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MICROBIOLOGY OF DIABETIC FOOT DETERMINED BY BIOPSY STUDY OF 100 PATIENT

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ABSTRACT

Objectives: The goal is to identify the microorganisms present in wounds on diabetic feet by analyzing biopsy samples. Materials and methods: Consecutive patients with diabetic foot and symptoms of infection were enrolled in the study. Biopsy samples were homogenized using a mortar and processed to identify aerobic organisms. When multiple isolates were found, only the two most common types of bacteria were identified. Bacterial identification was done using biochemical techniques, while the sensitivity to antibiotics was determined using the disk diffusion method. **Results:** A total of 100 biopsy samples were analyzed, with 52 of them taken from male patients (52%). Of the 102 isolates identified, 67% were Gram-negative bacilli, with Escherichia coli being the most common type, accounting for 21% of all isolates. Gram-positive cocci accounted for 28% of all isolates, while 6% were yeasts. Among the 68 Gram-negative bacilli, 35% were resistant to ciprofloxacin. The study found 55 Enterobacteriaceae isolates, 7% of which produced extended-spectrum beta-lactamases. Finally, the study identified 8 Staphylococcus aureus isolates, 38% of which were resistant to methicillin. **Conclusions:** When compared to reports from developed countries, our study identified a greater proportion of Gram-negative bacteria and antibiotic-resistant organisms.

KEYWORDS

Diabetes. Diabetic foot. Microbiology

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INTRODUCTION

Diabetes can cause a variety of acute and chronic complications. One common issue is the diabetic foot, which refers to any infection or ischemic lesion that occurs below the knee in a person with diabetes. This condition can cause ulcerations and necrosis of the feet, and it encompasses various specific problems such as abscesses and osteomyelitis. The diabetic foot is caused by multiple risk factors, including neuropathy, deformity, and vascular issues. Ulcers associated with the diabetic foot can be classified based on their underlying cause, such as neuropathy, ischemia, or infection.[1,2]

Once the protective layer of the skin is lost, the underlying tissues are exposed to bacterial colonization and the wound can become complicated with infection by various pathogens, mainly gram-positive aerobic cocci such as staphylococci and beta-hemolytic streptococci in the initial stages, while chronic ulcers have a more complex microbiota that includes enterococci, enterobacteria, Pseudomonas spp., and anaerobes.[3,4]

Due to the lack of a consensus, there are multiple antimicrobial regimens for managing diabetic foot. Clindamycin is commonly used in combination with a quinolone or third-generation cephalosporin. When parenteral administration is necessary, clindamycin or metronidazole is combined with a third-generation cephalosporin or a quinolone, as well as piperacillin/tazobactam or carbapenems.[5,10]

Given the complex pathophysiology of diabetic foot, its management requires the intervention of a multidisciplinary team that includes at least a surgeon, internist, nutritionist, and microbiologist. It is known that the rate of antibiotic resistance is high in community-acquired infections in Mexico, but the microbiology and antibiotic sensitivity of clinical isolates from diabetic foot patients in our setting undergoing standard recognized culture studies (tissue biopsy) is unknown. This knowledge is important for guiding the initial empirical management of our patients, which will help in the comprehensive management and preservation of the extremities.

MATERIAL & METHODS

This is a study that describes 100 patients with diabetic foot and their clinical data regarding active infections. The study was conducted at the Zydus Medical College, Dahod. Biopsy cultures were taken from all 100 patients who had diabetic foot and showed signs of infection during 2021-2023 period. The study also used the University of Texas classification system to determine the severity of foot lesions based on the depth of ulcers and the presence of infection or ischemia 14-16.

Sample processing for culture

The biopsy with forceps was used to collect the sample for culturing. The samples were then transported to the laboratory in sterile containers with Amies medium without charcoal. They were processed within 60 minutes. Aerobic bacteria were cultured, and tissue biopsies were processed in sterile mortar first. Gram staining and semi-quantitative culture were done. In case of polymicrobial flora, the two predominant germs were identified, while the rest were only described based on colony characteristics. Bacterial identification was performed using biochemical procedures and incubated for 18 to 24 hours at 35°C. Antibiotic sensitivity was determined using the agar plate diffusion method, following CLSI quality control procedures. Each new batch of Muller-Hinton agar was tested with ATCC strains E. coli 25922, S. aureus 25923, and P. aeruginosa 28753. Methicillin resistance was determined for S. aureus using the oxacillin disk test on agar. ESBL determination for enterobacteria was done using the double disk technique on agar.

