Jurnal Kedokteran dan Kesehatan: Publikasi Ilmiah Fakultas Kedokteran Universitas Sriwijaya
Volume 11, No 2. 2024/DOI: 10.32539/jkk.v11i2.295
p-ISSN 2406-7431; e-ISSN 2614-0411
Page: 200-208

ISOLATION AND IDENTIFICATION OF BACTERIA CAUSING SECONDARY WOUND INFECTIONS IN DIABETES MELLITUS PATIENTS IN JAMBI CITY

Qatrinnada Maulidya Akmal[1*], Hanina [2], Humaryanto[3], Lipinwati[4], Rita Halim[5]

[1] Medical Study Program, Faculty of Medicine and Health Sciences, Jambi University, Jambi, Indonesia
[2,4]Departement of Microbiology, Faculty of Medicine and Health Sciences, Jambi University, Jambi. Indonesia
[3]Departement of Surgery, Faculty of Medicine and Health Sciences, Jambi University, Jambi, Indonesia
[5]Departemen of Nutrition, Faculty of Medicine and Health Sciences, Jambi University, Jambi, Indonesia

ARTICLE INFO

***Corresponding author:**
Qatrinnada Maulidya Akmal
Medical Study Program
Faculty of Medicine and
Health Sciences
Jambi University
Jambi, Indonesia

Email:
qatrindamaulidya@gmail.com

Kata kunci:
Isolasi bakteri
Identifikasi bakteri
Luka infeksi sekunder
Diabetes melitus

Keywords:
Isolation of bacteria
Identification of bacteria
Secondary wound infections
Diabetes mellitus

Original submission:
October 20, 2023;
Accepted:
December 28, 2023;
Published:
April 20, 2024;

ABSTRAK

Pengetahuan tentang jenis bakteri penyebab luka infeksi sekunder pada pasien Diabetes Melitus (DM) sangat penting untuk mengembangkan strategi pengobatan yang efektif. Bakteri yang menginfeksi sangat beragam dan belum diketahui secara pasti bakteri yang paling banyak menginfeksi luka pada pasien DM. Jenis penelitian ini adalah deskriptif dengan rancangan *cross-sectional.* Sampel penelitian diambil melalui swab luka infeksi sekunder pada pasien DM di RSUD H. Abdul Manap Kota Jambi pada bulan Agustus hingga Oktober 2023, lalu dilakukan pewarnaan gram, kultur, dan uji enzimatik. Karakteristik pasien diperoleh melalui wawancara dan rekam medik. Sebanyak 21 sampel yang diisolasi dari swab luka infeksi sekunder pasien DM, didapatkan pasien terbanyak pada kelompok usia 45-59 tahun (61,9%), jenis kelamin laki-laki (81%), pekerjaan kategori risiko tinggi (42,9%), lokasi pada ekstremitas bawah (90,5%), diagnosis luka infeksi primer *vulnus scissum* dan *vulnus abrasio* (33,3%), diagnosis luka infeksi sekunder ulkus DM (66,7%). Hasil identifikasi bakteri dengan kultur, pewarnaan Gram, dan uji enzimatik didapatkan paling banyak bakteri *Staphylococcus aureus* (42,9%). Hasil identifikasi bakteri dari swab luka infeksi sekunder pasien DM didapatkan paling banyak bakteri *Staphylococcus aureus.*

ABSTRACT

Isolation and identification of bacteria causing secondary wound infections in diabetes mellitus patients in Jambi City. *The isolation and identification of secondary wound infection bacteria in diabetic patients in Jambi City are crucial for developing effective treatment strategies. The diversity of infecting bacteria underscores the importance of discerning the most prevalent types. This research aims to identify bacteria causing secondary wound infections in diabetic patients. Researchers obtained samples for this descriptive cross-sectional study by swabbing the incisions of diabetic patients at RSUD H. Abdul Manap in Jambi City between August and October 2023. Gram staining, culturing, and enzymatic tests were performed on the samples. Out of 21 samples, the age group most affected was 45-59 years (61.9%), predominantly male (81%), engaged in high-risk occupations (42.9%), with lower extremity wounds (90.5%), and primary wounds diagnosed as vulnus scissum and vulnus abrasio (33.3%). In contrast, DM ulcers constituted 66.7% of secondary wound infections in diabetic patients. Bacterial identification revealed Staphylococcus aureus as the predominant species (42.9%) responsible for secondary wound infections in diabetic patients. This study underscores the dominance of Staphylococcus aureus in secondary wound infections among diabetic patients.*

