AEROBIC CULTURE OF DIABETIC FOOT ULCERS:BACTERIAL PROFILE AND ANTIBACTERIAL RESISTANCE PATTERN AT LIAQUAT UNIVERSITY HOSPITAL, JAMSHORO.

Muhammad Aslam Rind[1], Maria Nazir Khyber[2], Muhammad Saleem Rind[3], Iffat Jamal[4], Shahzad Shaikh[5], Tariq Feroz Memon.[6]

ABSTRACT

Introduction: Antibiotics are commonly used in diabetic foot ulcers, it is the mainstay of treatment to avoid amputations. **Objective:** To evaluate the aerobic bacteriological profile and in-vitro resistance pattern to regularly used antibiotics at medical unit of Liaquat university hospital, Jamshoro. **Methods**: Cross sectional study was conducted at medical unit of Liaquat University hospital from September 2019 to January 2020. The University of Texas wound classification system was utilized for wound grading while tissue samples were collected using a sterile curette under aseptic measures. Moreover, bacteriological analysis and antibiotic sensitivity test was carried out in the diagnostic laboratory. SPSS ver. 24.0 was used to analyze the data. **Results:** Forty-one diabetic patients with mean age 59.2±14.68 participated in the study. Majority (73.17%) of participants were male while most (56%) of the study participants having grade II diabetic foot ulcers. Culture-positive specimens were found in 30 (73%) and the remaining 11 (27%) were found to be negative. Out of the 30 culture-positive patients, the total number of bacterial isolates was 89. Around two-third (70.8%) samples had gram positive bacteria. The most common species, among the Gram-positive bacteria, were Staphylococcus Aureus while amongst the Gram-negative bacterial isolates, the most common species found were Proteus and Enterobacter. **Conclusion:** Diabetic Foot Ulcers are most commonly infected by the gram-positive bacteria of which staphylococcus aureus species are not only dominant but also shows multi-drug resistance. The antibiotic like Imipenem is the most effective drug against the all type of bacteria.

Key Words: Aerobic Bacteria, Bacteriological Profile, Diabetic foot, Multi-Drug Resistance

1. Assistant Professor, Department of Medicine, LUMHS Jamshoro.
2. Senior Registrar, Department of Medicine, LUMHS, Jamshoro.
3. Assistant Professor, Department of Medicine, PUMHS, Nawabshah.
4. FCPS-II (Traniee) General Medicine, Department of Medicine, LUMHS, Jamshoro.
5. FCPS-II (Traniee) Plastic Surgery, Department of Surgery, LUMHS, Jamshoro.
6. Assistant Professor, Department of Community Medicine, Isra University, Hyderabad.

Corresponding Author; Dr. Tariq Feroz Memon, Assistant Professor, Department of Community Medicine, Isra University, Hyderabad.
Email; drtariqferoz@gmail.com

How to cite this article: Rind MA[1], Khyber MN[2], Rind MS[3], Jamal I[4], Shaikh S[5], Memon TF[6]. **AEROBIC CULTURE OF DIABETIC FOOT ULCERS: BACTERIAL PROFILE AND ANTIBACTERIAL RESISTANCE PATTERN AT LIAQUAT UNIVERSITY HOSPITAL, JAMSHORO. JPUMHS;2020;10(03)37-42.**
http://doi.org/10.46536/jpumhs/2020/10.02.223

Introduction

Diabetes, a chronic non-communicable disease defined as hyperglycemia secondary to the inadequacy of secretion or function of insulin or an amalgamation of both, is one of the most widespread public health burdens worldwide. [1,2] The overall prevalence of diabetes has been on a steady rise since the last forty years, and it was listed as one of the most common causes attributable to loss of life in 2015. [3,4] Regardless of the efforts of the World Health Organization (WHO) to impede the rise in the incidence of diabetes, the prognosis is notvery promising. Estimations from recent studies have indicated that the number of diabetic people will rise worldwide dramatically, even if the age-specific prevalence is kept constant, from 415 million in 2015 to 642 million in 2040.[5]

