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Original article

Culture and sensitivity pattern of micro-organism isolated from diabetic foot infections in a tertiary care hospital

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ABSTRACT

Introduction- Diabetes is the leading cause of non traumatic amputation in India and is commonly preceded by infection in diabetic foot. The aim to decrease the morbidity is by giving appropriate antibiotics based on proper knowledge of sensitivity of microorganisms to various antimicrobials. Materials and methods- A prospective study was conducted on 60 patients admitted with diabetic foot infections. The aim of the study was to find the pattern of culture and sensitivity of antimicrobials used to treat infected diabetic foot. . Empirical antimicrobials were administered after taking swab for pus culture and sensitivity. Results- The mean age of males and females were 56.69 ± 11.75 yrs and 55.38 ± 11.17 years respectively. Males were affected more than females. Majority of patients were suffering from diabetes for a period of 5 years. Patients hospitalized for previous foot infections were 20%. Ulcer, cellulitis and gangrene were the presenting features. Wound culture revealed predominance of polymicrobial organisms- Gram negative bacilli were common than gram positive cocci. Predominant organisms were E.coli, Staphylococci aureus, Pseudomonas and Klebsiella. Discussion- Low virulence colonizers such as S. epidermidis and diphtheroids were also isolated may be due to impaired host defenses at the site of the wound . Most of isolated strains of E.coli, Pseudomonas, and Klebsiella were resistant to ampicillin. MRSA accounted for 64% of isolated strains of S. aureus i.e. 15% of all samples and was commonly seen in patients with osteomyelitis. 4 patients had MRSA strains resistant to vancomycin. Amikacin is a better choice of drug in infection caused by E.coli, proteus and klebsiella. Pseudomonas infection responds better to imipenem. E.coli 60-70% was resistant to commonly used ceftriaxone, ciprofloxacin, and gentamicin. Enterobacteriacacae were resistant to ciprofloxacin. Conclusion- Combination of ciprofloxacin (fluoroquinolones) and amikacin is a better choice of drug combination for infection caused by Gram positive and negative organism in diabetic foot infections.

1. Introduction

Diabetes is a fairly common disease seen in India with a prevalence of almost 12% - 17% in the Indian urban population as per a study in 2001 with a prevalence of 2.5% in the rural population.[1] Foot infections are among the most common bacterial infections encountered in patients with diabetes mellitus.[2] These infections and their sequelae are also the most common cause for most hospital admissions among diabetic patients.[3] Diabetes is the leading cause of non traumatic lower extremity amputations and accounts for more than 50% of amputations.[4,5] More than 85% of lower extremity amputations in patients with diabetes are preceded by foot ulcers. [6] More than half of patients who have undergone lower extremity amputation will have a contralateral amputation within 5 years and half of

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those who undergo amputation will die within 3 years. [4] Early recognition of lesions and prompt initiation of appropriate antimicrobial therapy are essential for controlling the infection and preventing morbidity and improve the quality of life. Antibiotic susceptibility test is a prerequisite for the management of infections which can help to make better therapeutic choices. Hence this study was planned to evaluate the prevalence of microorganism in infected diabetic foot and its sensitivity pattern at a tertiary care hospital.

2. Materials and Methods

A prospective study was conducted on 60 patients admitted with infected diabetic foot. A proforma containing detailed information on each patient was prepared according to the protocol designed for the study. Ethical clearance was obtained from institutional ethics committee and informed consent was taken from all the patients. Patients of type 1 and type 2 diabetes mellitus with recent and recurrent infected diabetic foot were included in the study. Relevant data pertaining to demographic characteristics, duration of diabetes and past history of diabetic foot infections was taken from the patients who were admitted in the hospital. Patient was clinically examined for rise in temperature, sensations of lower extremities and local examination of the diabetic wound. If the wound had devitalized tissue, debridement was done and swab or pus or tissue after surgical procedure was taken and sent for culture and sensitivity tests. Laboratory investigation included total leukocyte count, glycated hemoglobin and mean blood sugar.