INTRODUCTION

Diabetes Mellitus (DM) is one of the most common metabolic diseases in the world. [1] The number of DM incidents continues to increase to date. According to the data from the International Diabetes Federation (IDF) in 2021, 537 million adults aged 20-79 years suffer from diabetes worldwide. Indonesia is in the fifth highest position, with the number of people with diabetes as many as 19.47 million, with a diabetes prevalence of 10.6%.[2] The increase in the prevalence rate of DM certainly intensifies the incidence of complications related to DM. Open wounds in DM patients become the entry point for bacteria that cause infection. The high incidence rate of secondary wound infections in DM patients globally, both type 1 and type 2, is associated with a high risk of infection. A retrospective study conducted in England revealed that DM caused 6% of infection-related hospitalizations and 12% of infection-related deaths.[3] Research in the United States indicated that DM patients had a 2-fold increased risk of hospitalization when they experienced an infection when they came to the emergency department, and up to 12% of hospitalized diabetes patients were caused by infection.[4] According to the Ministry of Health of the Republic of Indonesia in 2013, the prevalence of wounds causing secondary infections in Indonesia reached 8.2% and 4.5% in Jambi.[5,6] However, the bacteria that infect are very diverse and it is not yet known with certainty which bacteria most commonly infect wounds in DM patients.[7]

Elevated blood glucose levels in DM patients can create an environment that supports bacterial growth, increases the risk of infection, and prolongs the wound-healing process. Secondary wound infection causes damage to skin integrity followed by infection of the skin in the same location. This secondary infection is more susceptible to occurring in people with a poor immune system, such as DM patients. Not only that, dirty wound conditions, frequent exposure to moisture, dirty environments, and inadequate treatment can also trigger secondary infections. The presence of secondary infections can cause hospital stays to be longer, increasing mortality rates and medical costs.[8,9]

This research is important to conduct, to determine the results of isolating and eliminating bacteria that cause secondary wound infections in DM patients in Jambi City. Then to determine the type of bacteria that causes secondary wound infections in DM patients, so that appropriate and efficient therapy can be given according to the patient's condition and can prevent irrational and ineffective administration of antimicrobial drugs. Bacterial isolation aims to obtain pure cultures. Identification of secondary infected bacterial wounds in DM patients is carried out by observing the morphology of bacterial colonies, Gram staining, and enzymatic tests.[10]

According to research by Ekawati ER et al., in pus from skin-infected wounds, 2 types of bacteria were obtained, namely *S.aureus* and *P.aeruginosa*.[11] In research by Macdonald KE et al., in patients with diabetic foot infections at Scottish Tertiary Hospitals in 2020, 62% of *S.aureus* bacteria were found.[1] Based on research conducted by Rizqiyah et al., on diabetic ulcer patients in RSUD Dr. H. Abdul Moeloek in 2020, *S.aureus* bacteria was the dominant isolate (58%).[12] In Jambi City itself there has been no research that has identified the bacteria that cause infection in DM patients. Based on the explanation above, it is necessary to carry out research regarding the isolation and identification of bacteria that cause secondary wound infections in DM patients.

METHODS

This research was a descriptive study with a cross-sectional design, which was conducted at the Biomedical Laboratory, Faculty of Medicine and Health Sciences, Jambi University from August

to October 2023. The samples in this study were DM patients who experienced secondary wound infections in the Outpatient and Inpatient Installations for Internal Medicine and Surgery in H. Abdul Manap Hospital, Jambi City which met the inclusion criteria. The inclusion criteria, namely DM patients who had been diagnosed with secondary wound infections aged 44-69 years in H. Abdul Manap Hospital, Jambi City from August to October 2023. Secondary wound infections were characterized by inflammatory symptoms such as redness, swelling, pain, and warmth, and accompanied by pus, the infection lasted more than 7 days after the wound occurred, with the patient willing to become a research subject and fill out an informed consent form. The exclusion criteria in this research were post-operative patients or infected wounds that were sewn up (heating).