Diabetic foot ulcer (DFU), expected to occur in around 15% to 25% of patients once in their lifetime, is one of the most common complications associated with diabetes and the chief cause of hospitalization, morbidity, and mortality. [6-9] DFU is also one of the major causes of lower limb amputations, leading to more than 75,000 amputations per year in the United States of America as reported by the American Diabetes Association (ADA). [10] Since DFUs are true surgical emergencies, in an attempt to salvage the infected limb and prevent the need for amputation, empiric antibiotic therapy should be started promptly. [(11)]However, after the availability of sensitivity and culture results, empiric therapy should be replaced with specific targeted therapy against the pathogens to avoid extended use of broad-spectrum antibiotics. [11] Furthermore, organisms detected in DFU's vary not only from patient to patient, hospital to hospital but also from country to country (region to region). [12]

Therefore, the present study was designed with an objective to evaluate the aerobic bacteriological profile and in-vitro resistance pattern to regularly used antibiotics at medical unit of Liaquat university hospital, Jamshor

Methodology

The Cross sectional study was conducted at medical unit of Liaquat University Hospital, Jamshoro after being approved from the university ethical review board, and adhered to the Helsinki declaration doctrines. Patients of either sex, belongs to age 20 years and above with DFUs at the time of admission in the hospital from September 2019 to January 2020 were included in the study. While those who didn't fulfill the inclusion criteria or with any other complications and other diseases were excluded from the study.The details of the study were explained to all the patients in their native tongues before obtaining informed written consent. Patient demographic and medical datalike; age, gender, duration of DFU, medical history, details regardingthe location and features of DFUs were recorded. The University of Texas wound classification system was utilized for wound grading, at the time of admission of the patients, as grade 0 (healed wounds either pre or post-operative), grade I (superficial wound), grade II (extending to tendon or joint capsule), grade III (extending to bone or joint).[13, 14] Grade 0 patients were not included in the study. To determine the size of the DFU, the length and width of the ulcers were measured, multiplied, and expressed in squared centimeters. [15,16] Collection of tissue samples was done under aseptic measures using a sterile curette, after rinsing the wound area with saline, properly debriding the wounds of contamination and superficial exudates, and cleaning the base of the DFUs with the help of sterile cotton swab sticks. [16] The samples were fixed in Stuart medium to be taken to the laboratory for microbiological analysis. The analysis for the bacteriology culture staining was done as detailed in previous studies.[11, 16] Only aerobic bacteria were investigated on account of the limitation of resources and laboratory facilities. As mentioned in the Clinical and Laboratory Standards Institute (CLSI) guidelines, the testing of the aerobic isolates for sensitivity/resistance against commonly used antibiotics was done using the Kirby Bauer disk diffusion method. [17] Statistical analysis of data was performed in SPSS version 24. Demographic data was presented as mean ± standard deviation or number (percentage).

Results

The current study consisted of 41 diabetic patients with a mean age of59.2±14.68 (range, 34-73), out of which majority were male. Most of the study participants' having grade II DFUs. The details about the demographic and lesion characteristics are summarized in Table 1.

