

A STUDY OF AEROBIC AND ANAEROBIC BACTERIA IN DIABETIC FOOT ULCER AND IN VITRO SENSITIVITY OF ANTIMICROBIAL AGENT

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ABSTRACT

Background: Diabetes mellitus is a chronic disorder affecting large population and a major public health problem. India ranking first in the world for diabetes. Diabetic patients are vulnerable to foot ulcer with polymicrobial infection.

Aims & Objective: Study was aimed to know etiology as well as antibiotic sensitivity that helps clinicians to select appropriate antimicrobial therapy.

Materials and Methods: The study was done from July 2010 to August 2011, in department of Microbiology, Sir T hospital and government medical college, Bhavnagar. 120 patients included from surgery dept. and samples were collected for aerobic and anaerobic culture, further identification was done by microscopy, culture and biochemical reaction. The antibiotic sensitivity testing was performed by Kirby Bauer method of disk diffusion using NCCLS guidelines.

Results: Out of 120 cases, total 150 bacterial species isolated with maximum aerobes (84%) and *Staphylococcus aureus* and *Pseudomonas aeruginosa* were commonest aerobic bacteria responsible for infection. Out of 24 Anaerobic isolates, 62.5% was *Bacteroides* species. Gram positive bacteria were sensitive to Vancomycin (100%) and gram negative bacteria were sensitive to Imipenem, Meropenam and Levofloxacin.

Conclusion: The study concludes that diabetic foot ulcer infection is always polymicrobial in nature. There was resistant pattern of aerobic bacteria to cephalosporin group may be due to indiscriminate usage of antibiotics. So, it is mandatory to screen all elder patients for diabetes as well as risk factors. Periodic surveillance is necessary to know multidrug resistant strains of hospital and to apply proper antibiotic policy.

Key Words: National Committee for Clinical Laboratory Standards (NCCLS) Guidelines; Diabetic Foot Ulcer; Bacteria

Introduction

Diabetes mellitus is a chronic disorder affecting a large segment of population and also a major public health problem.^[1] Diabetes is rightly called a “disease of complications” and “Iceberg disease”. India homes 33 million diabetics, ranking highest in the world and has a prevalence of about 8% in urban India. According to one study, the number of people with diabetes is expected to rise from a current estimate of 150 million to 220 million in 2010 and 300 million in 2025.^[2]

20% of all diabetic complications involve feet and major factors considered important in the development of “Diabetic foot” are peripheral neuropathy -causing sensory impairment and weakness of intrinsic muscles of the foot and joint that leads to foot deformities, macro and microangiopathy occurring frequently and leading to ischemia of foot tissues and wounds become infected 5 times more often in diabetic than in nondiabetic patients and the rate of infection parallels blood glucose levels.

Selecting appropriate antimicrobial therapy for diabetic foot infections requires knowledge of likely etiological agents.^[3] So, present study was undertaken to assess the

role of aerobic and anaerobic bacteria in the causation of diabetic foot ulcers and the antimicrobial spectrum of these isolates would assist clinicians in the therapy of this dreaded complication of diabetes.

Materials and Methods

The present study was undertaken over a period of 1 year from July 2010 to August 2011 in the department of Microbiology, Government medical college and Sir Takhtsinhji Hospital Bhavnagar. 120 diabetic patients with foot ulcer admitted in the Sir Takhtsinhji Hospital under Surgery department were included in the study. A detail history of the patient regarding age and sex, duration of diabetes, types of diabetes, duration of foot ulcer, smoking, hypertension, peripheral neuropathy, peripheral vascular disease, antibiotic usage was taken and recorded. Foul smell, local rise of temperature, discharge and discoloration of surrounding area was noted. When osteomyelitis was suspected, the foot X-ray was done and the ulcer was graded according to the Wagner’s classification.

The patients were made to sit in a chair comfortably. The surrounding area of the ulcer was cleaned with spirit,

povidone iodine and sterile normal saline with a sterile cotton swab. 3 debrided tissue sample were taken from each patient and were subjected for smear preparation, aerobic & anaerobic culture. Transport media used were brain heart infusion broth and Robertson cooked meat media for aerobic and anaerobic culture respectively.