The first step in taking samples was washing hands and using sterile gloves, then cleaning the wound with gauze and 0.9% NaCl. Research samples were taken by carrying out a duplex swab (wipe twice) on secondary wound infections of DM patients using a sterile cotton swab, then putting it into a test tube containing sterile 0.9% NaCl with the tip of the cotton swab broken, labeled and put in a cooler box, then the sample had to be taken immediately to the laboratory in less than 2 hours for Gram staining and bacterial cultivation in appropriate media. The Gram staining procedure started with preparing a clean glass object. The round tube was heated until it glowed, 1 tube of bacterial suspension solution was taken and then placed on a glass object and stirred with a circular motion until evenly mixed, then dried, and fixed over a Bunsen burner. The smear preparation was first stained with gentian violet solution for 30 seconds, then flushed with distilled water. Next, it was dripped with Lugol's solution for 30 seconds and flushed again with distilled water. Then the smear preparation was dissolved with 95% alcohol, rinsed with distilled water, and given a second stain with fuchsin solution for one minute, rinsed again with distilled water, and dried. The morphology and color of the cells were observed under a microscope with 100x magnification which had previously been dripped with immersion oil. The bacteria would be classified as Gram-positive if they were purple and Gram-negative if they were red.

Next, the bacteria were planted using the scratch or streak plate method. The media used in this research were Nutrient Agar (NA), Mannitol Salt Agar (MSA), Blood Agar (BA), and Mac Conkey Agar (MCA). The stretch plate method was done by heating the hose until it glowed over a Bunsen burner, then cooling it. Then a tube of bacteria was taken and scratched on the surface of the media starting at one end, from the edge of the petri dish towards the center of the dish with a zig-zag movement. The loop needed to be lighted first every time you applied it for the next quadrant, and let it cool, then incubated at 37°C for 24 hours to grow microorganisms.[13] Next, an enzymatic test consisting of a catalase test, coagulase test, and oxidase test was carried out by adding one drop of 3% Hydrogen Peroxide (H2O2) to bacterial colonies on a clean glass slide. The suspension was mixed slowly using a hose, and the formation of gas bubbles indicated a positive catalase test result. Then, the coagulase test was carried out using the slide test method. The slide test was carried out by placing a drop of NaCl on a glass object, then taking a colony of bacteria to be tested with a slide, then suspending it. A drop of plasma was placed near the bacterial suspension, both were homogenized on a slide. A positive reaction occurs if a clot formed within two to three minutes. In the oxidase test, the bacterial culture was streaked on an oxidase strip using a loop. Changes in bacterial colonies were observed for approximately ± 5 seconds. If the colony changed color to deep blue/violet on the oxidase strip, it indicated a positive oxidase test result. A negative test result was indicated by no color change.[13] Patient characteristics were obtained through interviews and medical records.

RESULTS

It was found that the number of DM patients with secondary wound infections who met the inclusion criteria during the August-October 2023 period was 21 patients. Occupational risk categories were divided into 3 categories based on exposures and types of activities. High risk based on exposures, namely contact with infected animals/livestock, barefoot contact with contaminated soil, and contact with infected humans. Types of activities, such as farming, raising livestock, plowing, digging, environmental, air and water contamination, caring for/treating infected patients, and person-to-person transmission through physical contact. Medium risk was based on exposures, namely contact with work environments contaminated with bacteria, contact with public transportation, and public spaces contaminated with bacteria.

Table 1. Patient Characteristics

Patient Characteristics	Frequency (n)	Percentage (%)
Age		
19-44 years old	1	4.8
45-59 years old	13	61.9
> 60 years old	7	33.3
Gender		
Male	17	81.0
Female	4	19.0
Occupation		
High risk	9	42.9
Medium risk	8	38.1
Low risk	4	19.0
Secondary Wound Infection Location		
Head and neck		
Thorax	2	9.5
Back	0	0
Abdomen	0	0
Upper extremities	0	0
Lower extremities	0	0
Perianal	19	90.5
	0	0
Diagnosis of Primary Wound Infection		
Vulnus scissum		
Vulnus abrasion	7	33.3
Vulnus lacerated	7	33.3
Vulnus punctum	5	23.8
	2	9.5
Diagnosis of Secondary Wound Infection		
DM Ulcer		
Access	14	66.7
Cellulitis	4	19.0
	3	14.3

Types of activities, specifically working in dirty environments, using public transportation, and public spaces could increase the risk of bacterial transmission. Low risk based on exposure, particularly, contact with contaminated objects or surfaces. Types of activities, namely cleaning,

washing, and cooking.[19,21] Table 1 illustrates that patient characteristics were mostly found in the age group 45-59 years (61.9%), male (81%), high-risk work category (42.9%), located in the lower extremities (90.5%), with the diagnosis of primary wound infection of *vulnus scissum* and *vulnus abrasio*n (33.3%), and diagnosis of secondary wound infections of DM ulcer (66.7%).

