzing the differences in microbial patterns between tissue culture and pus culture examinations in diabetic foot ulcers. The study was conducted at Adam Malik Hospital from January 2024 to January 2025. Patient data and samples were collected directly from medical records that met the inclusion criteria. Based on the sample size calculation, the minimum required sample size for this study is 41. The population for this study consists of all patients diagnosed with DM and diabetic foot ulcers who underwent culture at Adam Malik Hospital. The study samples were medical record data from inpatients with DM and diabetic foot ulcers with Wagner grades 2, 3, and 4.

The inclusion criteria for this study were medical record data of patients diagnosed with DM and diabetic foot ulcers who had undergone both swab and tissue cultures. Demographic data such as age, gender, and ulcer grade were collected from medical records. Sample collection was performed by the researcher following the steps outlined below: [4,5]

Data were analyzed descriptively to illustrate the frequency distribution based on demographic characteristics and culture results. Statistical analysis was carried out using statistical software with the Kruskal-Wallis test to compare differences in microbial patterns between pus culture and tissue culture. Results were considered statistically significant if the p-value was less than 0.05. Research permission was granted by the supervisor, and the letter was issued by the Director of HR, Education, and Research at Adam Malik Hospital (DP.04.03/D.XXVIII/3115/2025) regarding research approval, along with approval from the Research Ethics Committee at the Universitas Sumatera Utara (USU).

3. Results

From the total data of medical records of patients with DM and diabetic foot ulcer complications who were hospitalized in the inpatient unit of Adam Malik Hospital, Medan, 41 patients met the inclusion and exclusion criteria. The average age of the study subjects was 55 years, with the youngest being 33 years old and the oldest being 87 years old. The majority of the study subjects were male, totaling 23 individuals (56%), and female, totaling 18 individuals (44%). The most common grade of pressure ulcer based on the Wagner classification was grade 3, found in 21 individuals (51.2%). The most frequently found comorbidity in diabetic foot ulcer patients was chronic kidney disease (CKD), with

18 subjects (43.90%). Additionally, there was 1 individual each with coronary artery disease (CAD), acute lymphocytic leukemia (ALL), myelodysplastic syndrome (MDS), and systemic lupus erythematosus (SLE) (2.43% each).

The results from the swab culture method showed that 13 samples (31.7%) had Gram-positive bacteria, 22 samples (53.7%) had Gram-negative bacteria, 5 samples (12.2%) showed the presence of both Gram-positive and Gram-negative bacteria, and 1 sample (2.4%) showed no bacterial growth. For tissue culture, 7 samples (17.1%) showed Gram-(+) bacteria, 28 samples (68.3%) showed Gram-negative bacteria, and 6 samples (14.6%) (Table 1).

Table 1. Demographic Characteristics

Results	n (%)
Gender	
Male	23 (56)
Female	18 (44)
Degree of Diabetic Ulcer	
Grade 2	16 (39)
Grade 3	21 (51.2)
Grade 4	4 (9.8)
Comorbidities	
CKD (Chronic Kidney Disease)	18 (43.90)
Hypertension	15 (36.58)
No comorbidity	4 (9.75)
Ischemic Stroke	2 (4.87)
CAD (Coronary Artery Disease)	1 (2.43)
ALL (Acute Lymphocytic Leukemia)	1 (2.43)
MDS (Myelodysplastic Syndrome)	1 (2.43)
SLE (Systemic Lupus Erythematosus)	1 (2.43)
Swab Culture	
Gram (+)	13 (31.7)
Gram (-)	22 (53.7)
Both Gram-(+) and (-)	5 (12.2)
No bacterial growth	1 (2.4)
Tissue Culture	
Gram (+)	7 (17.1)
Gram (-)	28 (68.3)
Both Gram (+) and (-)	6 (14.6)

The prevalence of culture isolates of DM specimens with diabetic ulcer complications is presented in Table 2. In the swab culture examination, 54 bacterial isolates were found, most of which were Gram-negative bacteria, 35 bacterial isolates with the most species being Pseudomonas ssp, followed by Klebsiella ssp, Escherichia coli, Acinetobacter ssp, Citrobacter freundii, Enterobacter ssp, Proteus ssp, Morganella morganii ssp morgani, Providencia stuartii, and Salmonella spp. There were 19 Gram-positive) bacterial isolates, with the most species being Staphylococcus spp, followed by Streptococcus spp and Enterococcus spp. In tissue culture examination, 61 bacterial isolates were found, with the most bacteria being Gram-negative bacteria, as many as 47 isolates, with the most species being Escherichia coli, followed by Klebsiella ssp, Pseudomonas ssp, Providencia ssp, Acinetobacter ssp, Enterobacter ssp, Proteus ssp, Serratia ssp, and Citrobacter freundii. Gram-positive bacterial isolates as many as 14, with the most species being Staphylococcus spp, followed by Enterococcus spp and Kocuria kristinae (Table 2).