Samples were first examined by gram staining and different characteristics were noted like presence of pus cells, bacteria with their size, shape, arrangement and spore if present. The inoculated Brain heart infusion broth was incubated overnight at 37 °C in an incubator. Smear was prepared from broth on next day and subculture was done on Blood agar, MacConkey agar and Chocolate agar. Blood agar and Chocolate agar were incubated under 5-10% CO₂. The growth of organisms was identified by colonial morphology, gram staining and biochemical tests as described in Practical Microbiology of Mackie MacCartney 14th volume.^[4] Antibiotic sensitivity testing was done by Kirby Bauer method of disk diffusion using CLSI guidelines.^[5]

Culture of Anaerobic Bacteria: The inoculated Robertson cooked meat broth was incubated till it was turbid, not earlier than 48 hours. Smear was made from broth and gram staining was performed to see bacteria. Subculture was done on to the Neomycin Blood agar plate and metronidazole disc was put to know anaerobic bacteria. The blood agar plate was incubated anaerobically for 48 hours at 37 °C in an anaerobic jar (Hi media Anaerobic System Mark II LE 0023.5L) with Gaspak (Anaerogas pack 3.5 L LE 002A-5NO). The organisms were identified using gram staining and colony morphology.

Results

Tissue samples were processed from 120 diabetic foot ulcers. Out of the 120 cases studied, most of cases belonged to seventh decades of life (68.33%). In 120 patients, 90 (75%) were male and 30 (25%) were female, male to female ratio was 3:1. The age ranged from 35-80 (average =62.23 years). The common findings seen were purulent discharge (94%), followed by fever (85%), cellulitis (74%), vasculopathy (36%), neuropathy (31%) and foul smell (30%).

Among gram positive aerobes, *Staphylococcus aureus* was the predominant isolate (16.66%). Among gram negative aerobes, *Pseudomonas aeruginosa* was the most common isolate (26.98%). 82.3% *Proteus mirabilis* were sensitive to Imipenam, Meropenam and Levofloxacin.

While it showed 64% sensitivity to Amikacin. It was resistant to ceftazidime, cefotaxime, cefaperazone, Gentamycin and ceftriaxone. 85.2% of *P. aeruginosa* were sensitive to Levofloxacin while 79% and 73% sensitive to Meropenam and Imipenam respectively.

Table-1: Numbers of aerobic and anaerobic organisms

Organisms	No. of organisms (n=150)
Aerobes	126 (84%)
Anaerobes	24 (16%)

Table-2: Number and percentage of Aerobes isolated

Aerobes	No. of aerobes (n=126)
<i>Staphylococcus aureus</i>	21 (16.66%)
<i>Enterococcus fecalis</i>	1 (0.79%)
Group A Streptococci	5 (3.9%)
<i>Proteus mirabilis</i>	15 (11.9%)
<i>Pseudomonas aeruginosa</i>	34 (26.98%)
<i>Klebsiella species</i>	21 (16.66%)
<i>Escherichia coli</i>	16 (12.69%)
<i>Proteus vulgaris</i>	6 (4.7%)
<i>Citrobacter freundii</i>	4 (3.17%)
<i>Acinetobacter species</i>	3 (2.38%)
<i>Enterobacter species</i>	0 (0%)

Table-3: Number of Anaerobes isolated

Anaerobes	No. of Anaerobes (n=24)
<i>Bacteroides species</i>	15 (62.5%)
<i>Peptostreptococcus species</i>	7 (29.16%)
<i>Clostridium species</i>	2 (8.3%)

Discussion

Worldwide, diabetic foot lesions are a major medical, social and economical problem and are the leading cause of hospitalization for patient with diabetes.^[15] Diabetic foot is considered one of the most threatening and disabling complication for a diabetic patient as the lesions of the extremities can become so severe that the patient may risk the amputation of the toe, foot or leg.^[6]

Joslin et al had noted that "it has been brought to me that diabetic gangrene is not heaven sent but earth born." So, foot problem are preventable and morbidity can be diminished by correct identification of organism with antibiotic sensitivity pattern.^[16]

When we compare our study with Mamtha P Samaga and Hyat A S et al in 2006 and 2011 respectively, it shows that the male population are predominant due to the fact that males are more involved in smoking and outdoor activities leading to injuries and prone to development of ulcers. The mean age of the patient was 59.5 years in Kahn et al.^[17] study, 58 years in Ramani et al.^[14] study and 61.9 years in our study were comparable. In the present study, peripheral neuropathy was seen in 31.6% and vasculopathy in 36.6% of patients. Purulent discharge was present in 94.1 % of patients.