Table 2. Identification of Bacteria that Cause Secondary Wound Infections in DM Patients

No. Sample	Gram Staining	Bacterial Colony	Enzymatic Test	Conclusion of Bacterial Type	Frequency (n)	Percentage (%)
5, 6, 7, 9, 13, 17, 19, 20, 21	Gram-positive Cocci	Yellowish colonies on NA media, yellow colonies on MSA media, beta hemolysis on BA Media	Positive catalase, positive coagulase	*Staphylococcus aureus*	9	42.9
8, 15, 16	Gram-positive Cocci	Yellowish colonies on NA media, no color change on MSA media, gamma hemolysis on BA Media	Positive catalase, negative coagulase	*Staphylococcus epidermidis*	3	14.3
11	Gram-positive Cocci	Yellowish colonies on NA media, no growth on MSA media, beta hemolysis on BA Media	Negative catalase	*Streptococcus pyogenes*	1	4.8
2,4	Gram-negative Bacilli	Yellowish colonies on NA media, lactose fermenters with mucoid colonies on MCA media	Positive catalase, negative oxidase	*Klebsiella pneumonia*	2	9.5
12, 14	Gram-negative Bacilli	Yellowish colonies on NA media, non-lactose fermenters on MCA media	Positive catalase, negative oxidase	*Proteus sp.*	2	9.5
1, 3, 10, 18	Gram-negative Bacilli	Greenish-yellow colonies on NA media, non lactose fermenters on MCA medium	Positive catalase, negative oxidase	*Pseudomonas aeruginosa*	4	19.0

Table 2 shows that the most Gram staining results were found to be Gram-positive bacteria in 13 samples (61.9%). Meanwhile, there were 8 samples (38.1%) of Gram-negative bacteria. Gram-

positive bacteria consisted of *S. aureus* (42.9%), *S. epidermidis* (14.3%), and *S.pyogenes* (4.8%). Whereas, Gram-negative bacteria consisted of *P.aeruginosa* (19%), *K.pneumonia* (9.5%), and *Proteus sp.* (9.5%).

DISCUSSION

In Table 1, based on age characteristics, the majority of patients were aged 45-59 years (61.9%). The majority of diabetic ulcer patients were found in the 50-59 year age group (45%).[14] Diabetic ulcer patients at RSUD Dr. Moewardi Surakarta, the highest number was found in the group of people aged 46-55 years (75.4%).[15] The occurrence of secondary wound infections in the 45-59-year age group could be caused by various factors, such as a decrease in the immune system and blood circulation, and an increased risk of injury. The immune system tends to decline as people age, making individuals susceptible to infections. In addition, decreased blood circulation can also slow down the wound-healing process, thereby increasing the risk of secondary infections. Chronic medical conditions such as diabetes can affect the body's ability to fight infections. In addition, changes in skin and tissue structures in old age can also make individuals more susceptible to injuries and infections.[16]

In this research, the male gender dominated with 17 samples (81%). Research conducted by Febiola R on diabetic ulcer patients in Yogyakarta City Hospital, found that the majority of patients were male (55.6%).[17] Diabetic ulcer patients in RSUD Dr. H. Abdul Moeloek it was found that the majority of patients were male (76.2%).[12] This situation was caused by men having a higher level of outdoor physical activities in hot and humid environments compared to women.[12]

In this research, based on the occupations, most patients were found in the high-risk category, namely 9 patients (42.9%). Diabetic foot ulcer patients in the Tikur Anbessa Specialist Hospital, Addis Ababa, Ethiopia, the patient's occupations, namely laborer and farmer, were in the high-risk category. In research by Rofiqi et al, farmers who suffered from diabetes were at risk of developing diabetic ulcers because farmers pay less attention to foot protection when working. Feet are at high risk of infection because they are often punctured by sharp agricultural tools, plant debris, and the lack of wearing footwear.[20] Patients who experience secondary wound infections, whether they are farmers, laborers, livestock breeders, or construction workers, their jobs are included in the high category. This categorization is based on exposure, namely contact with infected animals/livestock, barefoot contact with contaminated soil, and contact with infected humans. Based on the types of work activities, including farming, animal husbandry, plowing, digging, environmental, air and water contamination, caring/treating infected patients, and transmission from person to person through physical contact.[19,21]