Analysis and calculation of the minimum sample

We consider a consecutive sample to be reasonably equivalent to a random one because the samples were taken over several months and patients were not excluded. To estimate germs that occur in proportions of up to 5%, with an absolute error of ±5%, the calculated minimum sample was 85 patients. The isolated germs are reported in descriptive measures of frequency with their 95% confidence interval (CI95%). To analyze the significance of differences between medians for continuous variables, the Kruskal-Wallis test was used with a bilateral of 0.05.

Result

A total of 100 biopsies from 100 patients were analyzed. Of these, 47 specimens were from males (47%) and 53 were from females (53%); the average age of the patients was 64.2 years. Regarding the severity of the lesions according to the University of Texas classification, patients with severe lesions predominated, with 22 patients having diabetic foot lesions at Grade I (22%), 27 at Grade II (27%), and 51 at Grade III (51%).

A total of 112 clinical isolates were obtained from the 100 biopsies, as 62 of them had monomicrobial growth (62%; 95% CI, 52-72%), while

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25 had polymicrobial growth (25%; 95% CI, 16-34%). There was no growth in 11 biopsies (11%; 95% CI, 5-17%), and 2 had suggestive data of exclusive presence of strict anaerobes by Gram staining (2%; 95% CI, 0-5%). Table 1 lists the isolated microorganisms and their frequency.

Table 1: Microorganisms isolated from 100 diabetic foot tissue biopsies

Microorganism	Total number of samples	Percentage out of total samples
Gram-negative bacilli	68	61%
Gram-positive cocci	28	25%
Yeasts	16	14%
No growth	11	11%
Polymicrobial growth	25	25%
Exclusive anaerobes*	2	2%

*Presence of strict anaerobes suggested by Gram staining.

Table 2. Microorganisms isolated from 100 diabetic foot tissue biopsies

Microorganism	Number of positive cases	95% confidence interval
Escherichia coli	24	13-29
Enterobacter aerogenes	3	0-6
E. agglomerans	12	1-11
E. cloacae	1	0-3
Klebsiella oxytoca	3	0-6
K. ozaenae	1	0-3
K. pneumoniae	3	0-6
Proteus mirabilis	8	2-13
P. penneri	3	0-6
P. vulgaris	6	1-11
Acinetobacter spp	4	0-9
Pseudomonas aeruginosa	9	3-14
Enterococcus spp	11	5-17
Staphylococcus aureus	8	3-13
S. coagulasa negativa	4	0-8
Streptococcus Beta-hemolítico, Gr. A	1	0-3
Streptococcus Beta-hemolitico, Gr. B	2	0-5
S. milleri	1	0-3
Streptococcus sp	1	0-8
Candida albicans	4	0-8
C. no albicans	2	0-5
Total	112	

There was no significant difference in the proportions of isolated microorganisms when comparing them with the gender of the patients (2 2.448, p 0.28). Similarly, there was no significant difference when comparing the median ages of the patients with respect to the type of isolated microorganism (H 3.28, p 0.35) or the grade of lesion (H 1.9, p 0.385). On the other hand, there was a significant difference when comparing the isolates with the depth grades of the University of Texas classification (2 21.257, $p < 0.001$), as a higher proportion of gram-negative bacilli (41/68, 60%) were obtained in Grade III lesions. All isolated yeasts (6/6) were obtained from Grade III lesions, and the highest proportion of gram-positive cocci (23/28, 82%) were isolated from Grade I and II lesions.

