



BIOPSY-DECIDED MICROBIAL VARIETY IN DIABETIC FOOT DISEASES: AN EXTENSIVE EXAMINATION OF 100 CASES

Dr Sanjo Gupta

Assitant Professor Of Microbiology, Zydus Medical College & Hospital, Dahod, India.

Dr Madhukar Wagh

Associate Professor Of Surgery, Zydus Medical College & Hospital, Dahod, India.

ABSTRACT

Objectives: The objective is to distinguish the microorganisms present in injuries on diabetic feet by examining biopsy tests. **Materials and methods:** Successive patients with diabetic foot and side effects of contamination were signed up for the review. Biopsy tests were homogenized utilizing a mortar and handled to recognize high-impact living beings. When various disconnects were found, just the two most normal sorts of microscopic organisms were recognized. Bacterial distinguishing proof was finished utilizing biochemical procedures, while the aversion to anti-toxins was resolved utilizing the circle dissemination technique. **Results:** A sum of 100 biopsy tests were broke down, with 52 of them taken from male patients (52%). Of the 102 secludes recognized, 67% were Gram-negative bacilli, with *Escherichia coli* being the most well-known type, representing 21% of all confines. Gram-positive cocci represented 28% of all secludes, while 6% were yeasts. Among the 68 Gram-negative bacilli, 35% were impervious to ciprofloxacin. The review found 55 Enterobacteriaceae separates, 7% of which created broadened range beta-lactamases. At long last, the review recognized 8 *Staphylococcus aureus* secludes, 38% of which were impervious to methicillin. **Conclusions:** When contrasted with reports from created nations, our review recognized a more noteworthy extent of Gram-negative microbes and anti-microbial safe creatures.

KEYWORDS : Diabetes. Diabetic foot. Microbiology

I. INTRODUCTION

Diabetes can cause various intense and persistent complexities. One normal issue is the diabetic foot, which alludes to any contamination or ischemic sore that happens beneath the knee in an individual with diabetes. This condition can cause ulcerations and corruption of the feet, and it includes different explicit issues like abscesses and osteomyelitis. The diabetic foot is brought about by various gamble factors, including neuropathy, deformation, and vascular issues. Ulcers related with the diabetic foot can be characterized in view of their hidden reason, like neuropathy, ischemia, or infection.^{1,2}

When the defensive layer of the skin is lost, the fundamental tissues are presented to bacterial colonization and the injury can become convoluted with disease by different microorganisms, basically gram-positive high-impact cocci like staphylococci and beta-hemolytic streptococci in the underlying stages, while constant ulcers have a more complicated microbiota that incorporates enterococci, enterobacteria, *Pseudomonas* spp., and anaerobes.^{3,4}

Because of the absence of an agreement, there are different antimicrobial regimens for overseeing diabetic foot. Clindamycin is normally utilized in blend with a quinolone or third-age cephalosporin. At the point when parenteral organization is essential, clindamycin or metronidazole is joined with a third-age cephalosporin or a quinolone, as well as piperacillin/tazobactam or carbapenems.^{5,10}

Given the complex pathophysiology of diabetic foot, its administration requires the mediation of a multidisciplinary group that incorporates basically a specialist, internist, nutritionist, and microbiologist. It is realized that the pace of anti-infection obstruction is high in local area procured diseases in Mexico, however the microbial science and anti-toxin awareness of clinical disengages from diabetic foot patients in our setting going through standard perceived culture studies (tissue biopsy) is obscure. This information is significant for directing the underlying observational administration of our patients, which will help in the thorough administration and protection of the furthest points.

II. MATERIAL & METHODS

This is a review that depicts 100 patients with diabetic foot and

their clinical information in regards to dynamic diseases. The review was directed at the Zydus Clinical School, Dahod. Biopsy societies were taken from each of the 100 patients who had diabetic foot and gave indications of disease during 2021-2023 period. The concentrate additionally utilized the College of Texas grouping framework to decide the seriousness of foot injuries in light of the profundity of ulcers and the presence of contamination or ischemia¹⁴⁻¹⁸.

III. Sample processing

The biopsy with forceps was utilized to gather the example for refined. The examples were then shipped to the lab in sterile compartments with Amies medium without charcoal. They were handled in 60 minutes or less. Vigorous microorganisms were refined, and tissue biopsies were handled in sterile mortar first. Gram staining and semi-quantitative culture were finished. In the event of polymicrobial vegetation, the two prevalent microorganisms were recognized, while the rest were just depicted in view of settlement attributes. Bacterial recognizable proof was performed utilizing biochemical methods and brooded for 18 to 24 hours at 35°C. Anti-infection awareness was resolved utilizing the agar plate dissemination technique, following CLSI quality control strategies. Each new clump of Muller-Hinton agar was tried with ATCC strains *E. coli* 25922, *S. aureus* 25923, and *P. aeruginosa* 28753. Methicillin not entirely set in stone for *S. aureus* utilizing the oxacillin circle test on agar. ESBL assurance for enterobacteria was finished utilizing the twofold circle method on agar.