Table-4: Antibiotic sensitivity of gram positive aerobes

Aerobes	Cep	Cd	Cot	E	G	Of	P	Va	Ac	Cf	Ak	Cu	Ro	Ax	Ce
S. aureus	7 (35%)	12 (60%)	7 (35%)	0	4 (20%)	2 (10%)	4 (20%)	20 (100%)	1 (5%)	9 (45%)	5 (25%)	4 (20%)	5 (25%)	2 (10%)	2 (10%)
E. faecalis	1 (100%)	1 (100%)	1 (100%)	-	-	-	-	1 (100%)	-	-	1 (100%)	-	-	-	-
Group A Strepto	-	5 (100%)	-	5 (100%)	-	-	-	5 (100%)	5 (100%)	5 (100%)	-	-	-	-	-

Cep: Cefalothin; Cd: Clindamycin; Cot: Cotrimoxazole; E: Erythromycin; G: Gentamycin; Of: Ofloxacin; P: Penicillin; Va: Vancomycin; Ac: Amoxyclove; Cf: Ciprofloxacin; Ak: Amikacin; Cu: Cefuroxime

Table-5: Antibiotic sensitivity of gram negative aerobes

Aerobes	Ak	Lom	Cf	Sps	Net	Caz	Cip	Ctx	Gen	Cpz	A	Ctr	Ipm	Mrp	Tob	Mo	Of	Le
P. mirabilis(17)	11	3	2	5	0	0	2	0	0	0	0	0	14	14	4	5	8	14
P. aeruginosa(34)	1	3	22	4	3	0	9	2	3	2	0	5	25	27	14	15	13	29
Klebsiella(21)	8	2	5	3	1	2	11	4	1	1	1	0	18	16	2	11	11	17
E. coli(16)	1	2	11	2	0	3	3	3	0	2	0	4	13	12	6	2	2	11
P. vulgaris(6)	1	3	0	3	0	0	3	0	0	0	0	0	3	2	1	3	5	3
Citrobacter(4)	4	0	0	0	0	0	0	0	0	0	0	0	4	0	4	0	0	4
Acinetobacter(3)	1	0	2	1	0	0	1	1	1	1	0	0	2	2	0	0	2	2

Ak: Amikacin; Lom: lomefloxacin; Cf: Cefotaxime; Sps: Sparfloxacin; Net: Netillin; Caz: Ceftazidime; Cip: Ciprofloxacin; Ctx: cotrimoxazole; Gen: Gentamycin; Cpz: Cefaperazone; A: Ampicillin; Ctr: Ceftraxone; Ipm: Imipenam; Mrp: Meropenam; Tob: Tobramycin; Mo: Moxifloxacin; Of: Ofloxacin; Le: Levofloxacin

Neuropathy leads to three major complications – the neuropathic ulcer, the neuropathic joint and neuropathic oedema.^[7] Osteomyelitis was observed in 30% by Wheat et al.^[8], 34% by Kahn O et al.^[17] and 15% by Louie et al.^[9]. In the present study, osteomyelitis was present in 29% of cases. Gangrene was found in 16.25% by Vijaya et al.^[13], 61.5% by Sapico et al.^[18] and 1.6% in our study.

In our study, 126 aerobes were isolated constituting 84% of the total organisms. 85% and 84.7% aerobic isolation has been reported by Vijaya et al.^[13] and Ravisekar et al.^[15] which matches to our result. Total of 24(16%) anaerobic organisms were isolated which shows similar result with Dipali AC et al.^[6] (19%) and Anandi C et al.^[1] (20%). Anaerobic bacteria are always associated with aerobic organisms as mixed infection.

Pathare NA et al.^[12] study in 1998 showed Staphylococci species as commonest organism, while our study's result shows as Pseudomonas commonest organism in 2011. This is may be due to drug resistant pseudomonas infection is increasing in Hospitals. Amongst anaerobic bacteria, Bacteroids species (62.5%) is common as with Dipali AC et al.^[6] and Vijaya D et al.^[13] study.

In our study Staphylococcus aureus showed 60% sensitivity to clindamycin, 35% sensitive to cefalothin and 35%, 20% and 25% sensitive to Cotrimoxazole, Gentamycin and Amikacin respectively. They are 100% sensitive to Vancomycin.

In gram negative bacteria, Pseudomonas was sensitive to levofloxacin in 85.2% while 79% and 73% sensitive to Meropenam and Imipenam. They were resistant to cephalosporin group because of uncontrolled use of that

antibiotics earlier.