Based on the location of the secondary wound infections, in this research, the lower extremities were the location it was found the most, in 19 people (90.5%). Patients with diabetic ulcers it was mostly found on the feet (100%).[22] Patients with diabetic ulcers it was found the most on the lower extremities (100%).[20] High blood sugar levels in the long term can cause atherosclerosis which can result in narrowing and hardening of the blood vessels. This condition can occur in all arteries in the body, including the lower legs, resulting in reduced blood flow to the legs. In this condition, if an infection occurs in a wound on the foot, bacterial contamination can cause a secondary infection.[23]

Based on the diagnosis of primary wound infection in this research, it was found that the first infections occurred the most in the *vulnus scissum* in 7 people (33.3%) and the *vulnus abrasio*n

in 7 people (33.3%). The highest number of vulnus abrasions was found (70.9%) on DM patients[24]and DM patients with vulnus scissum were found.[25] When the skin is injured, whether because of a punctured wound, scratch, or other wounds, it becomes infected the second time by entering the skin through the wound, which can cause a secondary infection. Then, in primary wound infections where wound care is inadequate, exposure to a dirty environment can result in secondary infections, and the healing process will take longer.[8]

Based on the diagnosis of secondary wound infections in this study, the highest number of patients diagnosed with DM ulcers were 14 people (66.7%), 4 abscess patients (19%), and 3 cellulitis patients (14.3%). According to research on patients with ulcers, abscesses, and cellulitis in 2020 in PKU Muhammadiyah Hospital Surakarta, there were 17 ulcer patients (50%), 12 abscesses patients (35.3%), and 5 cellulitis patients (14 .7%).[26] Basuki R and Husen F's research on diabetes patients in Aghisna Sidareja General Hospital found that most of the patients were diagnosed with DM ulcers (22.41%).[27] DM patients who have primary wound infections will be more susceptible to secondary infections because of their weak immune system and the presence of high blood sugar that becomes a nutrient and a place for bacterial growth.[34]

Based on Table 2, from the results of this research, the bacteria most frequently identified in DM patients with secondary wound infections was *S.aureus* with 9 isolates (42.9%). According to research in RSUD Dr. H. Abdul Moeloek in 2020, *S.aureus* was the dominant isolate in Gram-positive cocci organisms (58%).[12] Patients with ulcers, abscesses, and cellulitis, the highest percentage of bacteria was *S. aureus* (44%).[26] Meanwhile, the most commonly found Gram-negative bacteria was *P. aeruginosa* with 4 isolates (19%). *S. aureus* bacteria is the most common cause of skin infections.[31,32] According to the International Working Group on the Diabetic Foot in 2020, *S.aureus* was the bacteria most often found in diabetic ulcer cultures.[33] Meanwhile, Pseudomonas bacteria are generally found in superficial wounds, especially in patients who have received antibiotic therapy.[7] In addition, *P.aeruginosa* tends to grow in humid environments.[11]

Apart from *S. aureus* bacteria, another Gram-positive bacteria obtained was *S. epidermidis* with 3 isolates (14.3%). Research in DM ulcer patients in RSUD Dr. H. Abdul Moeloek 2020, found *S.epidermidis* bacteria (4%).[12] *S.epidermidis* is not dangerous on intact human skin, but this bacteria can cause serious infections after skin penetration, especially in individuals with a weak immune system, such as diabetes.[12] Another Gram-positive bacteria that was also found in this study was *S.pyogenes* with 1 isolate (4.8%). On diabetic foot patients in Sanglah Central General Hospital 2020, 1 *S. pyogenes* bacteria was found (1.2%).[29] *S. pyogenes* is one of the bacteria commonly found in skin-infected wounds.[11]

Apart from *P.aeruginosa* bacteria, other Gram-negative bacteria obtained were *K. pneumonia* and *Proteus sp*. which had the same number of isolates, namely 2 isolates (9.5%). Based on research in DM ulcer patients, *K. pneumonia* (17.9%), and *Proteus sp.* (4.9%) bacteria were found.[30] DM ulcer patients in RSUD Dr. H. Abdul Moeloek, *Proteus sp.* as much as 28% was obtained.[12] *K.pneumonia* is normal flora found in the human upper respiratory tract, and can also be found in soil and water. It is also often associated with opportunistic infections and in hospitalized patients.[28,29] Meanwhile, *Proteus sp*. is one of the infectious agents that is often found in diabetic ulcers.[30]