Table 2 below demonstrating the details of the culture specimens and the bacterial isolates from the DFUs. Almost two third of DFU patients culture report were found positive for bacterial infections. Total 89 bacterial isolates of different species were found from the tissue culture.The most common species, among the Gram-positive bacteria, wereStaphylococcus Aureus (S. Aureus), followed by Streptococcus Saprophyticus (S. Saprophyticus), Streptococcus epidermidis (S. Epidermidis), and Streptococcus Pneumonia (S.Pneumonia). Whereas amongst the Gram-negative bacterial isolates, the most common species found were Proteusand Enterobacter, followed by Escherichiacoli (E. coli). The details of the different resistance patterns of the various species of the isolated gram-positive and gram-negative bacteria to some of the most commonly used antibiotics, acquired through the Kirby Bauer disk diffusion method, are given in Table 3 and Table 4 respectively. Majority of gram positive bacteria found to be resistant against drugs like penicillin, erythromycin and azithromycin. The most effective antibiotic against S.aureus, S.epidermidis, Strep.pneumonia, S. saprophyticus, and S.agalactiae was Imipenem. Amongst the gram-negative bacteria, the most effective antibiotic against E.coli, Proteus, and Citrobacter was Imipenem. Citrobacter was also resistant against Amoxicillin, Ampicillin, Cefotaxime, Cefoxitin, and Gentamicin.

Table 1. Demographic & lesion characteristics (n=41)

Variable	n	%
Gender		
- Male	30	73.17
- Female	11	27.83
Taking Diabetes Medication		
- Yes	39	95.12
- No	2	4.88
Duration of Diabetic foot ulcer		
- 1-30 days	26	63.41
- 31-60 days	7	17.07
- 61-90 days	2	4.87
- >90 days	6	14.65
Size of ulcer		
- ≤ 4 cm^2	9	21.96
- > 4 cm^2	32	78.04
Grade of ulcer*		
- Grade I	6	14.6
- Grade II	23	56.0
- Grade III	12	29.4
Location of the ulcer		
- Plantar surface	13	31.70
- Dorsal surface	7	17.08
- Toes (Right foot)	15	36.58
- Toes (Left foot)	6	14.63

University of Texas wound classification system.

Table 2. Bacteriological profile of cultural isolates (n=41).

Variable	n	%
Total patients with a positive culture	30	73.17
Total cultures with 1 pathogen isolated	7	23.33
Total cultures with 2 or more pathogens isolated	23	76.67
Total number of pathogens isolated	89	100
Gram-positive bacteria	63	70.78
Gram-negative bacteria	26	29.22
Bacterial Isolates		
- S. Aureus	29	32.58
- S. Saprophyticus	17	19.10
- Proteus spp.	10	11.27
- S. Epidermidis	9	10.11
- Enterobacter spp.	7	7.86
- Strep.Agalactiae	6	6.74
- E.Ccoli	4	4.49
- Pseudomonas spp.	3	3.37
- Strep. Pneumonia	2	2.24
- Citrobacter spp.	2	2.24

Table 3. Antibiotics resistance and sensitivity pattern against Gram-positive bacteria (n=63).

Antibiotic	S. Aureus (n=29)		S. Saprophyticus (n=17)		S. epidermidis (n=9)		S. Agalactiae (n=6)		S. pneumonia (n=2)	
	n	%	n	%	n	%	n	%	n	%
Azithromycin	16	55.1	10	58.8	9	100.0	4	66.6	1	50.0
Amoxicillin	13	44.8	10	58.8	6	66.6	4	66.6	1	50.0
Cefoxitin	9	31.0	10	58.8	4	44.4	6	100.0	2	100.0
Cefalexin/cefalotin	13	44.8	8	47.0	9	100.0	6	100.0	2	100.0
Erythromycin	18	62.0	16	94.1	9	100.0	6	100.0	2	100.0
Imipenem	0	0.0	0	0.0	2	22.2	0	0.0	0	0.0
Oxacillin	16	55.1	16	94.1	8	88.8	6	100.0	0	0.0
Penicillin	19	65.5	16	94.1	9	100.0	6	100.0	0	0.0
Trimethoprim-sulfamethoxazole	14	48.2	7	41.1	9	100.0	6	100.0	0	0.0
Vancomycin	7	24.1	ND		ND		5	83.3	0	0.0

Table 4. Resistance/sensitivity pattern of Gram-negative bacteria (n=26).