Table 3. Table 2. Antibiotic resistance rate of Escherichia coli isolated from cultured diabetic foot ulcers in different developing countries

Author Referance	County	n	Antibiotic Resistance in %												
			AN	AMC	AM	FEP	CTX	CAZ	CTB	CRO	CIP	GM	IPM	TYG	SXT
Gadepalli R, et al. 2006 (20)	India	22	50	55	ND	ND	55	55	ND	ND	50	ND	0	ND	ND
Bansal E, et al. 2008 (22)	India	26	10	73	100	ND	ND	18	ND	ND	63	70	0	ND	ND
Khosravi AD, et al. 2007 (23)	Pakistán	10	ND	ND	ND	ND	ND	80	ND	80	80	ND	ND	ND	ND
Raja NS. 2007 (24)	Malasia	14	0	21	93	ND	ND	14	ND	14	29	29	0	ND	ND
Our Study(100)	India	21	5	33	86	29	29	29	29	29	71	33	0	0	62

Of the total clinical isolates, 68 were gram-negative bacilli (61%; 95% CI, 51-71%), 28 were gram-positive cocci (25%; 95% CI, 17-34%), and 16 were yeasts (14%; 95% CI, 8-21%). Of the 68 gram-negative bacilli, 1 Acinetobacter spp. and 1 Pseudomonas spp. were imipenem-resistant (3%; 95% CI, 0-7%). Additionally, 24 gram-negative bacilli were resistant to ciprofloxacin (35%; 95% CI, 24-47%). Of the 55 enterobacteria isolated, four (7%; 95% CI, 0-14%) were extended-spectrum -lactamase producers. Of the 21 E. coli strains isolated, 18 were resistant to ampicillin (86%), 15 were resistant to ciprofloxacin (71%), and 13 were resistant to trimethoprim-sulfamethoxazole (62%). Of the 28 gram-positive cocci, 8 were S. aureus, of which 3 (38%; 95% CI) and 4 (71%) were methicillin-resistant.

Discussion

The current study presents a microbiological description of 100 biopsies from patients at Surgery Department OPD, representing one of the largest collections of cultures per biopsy in diabetic foot. The study found a high proportion of gram-negative bacilli (67%), which differs from previous microbiology reviews in diabetic foot where S. aureus is the prevalent isolation, followed by S. epidermidis, Streptococcus spp., P. aeruginosa, Enterococcus spp., and coliform bacteria. This could be due to the chronicity of the patients' conditions as acute lesions are initially colonized and infected by gram-positive bacteria. The study also found a significant association between gram-negative bacilli and yeast with grade III ulcers. The results are consistent with previous studies from India19, where gram-negative bacilli were the most frequent in biopsies and swabs of diabetic foot ulcers. The study recommends cautious use of antibiotics as some pathogens showed high resistance rates, particularly to ciprofloxacin. The study suggests that cephalosporins of the third generation could be used as the initial empirical treatment instead. The study also found two strains of gram-negative non-fermenting bacilli resistant to imipenem, reflecting the high rates of resistance to antibiotics observed in community infections in India. The proportion of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae (7%) was lower than that reported in India, but the proportion of methicillin-resistant Staphylococcus aureus (MRSA) (38%) was similar.20 The observed proportion of E. coli in the present study is considerably higher (21% vs. 4%).21

Our work has limitations that need to be acknowledged. Firstly, we did not analyze hospitalizations, previous treatments, or their duration, so we cannot determine the relationship between therapies and antimicrobial resistance profiles. Secondly, we did not assess how the study results impacted medical decision-making or patient outcomes. Nonetheless, our study provides significant data on the microbiota of diabetic foot in our setting, which is a valuable contribution to the complex task of salvaging the diabetic foot, and it provides important information for clinicians because appropriate medical management of the infection is critical for the salvage of the diabetic foot. We have one of the largest collections of biopsy cultures in the literature on diabetic foot and observed a higher proportion of gram-negative bacteria and antibiotic resistance compared to studies from industrialized countries.