CONCLUSION

Based on the research that was carried out, it can be concluded that the highest number of

sufferers of secondary wound infections in DM patients were in the age group 45-59 years, male, with a high-risk work category. The location of secondary wound infections in DM patients was often found the most in the lower extremities, with primary infections being *vulnus scissum* and *vulnus abrasio*n, and the diagnosis of secondary wound infections was DM ulcers. The result of the identification of bacteria that cause secondary wound infections in DM patients was mostly *S.aureus* bacteria (42.9%).

REFERENCES

1. Macdonald KE, *et al. A Retrospective Analysis of The Microbiology of Diabetic Foot Infections at A Scottish Tertiary Hospital. BMC Infectious Diseases.* 2020;20(1):1-7.
2. International Diabetes Federation. 2022. *Diabetes around the world in 2021* [Internet]. [cited December 22, 2023]. Available from: https://diabetesatlas.org/.
3. Carey IM, *et al*. *Risk of Infection in Type 1 and Type 2 Diabetes Compared With the General Population: A Matched Cohort Study. Diabetes Care*. 2018;41(3):513-521.
4. Korbel L, Spencer JD. *Diabetes mellitus and infection: an evaluation of hospital utilization and management costs in the United States. Journal Diabetes Complications.* 2015;29(2):192-5.
5. Wintoko R, Yadika ADN. Manajemen Terkini Perawatan Luka. Jurnal Kesehatan Universitas Lampung. 2020;4(2):183–9.
6. Departemen Kesehatan Republik Indonesia. Riset Kesehatan Dasar. 2013.
7. Suparwati, Sukarni, Fradianto I. Identifikasi Bakteri pada Luka Kaki Diabetes yang Mengalami Infeksi: Kajian Literatur. Fakultas Kedokteran Universitas Tanjungpura Pontianak. Jurnal Berkala Ilmiah Mahasiswa Ilmu Keperawatan Indonesia. 2022;10(1):35-41.
8. Hidayati A, dkk. Infeksi Bakteri di Kulit. Surabaya: Airlangga University Press; 2019.
9. Joegijantoro R. Penyakit Infeksi. Malang: Intimedia; 2019.
10. Kuswiyanto. Bakteriologi 2: Buku Ajar Analisis Kesehatan. Jakarta: EGC; 2022.
11. Ekawati ER, Husnul Y, Herawati D. Identifikasi Kuman pada Pus dari Luka Infeksi Kulit. Universitas Maarif Hasyim Latif Sidoarjo. Jurnal SainHealth. 2018;2(1):31.
12. Rizqiyah H, dkk. Pola Bakteri Ulkus Diabetikum pada Penderita Diabetes Melitus di RSUD Dr. H. Abdul Moeloek. Universitas Lampung. Jurnal Kedokteran Universitas Lampung. 2020;9(2):128-32.
13. Karolina ME, Lipinwati, Hanina, Wulandari PS. Penuntun Praktikum Mikrobiologi untuk Mahasiswa Kedokteran. Universitas Jambi. 2019;5-26.
14. Gaol YEL, dkk. Pola Resistensi Bakteri Aerob pada Ulkus Diabetik Terhadap Beberapa Antibiotika di Laboratorium Mikrobiologi RSUP Dr. M. Djamil Padang. Jurnal Kesehatan Andalas. 2017;6(1):164.
15. Fadlilah S. Faktor-Faktor yang Berhubungan dengan Derajat Ulkus Kaki Diabetik di RSUD Dr. Moewardi Surakarta. Jurnal Ilmiah Rekam Medis dan Informasi Kesehatan. 2018;8(1):37.
16. Prahasanti K. Gambaran Kejadian Infeksi pada Usia Lanjut. Universitas Muhammadiyah Surabaya. Jurnal Kedokteran Universitas Muhammadiyah Surabaya. 2019;3(1):81-90.
17. Febiola R. Identifikasi Bakteri Patogen dengan Hasil Uji Kultur pada Kasus Ulkus Diabetik di RSUD Kota Yogyakarta Tahun 2021. Universitas 'Aisyiyah Yogyakarta. *Digital Library at the Aisyiyah University of Yogyakarta*. 2022;9(2):3.
18. Yimam A, *et al. Prevalence of Diabetic Foot Ulcer and Associated Factors Among Diabetic Patients in Tikur Anbessa Specialized Hospital, Addis Ababa, Ethiopia*. *International Journal of Africa Nursing Sciences.* 2021;14(1):1-5.