Antibiotic	E. coli (n=4)		Enterobacter (n=7)		Proteus (n=10)		Pseudomonas (n=3)		Citrobacter (n=2)	
	n	%	n	%	n	%	n	%	n	%
Amoxicillin	3	75.0	7	100.0	9	90.0	2	66.6	2	100.0
Ampicillin	3	75.0	7	100.0	9	90.0	3	100.0	2	100.0
Cefotaxime	2	50.0	5	71.4	6	66.6	2	66.6	2	100.0
Cefoxitin	2	50.0	6	85.7	3	33.3	2	66.6	2	100.0
Gentamicin	1	25.0	7	100.0	3	33.3	2	66.6	2	100.0
Imipenem	0	0.0	3	42.8	0	0.0	1	33.3	0	0.0
Norfloxacin	2	50.0	7	100.0	4	44.4	2	66.6	1	50.0
Tetracycline	3	75.0	7	100.0	5	55.5	2	66.6	2	100.0

Discussion

DFUs are not only a serious complication of diabetes but also an expensive one owing to added hospitalization and treatment costs. [18] The current study shows the details of the bacteriological isolates from DFUs and their resistance pattern against commonly used antibiotics. Most of the patients with DFUs in the current study were elderly (mean age 59.2±14.68 years) which can be owed to the fact that DFUs occur mostly in those patients who have a comparatively greater age and sensory neuropathy. [11] The total number of male patients in the current study was greater as compared with female patients, which is in accordance with a previous study by Sivanmaliappan et al., which has stated that male patients are more susceptible to develop DFUs than their female counterparts. [19] DFUs render a person prone to develop lower limb infections which may lead to the destruction of host tissues or triggering of host immune response secondary to pathogen invasion and proliferation. [20] In this study, the most common presentation of the DFUs was recent i.e. (1 to 30 days), and the distribution of the majority of the DFUs was on the plantar surface as well as on the toes. These findings are consistent with those reported by Donoso et al. [21]

Previous studies have stated that the prevalence of gram-positive bacteria in DFUs is comparatively higher than gram-negative.[22] These findings are consistent with the current study in which the DFU infestation was predominantly by gram-positive bacteria. Some studies, however, have stated that gram-negative bacteria are more common in DFUs in some regions. [23] These differences in findings can be due to regional differences as well as variations in the sort and severity of infections. [24] Among the gram-positive bacteria, the most common isolates were S.aureus and S.saprophyticus, whereas the most common isolates among the gram-negative bacteria were proteus and enterobacter. These findings are consistent with Jia et al. who reported that in chronic patients or those who have been treated previously, the isolation of Gram-negative and Gram-positive aerobes is usually in conjugation. [25] The majority of DFUs had more than one type (polymicrobial) of bacterial isolate. This is consistent with the findings of Singh et al. and Perim at al. who reported the predominance of polymicrobial etiology in DFUs, but inconsistent with the findings of Dhanasekaran et al. who reported the higher prevalence of monomicrobial infections in DFUs.[11,23,26] These incongruous findings can be explained by the difference in the nature of the DFUs, with mild and severe infections having monomicrobial and polymicrobial isolates respectively. [11, 24]

The most common pathogen isolated in the current study was S.aureus (32.5%). Cefoxitin and oxacillin were used to confirm methicillin resistance. Methicillin resistant Staphylococcus aureus or MRSA,which has evolved into a ginormous problem for medical personnel, is often referred to as the “super bug” owing to its difficult treatment.[27] The prevalence of MRSA in DFUs is increasing significantly with studies identifying MRSA isolation in 15-30% of DFUs. [11]

In the present study, the pathogens belonging to the Enterobacteriaceae family showed significant resistance to most of the antibiotics that they were tested against, which is similar to the findings reported by Perim et al.[11]

In our study, the most effective antibiotic was Imipenem which is consistent with the findings of previous studies by Perim at al. and Umadevi et al. who also found Imipenem to be the most efficacious antibiotic against pathogens in DFUs. [11,28] According to previous study by Rajalakshmi et al., imipenem is the most efficacious drug against Pseudomonas aeruginosa, which is also consistent with the findings of the current study. [29]