The culture and sensitivity tests were was carried out as follows:

2.1.Pus culture

Pus samples were inoculated on sheep blood agar, Mac Conkey's agar and thioglycollate broth. Blood agar and Mac Conkey's agar were incubated at 37^0 C aerobically for 24 to 48 hours. Then colonies were identified using standard biochemical reactions.[7]

2.2.Antibiotic sensitivity

This test was carried out using Kirbey-Bauer disc diffusion method on Mueller Hinton agar plate. In this method filter paper discs were used, 6.0mm in diameter, charged with the concentrations of the drugs as in table 1.[8]

The discs were dried and stored in the refrigerator. A suitable dilution of a broth culture was spread on Mueller Hinton agar plate using sterile swabs. After drying the plate at 370 c for 30 mins, antibiotic discs (4 or 5 per 10cm plate) were applied with sterile forceps. After overnight incubation, the degree of sensitivity was determined by measuring the zones of inhibition of growth around the discs. Growth was inhibited around discs containing antimicrobials to which the bacterium was susceptible but not around those to which it was resistant.[8]

The data obtained were subsequently analyzed using descriptive statistics. The results were expressed as mean $\pm$ standard deviation.

Table 1.

Antimicrobials	Disc content	Antimicrobials	Disc content
Amikacin	30 mcg	Doxycycline	30 mcg
Amoxicillin + clavulanic acid	30 (20+10) mcg	Erythromycin	15 mcg
Ampicillin	10 mcg	Gentamicin	10 mcg
Cephalexin	30mcg	Imipenem	10 mcg
Cefepime	30 mcg	Levofloxacin	5 mcg
Cefoperazone	75 mcg	Netilmicin	30 mcg
Cefotaxime	30 mcg	Ofloxacin	5 mcg
Cefpirome	30 mcg	Oxacillin	1 mcg
Ceftazidime	30 mcg	Piperacillin	100 mcg
Ceftriaxone	30 mcg	Piperacillin +tazobactum	100 + 10 mcg
Cefuroxime	30 mcg	Penicilling	10 mcg
Chloramphenicol	30 mcg	Tetracycline	30 mcg
Ciprofloxacin	5 mcg	Ticarcillin	75 mcg
Clindamycin	2 mcg	Tobramycin	10 mcg
Cloxacillin	5 mcg	Vancomycin	30 mcg
Cotrimoxazole (trimethoprim+ Sulphamethoxazole)	1.25 + 23.75 mcg		

3.Results

3.1.Demographic data

60 patients admitted to medicine and surgery wards were analysed of which 42 were males and 18 were females. The mean age of males was 56.69 $\pm$ 11.75 years and that of females was 55.38 $\pm$ 11.77 years. The systemic manifestation of infection were fever and leucocytosis. Fever was seen in 5 patients. Nineteen patients have leucocytes more than 10,000/mm3 which indicates the presence of infection. Polymorphs(P) were more in number as compared to lymphocytes (L) in 35 patients.

3.2.Previous foot infection

12 patients had history of previous foot infection. Out of these , 6 patients had undergone amputation ; 2 had disarticulation at the level of metatarso-phalangeal joint and 3 had undergone forefoot amputation and 1 below knee amputation . Of these amputations, 2 were performed on contra-lateral extremity.

3.3.Characteristics of foot lesion

Based on etio-pathogenesis, 18(30%)patients (neuropathic ulcer, abscess, osteomyelitis) had underlying neuropathic features like loss of protective sensation while 18 (30%) patients (including ischemic ulcer, gangrene)had ischemic manifestations like dry, black and shriveled skin with loss of hair. Ten(17%) patients had clinical manifestation of both ischemic and neuropathic changes in foot. Fourteen patients had cellulitis and infection in amputation stump. (Table 3) Left leg was more frequently involved 42 (70%) patients as compared to right 18(30%).

3.4.Wound microbiology

TABLE 3 represents 23(38.33%) cultures revealing monomicrobial isolates whereas 30(50 %) showed 2 organisms per report. Seven (11.66%) specimens revealed > 2 organisms per report. Cellulitis and neuropathic ulcer patients have more than 2 organism per culture Microbiological culture identified 99 bacterial isolates with an average of 1.65 organisms per culture. Gram negative bacilli predominated in 59 isolates and gram positive cocci in 40 isolates. The commonest organisms isolated were E. coli, Staph. aureus, pseudomonas, klebsiella, Staph. epidermidis and proteus vulgaris. (figure 1 & 2). Cellulitis and neuropathic ulcer patients had more than 2 organism per culture.