Table 2. Specimen Culture Results of Isolates of Specimens Diabetic Ulcer

Swab Culture		**Tissue Culture**		
		Gram (-)		
Bacterial Isolates	n (%)	Bacterial Isolates	n	Isolates
Escherichia coli	6	*Escherichia coli*	10	4
Pseudomonas spp	8	*Pseudomonas spp*	7	-1
Klebsiella ssp	7	*Klebsiella ssp*	8	1
Acinetobacter ssp	3	*Acinetobacter ssp*	5	2
Enterobacter ssp	3	*Enterobacter ssp*	4	1
Providencia ssp	1	*Providencia ssp*	6	5
Proteus ssp	2	*Proteus ssp*	4	2
Citrobacter freundii	3	*Citrobacter freundii*	1	-2
Serratia spp	0	*Serratia spp*	2	2
Morganella morganii ssp morgani	1	*Morganella morganii ssp morgani*	0	-1
Salmonella spp	1	*Salmonella spp*	0	-1
Total	35 (64.81)		47 (77.05)	12 (26.67)
		Gram (+)		
Staphylococcus spp	13	*Staphylococcus spp*	12	-1
Enterococcus spp	3	*Enterococcus spp*	1	-2
Streptococcus spp	3	*Streptococcus spp*	0	-3
Kocuria kristinae	0	*Kocuria kristinae*	1	1
Total	19 (35.19)		14 (22.95)	-5 (35.71)
Overall Total	54 (100)		61 (100)	7 (11.48)

There was a significant difference in the bacterial pattern in tissue culture and pus culture examinations of diabetic foot ulcers (p=0.002). More complete results are presented in Table 3.

Table 3. Relationship between swab culture and tissue culture

	Tissue Culture			
Swab Culture	Gram (+)	Gram (+)	Gram (+) and (-)	p
Gram (+)	5	6	2	0.002
Gram (-)	0	20	2	
Gram (+) and (-)	1	2	2	
No growth	1	0	0	

4. Discussion

Research shows that the global prevalence of diabetic foot ulcers is 6.3%, higher in men, and common in T2DM. A study in India by Debarath et al. concluded that gender and age do not affect the agreement between swab and tissue culture methods in diagnosing diabetic foot infections [5]. In this research, there were 56% men and 44% women, Shah et al. (2019 - 2021) in India, where out of 50 diabetic foot patients, 21 (42%) subjects had grade II Wagner lesions, followed by 17 (34%) with grade III lesions and 6 (12%) with grade IV lesions. Stratification of diabetic foot patients and proper management based on Wagner scores certainly helps reduce amputation and mortality rates. Increasing Wagner stratification indicates an increased risk of amputation and management [6]. Chronic hyperglycemia in DM contributes to the emergence of various complications, long-term damage, dysfunction, and failure of various organs, one of which is the kidneys. One of the causes of kidney damage (kidney failure) is diabetic nephropathy due to uncontrolled DM, and it is the leading cause of death in people with DM [7]. Research by Debarath et al (2016-2017) found that more bacterial isolates were found in tissue than swabs [8]. Research by Gurulingaiah et al (2018-2020) showed that Gram-positive bacteria were more commonly found with swabs, while Gram-negative bacteria were more commonly found in tissue, with swab and tissue sensitivity of 82.27% and 78.48%

respectively, with specificity of 52.38% and tissue of 71.43% [8]. Research by N Sankar et al (2020) also revealed that more bacterial isolates were found in tissue than swabs [9]. A study in the UK by Nelson et al (2018) found that bacterial isolates were more commonly found in tissue in a multicentre study with 395 subjects (P<0.01) [9]. A study in China by Huang et al (2016) showed that Gram-positive and Gram-negative bacteria were more commonly detected in tissue in a prospective study with 56 subjects [10,11]. A study in Turkey by Mutluoglu et al (2012) concluded that Gram-negative bacteria were more commonly found in tissue, while Gram-positive) Bacteria were more commonly found in swabs in a review study with 54 subjects [11]. For a study in Indonesia, Darwis et al (2017-2019): Of the 131 subjects, 85.5% of bacterial isolates were Gram-negative and 14.5% were Gram-positive. There are no clear data regarding the comparison of swab vs tissue [12]. Radji et al (2012): In 35 subjects, 59 bacterial isolates were found via swabs, dominated by Gram-(+) [13]. Tangion, et al (2018-2020): From 44 subjects, 54 bacterial isolates were found, with a predominance of Gram-negative [14]. Bulolo et al (2018): In 33 subjects, Gram-negative was more dominant than Gram-positive. Data on tissue culture in Indonesia is still limited [15].

This study used the Kruskal-Wallis Test to compare the results of swab and tissue cultures, with a P value (0.002) indicating a significant difference. Tissue culture is considered more accurate because it removes necrotic tissue to avoid contamination, while swab culture has a higher risk of contamination. According to the recommendations of the Infectious Diseases Society of America (IDSA), tissue culture is recommended. The Infectious Diseases Society of America (IDSA), 2012, stated that in temperate climates (North America and Europe), the main pathogens in diabetic foot ulcers are aerobic Gram-positive. Cocci, such as Staphylococcus aureus and Streptococcus. In tropical/subtropical areas (Asia and North Africa), the main cause is Gram-negative aerobic bacilli, either alone or in combination with Gram-positive bacilli. Cocci [16,17]. Variations in the bacterial profile of diabetic ulcers are influenced by geographical factors, climate, culture, and specimen collection methods, so that international clinical guidelines are difficult to apply in developing countries. Therefore, each region needs to have its own guidelines regarding bacterial patterns and antibiotic selection [18-20]. The strength of this research is that new data obtained in this research show that there is a significant difference between tissue culture and pus culture in diabetic foot ulcers, where the difference lies in the quantity and types of bacterial isolates obtained. The weakness of this study is the presence of confounding caused by the administration of antibiotics before the patients underwent culture.

4. Conclusion

There was a significant difference between tissue culture and swab in diabetic foot ulcers, especially in the number and type of bacterial isolates (p=0.002), with tissue culture being more recommended. The majority of patients were male with comorbid CKD and Wagner grade 3. Gram-negative bacteria predominated, with Pseudomonas spp in swabs and Escherichia coli in tissues, while the most common Gram-positive bacteria were Staphylococcus spp. A weakness in this study is the presence of confounders in the form of antibiotics given before the patient was cultured.

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Conflict of Interest

All the authors declare that there are no conflicts of interest.

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