With strengths there were certain limitations to this present study. The cultures for anaerobic organisms weren’t performed due to deficiency of funds and laboratory resources. The study was also limited in terms of the small sample size. Although the role of anaerobic pathogens in DFUs is still uncertain with studies stating they only

play a slight role in such conditions,[30] further studies are recommended to probe further into this topic so as to either confirm or deny this assumption. Furthermore, culture studies of DFUs and other infections should be carried out in further detail, offering better pathogenic characterization and targeted treatment. This knowledge is critical for proper treatment plan, minimizing monetary healthcare burden.

Conclusion

Based on the findings of present study it is concluded that Diabetic Foot Ulcers are most commonly infected by the gram-positive bacteria of which staphylococcus aureus species are not only dominant but also shows multi-drug resistance. The antibiotic like Imipenem is the most effective drug against the all type of bacteria.

References

1. 1.Talpur RA, Meghji KA, Uqaili AA, Kazi N, Nizammani GS, Qazi S. Anti-hyperglycemic and anti-oxidative effects of l-carnitine administration in alloxan induced diabetic albino wistar rats. Khyber medical university journal. 2019;11(4):204-8.
2. 2. Bommer C, Sagalova V, Heesemann E, Manne-Goehler J, Atun R, Bärnighausen T, et al. Global economic burden of diabetes in adults: projections from 2015 to 2030. Diabetes care. 2018;41(5):963-70.
   3. Zhou B, Lu Y, Hajifathalian K, Bentham J, Di Cesare M, Danaei G, et al. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4 & #xb7;4 million participants. The Lancet. 2016;387(10027):1513-30.
4. Wang H, Naghavi M, Allen C, Barber RM, Bhutta ZA, Carter A, et al. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980 & #x2013;2015: a systematic analysis for the Global Burden of Disease Study 2015. The Lancet. 2016;388(10053):1459-544.
5. Bommer C, Heesemann E, Sagalova V, Manne-Goehler J, Atun R, Barnighausen T, et al. The global economic burden of diabetes in adults aged 20-79 years: a cost-of-illness study. The lancet Diabetes & endocrinology. 2017;5(6):423-30.
6. Embil JM, Albalawi Z, Bowering K, Trepman E. Foot Care. Canadian journal of diabetes. 2018;42 Suppl 1:S222-s7.
7. Yazdanpanah L, Nasiri M, Adarvishi S. Literature review on the management of diabetic foot ulcer. World journal of diabetes. 2015;6(1):37-53.
8. Zubair M. Prevalence and interrelationships of foot ulcer, risk-factors and antibiotic resistance in foot ulcers in diabetic populations: A systematic review and meta-analysis. World journal of diabetes. 2020;11(3):78.
9. Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW, et al. Diagnosis and treatment of diabetic foot infections. Plastic and reconstructive surgery. 2006;117(7 Suppl):212s-38s.
10. Sanchez-Sanchez M, Cruz-Pulido WL, Bladinieres-Camara E, Alcala-Duran R, Rivera-Sanchez G, Bocanegra-Garcia V. Bacterial Prevalence and Antibiotic Resistance in Clinical Isolates of Diabetic Foot Ulcers in the Northeast of Tamaulipas, Mexico. The international journal of lower extremity wounds. 2017;16(2):129-34.
11. Perim MC, Borges JdC, Celeste SRC, Orsolin EdF, Mendes RR, Mendes GO, et al. Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections. Revista da Sociedade Brasileira de Medicina Tropical. 2015;48(5):546-54.
12. El-Tahawy AT. Bacteriology of diabetic foot. Saudi medical journal. 2000;21(4):344-7.
13. Lavery LA, Armstrong DG, Harkless LB. Classification of diabetic foot wounds. The Journal of Foot and Ankle Surgery. 1996;35(6):528-31.
    Sabir M, Kanwal R, Shahzad MN, Khan MMUR. Wagner Versus The University Of Texas Classification Of Diabetic Foot Ulcers: A Descriptive Study In Nishter Hospital Multan. Annals of PIMS-Shaheed Zulfiqar Ali Bhutto Medical University. 2018;14(1):19-24.
14. Slater R, Lazarovitch T, Boldur I, Ramot Y, Buchs A, Weiss M, et al. Swab cultures accurately identify bacterial pathogens in diabetic foot wounds not involving bone. Diabetic medicine. 2004;21(7):705-9.
15. Zubair M, Malik A, Ahmad J. Clinico-microbiological study and antimicrobial drug resistance profile of diabetic foot infections in North India. The Foot. 2011;21(1):6-14.
16. Taylor CR. New revised clinical and laboratory standards institute guidelines for immunohistochemistry and immunocytochemistry. Applied Immunohistochemistry & Molecular Morphology. 2011;19(4):289-90.
17. Lipsky BA. A report from the international consensus on diagnosing and treating the infected diabetic foot. Diabetes/metabolism research and reviews. 2004;20(S1):S68-S77.
18. Sivanmaliappan TS, Sevanan M. Antimicrobial susceptibility patterns of Pseudomonas aeruginosa from diabetes patients with foot ulcers. International journal of microbiology. 2011;2011.
19. Thakral G, LaFontaine J, Najafi B, Talal TK, Kim P, Lavery LA. Electrical stimulation to accelerate wound healing. Diabetic foot & ankle. 2013;4(1):22081.
20. Donoso MTV, Rosa EG, Borges EL. Perfil dos pacientes com pé diabético de um