IV. Analysis and calculation of the minimum sample

We believe a successive example to be sensibly comparable to an irregular one in light of the fact that the examples were assumed control more than a while and patients were not rejected. To gauge microbes that happen in extents of up to 5%, with an outright mistake of $\pm 5\%$, the determined least example was 85 patients. The detached microorganisms are accounted for in enlightening proportions of recurrence with their 95% certainty span (CI95%). To dissect the meaning of contrasts between medians for consistent factors, the Kruskal-Wallis test was utilized with a reciprocal α of 0.05.

V. RESULT

A sum of 100 biopsies from 100 patients were examined. Of these, 47 examples were from guys (47%) and 53 were from

females (53%); the typical age of the patients was 64.2 years. Concerning seriousness of the sores as per the College of Texas characterization, patients with extreme sores prevailed, with 22 patients having diabetic foot injuries at Grade I (22%), 27 at Grade II (27%), and 51 at Grade III (51%). A sum of 112 clinical separates were gotten from the 100 biopsies, as 62 of them had monomicrobial development (62%; 95% CI, 52-72%), while 25 had polymicrobial development (25%; 95% CI, 16-34%). There was no development in 11 biopsies (11%; 95% CI, 5-17%), and 2 had interesting information of select presence of severe anaerobes by Gram staining (2%; 95% CI, 0-5%). Table 1 records the disengaged microorganisms and their recurrence.

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 intervals
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. coagulans negativa	4	0-8
Streptococcus Beta-hemolítico, Gr. A	1	0-3
Streptococcus Beta-hemolítico, 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 tremendous contrast in the extents of disengaged microorganisms while contrasting them and the orientation of the patients (χ^2 2.448, p 0.28). Additionally, there was no tremendous contrast while looking at the middle times of the patients regarding the sort of disengaged microorganism (H 3.28, p 0.35) or the grade of injury (H 1.9, p 0.385). Then again, there was a tremendous contrast while contrasting the disengages and the profundity grades of the College of Texas grouping (χ^2 21.257, p < 0.001), as a higher extent of gram-negative bacilli (41/68, 60%) were gotten in Grade III sores. Every disengaged yeast (6/6) were acquired from Grade III sores, and the most elevated extent of gram-positive cocci (23/28, 82%) were separated from Grade I and II sores.

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

Author Reference	Country	n	Antibiotic Resistance in %											
			AN	AM	FEP	CTX	CAZ	CTB	CRO	CIP	GM	IPM	TYG	SXT
Gadgil R, et al. 2006 (20)	India	22	50	55	ND	55	55	ND	ND	50	ND	0	ND	ND
Bansal E, et al. 2008 (22)	India	26	10	75	100	ND	ND	18	ND	ND	63	70	0	ND
Khosravi AD, et al. 2007 (23)	Pakistan	10	ND	ND	ND	ND	80	ND	80	80	ND	ND	ND	ND
Raja NS. 2007 (24)	Malasia	14	0	21	33	ND	ND	14	ND	14	29	29	0	ND
Our Study(100)	India	21	5	33	36	29	29	29	29	29	71	33	0	62

Of the absolute clinical separates, 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. also, 1 Pseudomonas spp. were imipenem-safe (3%; 95% CI, 0-7%). Also, 24 gram-negative bacilli were impervious to ciprofloxacin (35%; 95% CI, 24-47%). Of the 55 enterobacteria confined, four (7%; 95% CI, 0-14%) were expanded range β -lactamase makers. Of the 21 E. coli strains confined, 18 were impervious to ampicillin (86%), 15 were impervious to ciprofloxacin (71%), and 13 were impervious 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-safe.

VI. DISCUSSION

The ongoing review presents a microbiological portrayal of 100 biopsies from patients at Medical procedure Division OPD, addressing one of the biggest assortments of societies per biopsy in diabetic foot. The review tracked down a high extent of gram-negative bacilli (67%), which contrasts from past microbial science surveys in diabetic foot where S. aureus is the predominant detachment, trailed by S. epidermidis, Streptococcus spp., P. aeruginosa, Enterococcus spp., and coliform microscopic organisms. This could be because of the chronicity of the patients' circumstances as intense sores are at first colonized and tainted by gram-positive microbes. The concentrate likewise tracked down a huge relationship between gram-negative bacilli and yeast with grade III ulcers. The outcomes are steady with past examinations from India 19, where gram-negative bacilli were the most continuous in biopsies and swabs of diabetic foot ulcers. The review suggests wary utilization of anti-microbials as certain microorganisms showed high opposition rates, especially to ciprofloxacin.