Figure No. 1 : Gram positive bacteria

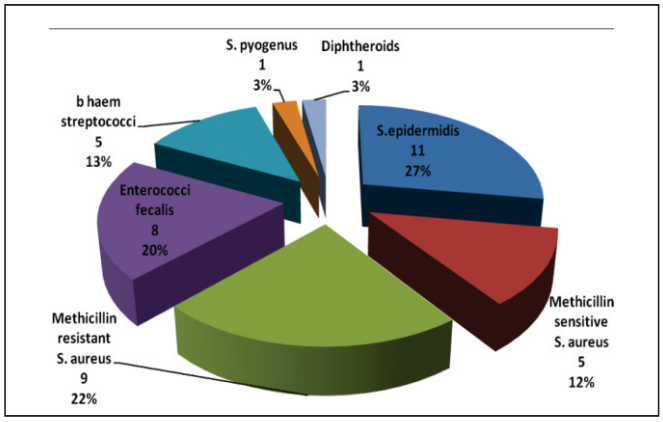
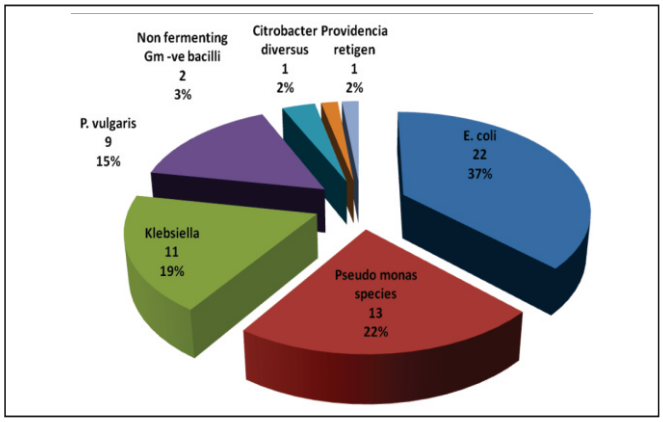


Figure No. 2 : Gram negative bacteria



The gram positive cocci response , in accordance with antibiogram from table 5; are as follows :

1. 90% of S.epidermidis were sensitive to amikacin, and 54 % to chloramphenicol and erythromycin
2. All MSSA were sensitive were sensitive to oxacillin, 60% to chloramphenicol, erythromycin, gentamicin, and imipenem.
3. 55% of MRSA were sensitive to vancomycin, and 33% to amikacin and 77 % to chloramphenicol.
4. About 62% of non-hemolytic streptococci [enterococci] were sensitive to chloramphenicol, 50% to vancomycin, and 37% to amikacin and 75% to gentamicin.
5. All β hemolytic streptococci were sensitive to chloramphenicol, 80 % to erythromycin and 40% to gentamicin.

The gram negative bacilli response, in accordance with antibiogram from table 6; are as follows

1. 86% of E.coli was sensitive to amikacin, 45% to chloramphenicol, and 36 % to gentamicin.

2. 99% of proteus species were sensitive to amikacin, and 66% to Ceftriaxone, and ciprofloxacin and 88% to chloramphenicol.

3. 72% of klebsiella species were sensitive to amikacin, 45% to Ceftriaxone and gentamicin.

4. 68% pseudomonas species were sensitive to imipenem while 53% to amikacin and 15% to ciprofloxacin.

5. Citrobacter were sensitive to amikacin and chloramphenicol.

6. Providencia retigen were sensitive to amikacin, ceftraixone and gentamicin.

7. Non fermenting gram negative bacilli were sensitive to imipenem.

4.Discussion

Diabetic foot ulcer is one of the most common complications requiring hospitalization among diabetic patients. A diabetic foot infection is defined as any inframalleolar infection in a diabetic. These include paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendinitis, and osteomyelitis. The most common and classical lesion, however, is the infected diabetic "mal-perforans" foot ulcer.[9] Neuropathy predisposes the foot to infection and angiopathy and infection influences the outcome.

Table 2 shows the period for which the patients were suffering from diabetes . In our study, majority (42%) of the patients had history of diabetes for a period of 5 years, while 14 and 11 patients suffered for 5-10 years and more than 10 years respectively. longer the duration of diabetes with poor blood glucose control , greater the incidence of foot infection. This study also shows that males had prolonged history of diabetes. 20% of patients had previous history of hospital admission for foot infections and surgical interventions.

Table No. 2 : Duration of diabetes

Duration (Years)	No.	percentages
1-5 yrs	18	30
5-10yrs	14	28.33
>10 yrs	11	18.33
Not known	17	28.33

In our study, all patients presented with ulcers of the foot or cellulitis or gangrene. Only 8.33% of patients presented with fever, although an infrequent, but ominous sign of widespread disease. Patients with osteomyelitis, deep abscesses and extensive destruction had leukocyte counts more than 10000 / mm3, indicating presence of infection.