serviço público de saúde. Rev Enferm UFPE. 2013;7(7):4740-6.
21. Wang S-H, Sun Z-L, Guo Y-J, Yang B-Q, Yuan Y, Wei Q, et al. Meticillin-resistant Staphylococcus aureus isolated from foot ulcers in diabetic patients in a Chinese care hospital: risk factors for infection and prevalence. Journal of medical microbiology. 2010;59(10):1219-24.
22. Singh SK, Gupta K, Tiwari S, Shahi SK, Kumar S, Kumar A, et al. Detecting aerobic bacterial diversity in patients with diabetic foot wounds using ERIC-PCR: a preliminary communication. The international journal of lower extremity wounds. 2009;8(4):203-8.
23. Citron DM, Goldstein EJ, Merriam CV, Lipsky BA, Abramson MA. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. Journal of clinical Microbiology. 2007;45(9):2819-28.
24. Jia L, Parker CN, Parker TJ, Kinnear EM, Derhy PH, Alvarado AM, et al. Incidence and risk factors for developing infection in patients presenting with uninfected diabetic foot ulcers. PloS one. 2017;12(5).
25. Dhanasekaran G, Sastry G, Viswanathan M. Microbial pattern of soft tissue infections in diabetic patients in South India. Asian J Diabet. 2003;5:8-10.
26. Sabir R, Alvi SFD, Fawwad A. Antimicrobial susceptibility pattern of aerobic microbial isolates in a clinical laboratory in Karachi-Pakistan. Pakistan journal of medical sciences. 2013;29(3):851.
27. Umadevi S, Kumar S, Joseph N, Easow JM, Kandhakumari G, Sreenivasan S, et al. Microbiological Study of Diabetic Foot Infections. INDIAN JOURNAL OF MEDICAL SPECIALITIES. 2011;2:12-7.
28. Rajalakshmi V, Amsaveni V. Antibiotic susceptibility of bacterial pathogens isolated from diabetic patients. International Journal of Microbiological Research. 2011;2(3):273-5.
29. Senneville E, Melliez H, Beltrand E, Legout L, Valette M, Cazaubie M, et al. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. Clinical infectious diseases. 2006;42(1):57-62.