Referances

1. Caputo GM, Cavanagh PR, Ulbrecht JS, Gibbons GW, et al. Assessment and management of foot disease in patients with diabetes. N Engl J Med 1994; 331: 854 60.
2. Frykberg RG. Diabetic foot ulcers: current concepts. J Foot Ankle Surg 1998; 37: 440 6.
3. Joshi N, Caputo G, Weitekamp M, Karchmer A. Infections in patients with diabetes mellitus. N Engl J Med 1999; 341: 1906 12.
4. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Micro- biol Rev 2001; 14: 244 69.
5. Aragon SFJ, Ortiz RPP. Infección en el pie diabético. En: Mar tínez de Jesús FR (ed.). Pie diabético. Atención Integral. 2a. Ed. México: McGraw Hill; 2003, p. 143 53.
6. Gilbert DN, Moellering RC, Sande MA. The Sanford guide to antimicrobial therapy. 36th Ed. Antimicrobial Therapy Inc. Hyde Park; 2006.
7. Lipsky BA, Armstrong DG, Citron DM, Tice AD, et al. Ertape nem versus piperacillin/tazobactam for diabetic foot infections (SIDESTEP): a prospective, randomized, controlled, double blinded, multicentre trial. Lancet 2005; 366: 1695 703.
8. Cunha BA. Antibiotic selection for diabetic foot infections: a review. J Foot Ankle Surg 2000; 39: 253 7.
9. Cavanagh PR, Lipsky BA, Bradbury AW, Botek G. Treatment of diabetic foot ulcers. Lancet 2005; 366: 1725 35.
10. Lipsky B. Medical Treatment of diabetic foot infections. Clin Infect Dis 2004; 39: S104 S114.
11. Martinez GD. Cuidados del pie diabético. Un enfoque multidi ciplinario. 1st. Ed. España; Arán; 2001.
12. Arreguin V, Cebada M, Simon JI, Bobadilla M, et al. Micro biología de las infecciones urinarias en pacientes ambulatorios. Opciones terapéuticas en tiempos de alta resistencia a los anti bioticos. Rev Invest Clin 2007; 59: 239 45.
13. Sader HS, Gales, AC, Granacher TD, Pfaller MA, Jones RN. Pre valence of antimicrobial resistance among respiratory tract isolates in Latin America: results from SENTRY

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antimicrobial surveillance program (1997 98). Braz J Infecti Dis 2000; 4: 245 54.
14. Armstrong DG, Lavery LA, Harkless LB. Validation of a dia betic wound classification system. Diabetes Care 1998; 21: 855 9.
15. Lavery LA, Armstrong DG, Harkless LB. Classification of dia betic foot wounds. J Foot Ankle Surg 1996; 35: 528 31.
16. Pittet D, Wysa B, Herter Clavel C, Kursteiner K, et al. Outcome of diabetic foot infections treated conservatively: a retrospecti ve cohort study with long term follow up. Arch Intern Med 1999; 159: 851 6.
17. Clinical and Laboratory Standards Institute. Performance stan dards for antimicrobial susceptibility testing. Fifteenth Interna- tional Supplement. M2-A8. 2005; 25: 11 58.
18. Lwanga SK, Lemeshow S. Sample size determination in health studies. A practical manual. 1st. Ed. Geneva: World Health Or ganization; 1991.
19. Senneville E, Melliez H, Beltrand E, Legout L, et al. Culture of percutaneous bone biopsy specimens for diagnosis of diabetic foot osteomyelitis: concordance with ulcer swab cultures. Clin Infect Dis 2006; 42: 57 62.
20. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, et al. A Clinico microbiological study of diabetic foot ulcers in an indian ter tiary Care Hospital. Diabetes Care 2006; 29: 1727 32.
21. Citron DM, Goldstein EJ, Merriam CV, Lipsky BA, et al. Bac teriology of moderate to severe diabetic foot infections and in vitro activity of antimicrobial agents. J Clin Microbiol 2007; 45: 2819 28.
22. Bansal E, Garg A, Bhatia S, Atri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Indian J Pathol Micro- biol 2008; 51: 204 8.
23. Khosravi AD, Alavi SM, Sarami A, Montazeri EA, et al. Bacte riologic study of diabetic foot ulcer. Pak J Med Scien 2007; 23: 681 4.
24. Raja NS. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: A Retrospective Study of 194 cases. J Mi- crobiol Immunol Infect 2007; 40: 39 44.