Left leg was more frequently involved than right. Ulcers were commonly seen on the toes, dorsum of the foot and plantar surface.

The mean random blood sugar levels were 192 to 221 mg/dl. The glycated hemoglobin levels were 6.73 to 9.19 suggestive of poor control of blood sugar levels for past 3 months. Hyperglycemia should be monitored closely and controlled because it may increase the virulence of microorganisms. It may contribute to development of infection, and its severity with an increase in the susceptibility to tissue impaired function of neutrophils and macrophages including chemotaxis and adherence phagocytosis.

During hyperglycemia, there may be decreased tissue oxygen utilization; this may shunt glucose through sorbitol pathway instead of glycolytic pathways, thereby decreasing mitochondrial pyruvate utilization and decreased energy utilization.[10] Good glycemic control has a positive impact on control of infection and wound healing as shown by Pearson's correlation analysis.[11

Wound cultures revealed 37 patients had polymicrobial isolates(both Gram positive and negative organism) and 23 had monomicrobial infection (Table 3).[12] In some studies, monomicrobial isolates were most common. [13[14]] This kind of discrepancy could be because of geographical variations, or the types and severity of infection included in the studies.[15,16,17]

E.coli was most common isolated organism in 37% of samples. In some studies , gram-positive bacteria were most common i.e 55% of the samples, with Staphylococcus aureus (33%) in the first position.[13] A number of studies have found that Staphylococcus aureus is the main causative pathogen [12,14,18,19] but recent investigations reported a predominance of gram-negative aerobes.[15,16,20] Staph.

Table No. 3 : Bacterial load per culture specimen

	No. of patients	Positive culture for bacteria		
		single	two	>2
Gangrene	8	2	6	-
Neuroischemic ulcer	10	2	7	1
Ischemic ulcer	10	3	7	-
Neuropathic ulcer	10	5	3	2
Cellulitis	11	4	4	3
Infection in amputation stump	3	2	-	1
Abscess	4	1	3	-
Osteomyelitis	4	4	-	-
Total	60	23	30	7

epidermidis and diptheroids are low virulent skin pathogens and were responsible to produce infection in patients with impaired host defenses around necrotic soft tissue. (Figure 1). Although Staph. epidermidis is innocuous on intact human skin, it can cause severe infections after it penetrates anatomic barriers, because it produces various proteases, peptidases, biofilms, and surface lipoproteins that promote host tissue adherence.[21] Enterococci and Gram-negative Citrobacter, providencia are considered commensals with low virulence whereas they may cause severe tissue damage in diabetic patients. [22]

Hospitalization, surgical procedures, and prolonged or broad-spectrum antibiotic therapy may predispose patients to colonization and infection with antibiotic-resistant organisms (e.g., MRSA or Vancomycin-resistant S.aureus [VRSA], Vancomycin-resistant enterococci [VRE]. [23] MRSA accounted for 64% of isolated strains of S. aureus i.e. 15% of all samples and was commonly seen in patients with osteomyelitis. 4 patients had MRSA strains resistant to vancomycin. (Table 3)MRSA were 77% sensitive to choloramphenicol , therefore under controlled conditions chloramphenicol can be used for Vancomycin resistant S.aureus. This is in contrast to one study where the MRSA accounts for 38% of isolated S.aureus. In this study, VRE accounted for 50% of MRSA isolates. Amikacin is a better choice of drug in infection caused by E.coli, proteus and klebsiella. Pseudomonas infection can respond better to imipenem. Imipenem resistance is seen in E.coli and proteus in 80% of samples. 60-70% E.coli were resistant to commonly used ceftriaxone, ciprofloxacin, gentamicin. Enterobacteriacacae were resistant to ciprofloxacin. (Table 4)

TABLE 4 -Duration of antibiotics used ,glycemic control and common bacterial isolates