19. Aw TC, Blair I, Babcock HM. *Occupational Infections: Infectious Diseases.* 5th edition. United States: Elsevier; 2017.
20. Rofiqi, dkk. Risiko Ulkus Kaki Diabetes pada Petani di Wilayah Kerja Puskesmas Palengaan Kabupaten Pamekasan Madura. Jurnal Pustaka Kesehatan. 2022;10(3):162-6.
21. Anies. Kedokteran Lingkungan : Penyakit Akibat Lingkungan. Yogyakarta: Ar-Ruzz Media; 2018.
22. Fitria E, Nur A, Marissa N, Ramadhan N. Karakteristik Ulkus Diabetikum pada Penderita Diabetes Mellitus di RSUD dr. Zainal Abidin dan RSUD Meuraxa Banda Aceh. Buletin Penelitian Kesehatan. 2017;45(3):156.
23. Julianti IMD. Hubungan antara Kadar Gula Darah dengan Tekanan Darah pada Pasien Diabetes Melitus Tipe II. Jurnal Penelitian Kedokteran. 2021;4(2):1-7.
24. Simanungkalit C, dkk. Manajemen Perawatan Luka pada Pasien DM di Pasir Bidang Tahun 2019. TRIDARMA: Pengabdian Kepada Masyarakat. 2019;2(2):119.
25. Wirawan, Saftarina. Penatalaksanaan Perawatan Luka yang Buruk pada Pasien dengan Diabetes Melitus Tipe II dan Neuropati Perifer. Medula: Jurnal Profesi Kedokteran Universitas Lampung. 2017;7(3):30-5.
26. Puspaningrum Y, Wibowo WA. Gambaran Pola Bakteri pada Ulkus, Abses dan Selulitis di RS PKU Muhammadiyah Surakarta. *Medical Research for Better Health*. 2020;1(2):472-82.
27. Basuki R, Husen F. Karakteristik dan Gambaran Diagnosa Komplikasi Pasien Diabetes di Rumah Sakit Umum Aghisna Sidareja. Jurnal Bina Cipta Husada. 2022;18(2):1.
28. Novelni R, Irwandi, Pratiwi D. Identifikasi dan Uji Resistensi Bakteri pada Pasien Ulkus Diabetikum di Bangsal Interne RSUP Dr. M. Djamil Padang. Jurnal Penelitian Farmasi. 2019;8(2):69.
29. Salim SE, dkk. Pola Bakteri pada Pasien Kaki Diabetik dan Resistensinya Terhadap Antibiotik di Rumah Sakit Umum Pusat Sanglah. Universitas Udayana. Jurnal Medika Udayana. 2020;9(10):102.
30. Patil Y, *et al. Bacteriological Profil of Diabetic Foot Infections, European Journal of Pharmaceutical and Medical Research.* 2018;5(6):631-5.
31. Khairunnisa R, dkk. Identifikasi dan Uji Resistensi *Staphylococcus aureus* pada Ulkus Diabetik di Instalasi Penyakit Dalam RSUD Dr. H. Abdul Moeloek. Jurnal Kedokteran Universitas Lampung. 2020;7(1):2-6.
32. Hanina, Humaryanto, dkk. Peningkatan Pengetahuan Siswa Pondok Pesantren Nurul Iman Tentang Infeksi *Staphylococcus aureus* di Kulit dengan Metode Penyuluhan. Fakultas Kedokteran dan Ilmu Kesehatan Universitas Jambi. *Medical Dedication*. 2022;5(2):426-30.
33. Lipsky BA, *et al. IWGDF Guidelines on The Diagnosis and Treatment of Foot Infection in Persons with Diabetes. IWGDF Working Group on Foot Infections. Diabetes/Metabolism Research and Reviews.* 2020.
34. Sugireng, Rosdarni. Deteksi MRSA (*Methicilin Resistant Staphylococccus aureus*) dengan Metode PCR pada Pasien Ulkus Diabetikum. Rumah Jurnal Universitas Islam Negeri Alauddin Makassar. 2020;6(1):31.