The review proposes that cephalosporins of the third era could be utilized as the underlying experimental treatment all things being equal. The concentrate likewise found two types of gram-negative non-maturing bacilli impervious to imipenem, mirroring the high paces of protection from anti-infection agents saw in local area diseases in India. The extent of broadened range beta-lactamase (ESBL)- creating Enterobacteriaceae (7%) was lower than that detailed in India, however the extent of methicillin-safe Staphylococcus aureus (MRSA) (38%) was similar. 20 The noticed extent of E. coli in the current review is extensively higher (21% versus 4%).²¹

Our work has impediments that should be recognized. First and foremost, we didn't dissect hospitalizations, past medicines, or their span, so we can't decide the connection among treatments and antimicrobial obstruction profiles. Furthermore, we didn't survey what the review results meant for clinical direction or patient results. In any case, our review gives huge information on the microbiota of diabetic foot in our setting, which is a significant commitment to the complicated undertaking of rescuing the diabetic foot, and it gives significant data to clinicians in light of the fact that proper clinical administration of the contamination is basic for the rescue of the diabetic foot. We have one of the biggest assortments of biopsy societies in the writing on diabetic foot and noticed a higher extent of gram-negative microorganisms and anti-infection opposition contrasted with studies from industrialized nations.

CONCLUSION

All in all, this study expected to give bits of knowledge into the microbiological qualities and anti-microbial opposition examples of diabetic foot diseases in a particular setting. The discoveries of this review uncovered a high commonness of gram-negative bacilli and yeast in diabetic foot biopsies, especially in Grade III ulcers. This differences with past examinations that detailed *Staphylococcus aureus* as the predominant microbe. The outcomes feature the significance of wary anti-infection use, as high opposition rates, particularly to ciprofloxacin, were seen among the disengaged microorganisms. The review proposes thinking about third-age cephalosporins as beginning experimental treatment choices. Also, the presence of gram-negative non-maturing bacilli impervious to imipenem shows the disturbing paces of anti-microbial obstruction in local area obtained contaminations. The extents of expanded range beta-lactamase-creating Enterobacteriaceae and methicillin-safe *Staphylococcus aureus* were predictable with past reports from comparable settings. Be that as it may, this study has limits, like the absence of data on hospitalizations and past medicines, as well as the shortfall of an investigation of the effect on persistent results. Regardless, the review gives significant information on the microbiota of diabetic foot contaminations in the particular setting, adding to the exhaustive administration of these perplexing diseases. The higher predominance of gram-negative living beings and anti-toxin opposition saw in this study underlines the requirement for customized treatment procedures and features the significance of progressing reconnaissance of antimicrobial obstruction in diabetic foot diseases.

REFERENCES

- 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 Microbiol Rev* 2001; 14: 244-69.
- 5) Aragon SFJ, Ortiz RPP. Infección en el pie diabético. En: Martínez 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. Ertapenem 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) Martínez GD. *Cuidados del pie diabético. Un enfoque multidisciplinario*. 1st. Ed. España; Arán; 2001.
- 12) Arreguin V, Cebada M, Simon JI, Bobadilla M, et al. Microbiología de las infecciones urinarias en pacientes ambulatorios. Opciones terapéuticas en tiempos de alta resistencia a los antibióticos. *Rev Invest Clin* 2007; 59: 239-45.
- 13) Sader HS, Gales AC, Granacher TD, Pfaller MA, Jones RN. Prevalence of antimicrobial resistance among respiratory tract isolates in Latin America: results from SENTRY antimicrobial surveillance program (1997-98). *Braz J Infect Dis* 2000; 4: 245-54.
- 14) Armstrong DG, Lavery LA, Harkless LB. Validation of a diabetic wound classification system. *Diabetes Care* 1998; 21:855-9.
- 15) Lavery LA, Armstrong DG, Harkless LB. Classification of diabetic foot wounds. *J Foot Ankle Surg* 1996; 35: 528-31.
- 16) Pitt D, Wysa B, Herter Clavel C, Kursteiner K, et al. Outcome of diabetic foot infections treated conservatively: a retrospective cohort study with long term follow up. *Arch Intern Med* 1999; 159: 851-6.
- 17) Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Fifteenth International 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 Organization; 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 Clinicomicrobiological study of diabetic foot ulcers in an indian tertiary Care Hospital. *Diabetes Care* 2006; 29: 1727-32.
- 21) Citron DM, Goldstein EJ, Merriam CV, Lipsky BA, et al. Bacteriology 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 Microbiol* 2008; 51: 204-8.
- 23) Khosravi AD, Alavi SM, Sarami A, Montazeri EA, et al. Bacteriologic study of diabetic foot ulcer. *Pak J Med Sci* 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 Microbiol Immunol Infect* 2007; 40: 39-44.