Diagnosis	No of patients	Empirical antibiotics administered. mean duration (days)	Glycemic control mean HbA1c	Mean blood sugar (mg/dl)	Most common bacterial isolates
Gangrene	8	9.25 ± 9.67	8.23 ± 1.59	207±57.39	Enterococci fecalis , E.coli, Staph epidermidis
Neuro-ischemic ulcer	10	12.6 ± 5.01	9.19 ± 1.65	215±51.73	E.coli, diphtheroids
Ischemic ulcer	10	9.7 ± 4.80	6.73 ± 2.00	192±68.3	E.coli, Proteus, MSSA, MRSA
Neuropathic ulcer	10	9.6 ±6.5	7.3 ± 39	169±57.3	E.coli, Pseudomonas, Klebsiella Staph
Cellulitis	11	8.27 ± 3.22	8.44 ± 1.22	221±42.8	epidermidis, E.coli
Infection in amputated stump	3	10.66± 0.57	7.69 ± 2.02	152.5±12.02	Proteus
Abscess	4	8.75 ± 2.62	7.8 ± 0.707	197±4.24	E.coli, proteus
Osteomyelitis	4	9.5 3.69	7.05± 1.52	196±29.1	MRSA, pseudomonas

MSSA- methicillin sensitive staphylococci , MRSA- methicillin resistant staphylococci

Thus, in the current scenario, there is no antibiotic which can cover all the organisms and thus combination of drugs have to be used as the multi-drug resistance is wide spread. The emergence of resistant strains represents a compounding problem standing against the efforts to prevent amputation as infection is the single most common cause of amputation. Even if the microorganism is sensitive to one particular antimicrobial , the drug is unlikely to attain therapeutic concentration at the site of infection because of virulence factors, such as hemolysins, proteases, and collagenases, as well as short-chain fatty acids, that cause inflammation, impede wound healing, and contribute to the chronicity of the infection.[21,24,25] Biofilms that impede the penetration of antimicrobial agents into the infected site may also be formed. Thus, the treatment of polymicrobial infection is difficult to treat in the above circumstances. To conclude , combination of ciprofloxacin (fluoroquinolones) and amikacin is a better choice of drug combination for infection caused by Gram positive and negative organisms.

5.Conclusion

Foot infections of diabetic patients are initially treated by empirical method directed at known causative organisms which may improve the outcome. If all diabetic foot infections are recognized early and treated rigorously, then the incidence of osteomyelitis and amputation of limb will decrease drastically. A patient who has one episode of foot infection is more likely to have another, so preventive action at early stage can reduce the risk. Empiric antibiotic should be guided by category of foot infection, reliable microbiological data and consideration of host factors (neuropathy and angiopathy). Correlating the antimicrobials used with culture reports indicate the growing incidence of drug resistance among bacteria and the need to modify standard treatment regimen. Judicious use of antimicrobials and insulin is necessary for glycemic control and healing in diabetic foot infection.

Conflict of Interest- There Is No Conflict of Interest .
Source of Support –NIL.

TABLE 5. Antibogram

GRAM POSITIVE COCCI Percent sensitive										
Organisms	**No. of isolates**	**Amikacin**	**Chloramphenicol**	**Ciprofloxacin**	**Clindamycin**	**Erythromycin**	**Gentamicin**	**Imipenem**	**Ceftriaxone**	**Vancomycin**
Staph epidermidis	11	90	54	45	9	54	45	===	45	45
MSSA	5	60	60	40	===	60	60	60	20	20
MRSA	9	33	77	===	22	11	11	===	==	55
Enterococci fecalis	8	37	62	75	===	25	75	===	25	50
β haemolytic streptococci	5	===	100	60	===	80	40	===	40	20
Streptococcus pyogenus	1	===	==	100	===	100	100	===	===	===
Gram positive cocci and bacilli (Diphtheroids)	1	100	100	===	===	100	==	===	100	===

TABLE 6. Antibiogram

GRAM POSITIVE COCCI Percent sensitive							
Organisms	**No. of isolates**	**Amikacin**	**Ceftriaxon**	**Chloramphenicol**	**Ciprofloxacin**	**Gentamicin**	**Imipenem**
E . coli	22	86	27	45	27	36	13
P. vulgaris	91	99	66	88	66	33	===
Klebsiella species	11	72	45	36	36	45	18
Pseudomonas species	3	53	7.6	===	15	7.6	68
Citrobacter diversus	1	100	==	100	===	===	===
Providencia retigen	1	100	100	===	===	100	===
Non fermenting gram negative bacilli	2	===	===	===	===	===	100

6.References

[1] Vasista SG. Epidemiology of diabetes mellitus urban-rural –A paradox. In:Jayaram BM, editor. Type II Diabetes: Urban–Rural. 1sted. Bangalore : Microlabs Ltd; 2004.p 24-25.