Conclusion

From present study we concluded that Foot ulcers were associated with purulent discharge, fever, vasculopathy, neuropathy, foul smell, osteomyelitis, cellulitis, gangrene and crepitus. Polymicrobial nature of the infection was noted and it represents an average of 1.5 organisms per case. Pseudomonas aeruginosa (26.99%) predominated the study followed by Staphylococcus aureus and Klebsiella species (16.66%). Increased incidence of hospital acquired infection may be one of the reason for predomination of Pseudomonas aeruginosa in this study. Highest sensitivity with gram negatives aerobes was seen with Imipenam, Meropenam and levofloxacin and they were resistant to cephalosporin group. These high level of resistant was observed in present study may be due to wide spread use of broad spectrum antibiotics leading to survival advantage of resistant organisms. Hence surveillance about the prevalence of the multidrug resistant organisms should be done at regular interval to assess the susceptibility pattern of the local strains in order to adapt proper antibiotic policy to keep a check on the increasing drug resistance. It is most important to screen all elderly patient for diabetes and educate them about foot care. Early identification of the risk factors and timely institution of appropriate antibiotics is indispensable to avoid amputations.

References

1. Anandi C, Alaguraja D, Natrajan V, Ramnathan M, Subramanian CS, Thulasiram M, Sumithra S. Bacteriology of diabetic foot lesions. Indian J Med Micro 2004 ; 22: 175-178.
2. Buse J, Polansky K, Burant C. Disorders of carbohydrate and lipid

- metabolism. In: Larsen R, Kronenbrey H, Melmed S, Polansky K, editors. Williams's textbook of endocrinology. 10th ed. Philadelphia: Saunders; 2003. pp. 1427-9.
3. Lipsky BA. Medical treatment of diabetic foot infections. Clin Infect Disease 2004;39;S104-114.
 4. Sathe SR, Bhorankar A. The diabetic foot. Its management in developing countries. J Diab Assoc India 1994; 34:12.
 5. Kahn O, Wanger W, Bessman AN, Downey. Mortality of Diabetic patients treated surgically for lower limb infection and/or Gangrene. Diabetes 1974;23:287-292.
 6. Dipali AC, Pal RB. Study of fungal and bacterial infections of the diabetic foot. Indian J Pathol Microbiol 2002; 45(1): 15-22.
 7. Edmonds ME. The Diabetic foot: Pathophysiology and Treatment. Clin Endocrinol Metab. 1986;15(4):889-916.
 8. Wheat J, Allen SD, Henry M, Kernek CB. Diabetic foot infections- Bacteriological analysis. Arch Intern Med 1986;146: 1935-1940.
 9. Louie TJ, Bartlett JG, Tally FP, Gorbach SL. Aerobic and Anaerobic bacteria in Diabetic Foot Ulcers. Ann Intern Med 1976;85:461-463.
 10. Bamberger DM, Daus GP, Gerding DN. Osteomyelitis in the Feet of Diabetic Patients. Am J Med 1987; 83: 653-660.
 11. Ramani A, Ramani R, Shivananda PG, Kundaje GN. Bacteriology of Diabetic Foot Ulcers. Indian J Pathol Microbiol 1991;34(2):81-87.
 12. Pathare NA, Bal A, Tavalkar GV, Antani DU. Diabetic foot infections: A study of microorganisms associated with the different Wagner grades. Indian J Pathol Microbiol 1998; 41(4): 437-441.
 13. Vijaya D, Sheshadri L. Bacteriology of diabetic foot infection. Biomedicine, 2000;20(3): 176-179.
 14. Kelkar U, Kagal A. Bacteriology of diabetic ulcers: effect of sample collection method. The Diabetic Foot Journal 2004; 7(3):124-128
 15. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhary R. A Clinico-microbiological Study of Diabetic Foot Ulcers in an Indian Tertiary Care Hospital". Diabetes Care 2006;29(8): 1727-1731.
 16. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. Mackie and McCartney Practical Medical Microbiology, 14th Ed. Singapore: Churchill Livingstone 1989.
 17. Wayne PA. National committee for clinical laboratory standards. Performance standards for antimicrobial disc susceptibility testing; 12th information supplement, 2002;M100-S12.
 18. Sapico FL, Canawati HN, Witte JL, Montgomerrie JZ, Wagner FW, Bessman AN. Quantitative aerobic and anaerobic bacteriology of infected diabetic feet" J Clin Microbiol 1980;12(3):413-420.

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