[2] Shea KW. Antimicrobial therapy for diabetic foot infections: a practical approach. Postgrad Med. 1999; 106(1):85-94.

[3] Young MJ, Veves A, Boulton AJM. The diabetic foot: etiopathogenesis and management . Diabetes Metab Rev. 1993; 9:109-127.

[4] Smith SR, Reed JF. Prevalence of mixed infections in the diabetic pedal wound: a perspective based on a national audit. . Int J Low Extrem Wounds 2002; 1(2):125–128.

[5] Bengalorkar GM, Kumar T.N. Diabetic foot infections: A review. Int J Biol Med Res. 2011; 2(1): 453-460.

[6] Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation :basis for prevention . Diabetes Care. 1990; 13:513-521.

[7] Vandepitte J, Engbaek K, Piot P,Heuck CC,editors. Basic laboratory procedures in clinical bacteriology. New Delhi : Jaypee Brothers;1992.p.62-72.

[8] Vandepitte J, Engbaek K, Piot P,Heuck CC, editors. Basic laboratory procedures in clinical bacteriology. New Delhi : Jaypee Brothers;1992.p.78-95.

[9] Lipsky BA, Berendt AR, Deery HG, et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2004;39(7):885–910.

[10] Llanes LRI, Pena AC ,Valera RC. Clinical, microbiological profile and outcome of diabetic patients with foot ulcers admitted at the Quirino memorial medical center: january 2000-may 2001. Phil J Microbiol Infect Dis. 2001; 30(3):101-107.

[11] Girish M.B, Kumar T.N, Srinivas R. Pattern of antimicrobials used to treat infected diabetic foot in a tertiary care hospital in Kolar. International Journal of Pharmaceutical and Biomedical Research. 2010, 1(2), 48-52.

[12] 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. J Clin Microbiol. 2007 ;45(9):2819-28.

[13] Martínez-Gómez Dde A, Ramírez-Almagro C, Campillo-Soto A, Morales-Cuenca G, Pagán-Ortiz J, Aguayo-Albasini JL. Diabetic foot infections. Prevalence and antibiotic sensitivity of the causative microorganisms. Enferm Infecc Microbiol Clin. 2009; 27:317-322.

[14] Yoga R, Khairul A, Sunita K, Suresh C. Bacteriology of diabetic foot lesions. Med J Malaysia. 2006;61 Suppl A:14-16.

[15] Gadepalli RB, Dhawan V, Sreenivas A, Kapil AC, Ammini C and R. Chaudhry R. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care.2006; 29:1727–32.

[16] Shankar EM, Mohan V, Premalatha G, Srinivasan RS, Usha AR. Bacterial etiology of diabetic foot infections in South India. Eur. J. Intern. Med.2005; 16:567–70.

[17] Viswanathan V , Jasmine JJ, Snehalatha C, Ramachandran A . Prevalence of pathogens in diabetic foot infection in South Indian type 2 diabetic patients. J. Assoc. Physicians India.2002; 50:1013–16.

[18] Dang CN, Prasad YD, Boulton AJ, Jude ED. Methicillin resistant Staphylococcus aureus in the diabetic foot clinic: a worsening problem. Diabet. Med. 2003; 20:159–61.

[19] Lipsky BA, Berendt AR, Deery HG, Embil JM, Joseph WS, Karchmer AW et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis.2004; 39:885–910.

[20] Chang A, Eslao E, Panilagao G, Quimpo J. Diabetic foot ulcers: experience at the Philippine general hospital. Phil J Intern Med. 1996; 34:205-209.

[21] Yao Y, Sturdevant DE, Villaruz A, Xu L, Gao Q, Otto M. Factors characterizing Staphylococcus epidermidis invasiveness determined by comparative genomics. Infect Immun. 2005; 3:1856–60.

[22] Edmonds M. Foster A, Pod DM. The use of antimicrobials in the diabetic foot . Am J Surg. 2004; 187 (Supp l to May): 25S–28S.

[23] Hartemann-Heurtier A, Robert J, Jacqueminet S, Ha Van G, Golmard JL, Jarlier V, et al. Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact. Diabet Med. 2004; 21:710–5.

[24] Bowler PG, Davies BJ. The microbiology of infected and noninfected leg ulcers. Int. J. Dermatol. 1999; 38:573–78.

[25] VonEiff C, Peters G, Heilmann C. Pathogenesis of infections due to coagulase-negative staphylococci. Lancet Infect. Dis.2002; 2:677–85.