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Medical Microbiology

BACTERIOLOGICAL PROFILE OF DIABETIC FOOT IN A TERTIARY CARE HOSPITAL

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ABSTRACT **Background:** The majority of Diabetic foot ulcers (60-80%) will heal, whereas 10-15% of them will remain active, and up to 24% of them will finally lead to Lower extremity amputation. **Objective:** To isolate and identify bacterial pathogens by using standard laboratory techniques causing Diabetic foot. Antibiotic sensitivity testing of bacterial isolates as recommended by clinical and laboratory standard institute (CLSI). **Materials & Methods:** This present cross-sectional study was done in a tertiary health care teaching hospital during periods of January 2020 to June 2021 on 144 study subjects. **Results:** After following inclusion and exclusion criteria, 144 patients were considered for this study. In present study, among the total of 144 patients 74% were male and 26% were female. Majority of patients in present study belongs to Wagner's Grade-2 ulcers 64 (44.4%), Total 223 organisms isolated, among which aerobic isolates were 209(93.7%) and anaerobic isolates were 14 (6.3%). Out of 209 aerobic isolated, most common isolates were Pseudomonas aeruginosa 48(21.5%), followed by Staphylococcus aureus 45(20.2%) and Klebsiella pneumoniae 43(19.3%). Among Anaerobes Peptostreptococcus species and Clostridium species most commonly isolated. Among the 223 isolates, Aerobic gram positives isolates were most susceptible to Linezolid (100%) and Vancomycin followed by Fluroquinolones. Aerobic Gram-negative isolates were most susceptible to Aminoglycosides and Carbapenems. Anaerobic organisms are sensitive to Piperacillin/Tazobactam and Vancomycin followed by Metronidazole. **Conclusion:** The high rates of antibiotic resistance shown in the present study due to such factors including prolonged hospitalization, chronic wounds, irrational use of antibiotics, and the transfer of resistance genes by transport means. The present study This study could help clinician to know the sensitivity pattern of organism. Empirical treatment of diabetic foot infection should be based on recent report of articles of same geographical region.

KEYWORDS : Diabetic foot, Bacteriological profile, Anaerobic Antibiotic susceptibility testing.

INTRODUCTION

The number of people with diabetes is increasing due to population growth, aging, urbanization, and the increasing prevalence of obesity and physical inactivity[1].Diabetes in India with a more than 62 million diabetics population, is rapidly gaining the status of a potential epidemic[2]. Screening of persons for diabetes is a must as one in two (50.1%) people living with diabetes do not know that they have diabetes[3]. Prevalence of diabetes in India differs according to region varying from 5.3% in central India to 13.6% in Northern India[4]. Nearly 15-20% of people suffer from diabetic foot ulcers during their lifetime[5]. The global diabetes prevalence in 2021 is estimated to be 537 million people[6].

Diabetic foot ulcers are defined as Infections, ulceration, and destruction of deep tissue associated with neurological abnormalities and various degrees of peripheral vascular disease in the lower limb[7]. The diabetic foot follows a common pathway that begins with a small ulcer or surgical wound. The majority of Diabetic foot ulcers (60-80%) will heal, whereas 10-15% of them will remain active, and up to 24% of them will finally lead to Lower extremity amputation[8]. These amputations in diabetes patients can be prevented with a team approach to wound care[9]. It leads to complications like gas gangrene. Diabetic foot infection includes cellulitis, abscess, necrotizing fasciitis, pyogenic or suppurative arthritis osteomyelitis, tendinitis[10].Diabetic foot infection is polymicrobial, it involves both aerobic and anaerobic organism.

Aerobic bacteria most commonly seen in diabetic foot infection are Staphylococcus aureus, Staphylococcus saprophyticus, Staphylococcus epidermidis, Streptococcus pyogenes, Streptococcus mutans, Pseudomonas aeruginosa, Proteus species, Escherichia coli, and Klebsiella pneumoniae. The anaerobic bacteria include Peptostreptococcus species, Anaerobic Streptococci, Bacteroides fragilis, and Clostridium species[11].

This study was planned to isolate and identify bacterial pathogens and Antibiotic sensitivity testing of isolates causing diabetic foot.

MATERIALS METHODS

Cross-sectional study conducted in Department of Microbiology Government medical college Aurangabad. Clinically suspected cases of 144 patients with diabetic foot infection in Outpatient Department and admitted in Wards during the period of January 2020 to June 2021 were included in this study. Patient's consent as well as ethical clearance for the study was obtained. These patients with Diabetic foot were known diabetic or detected diabetic by estimated blood glucose estimation at the time to visit the hospital for treatment of foot ulceration/foot infection. In this study, samples collected according to Wagner's grading.[12]

Grade 0: No obvious ulcer but thick callus prominent metatarsal heads, claw toes or any bony abnormality,
Grade 1: Superficial ulcer, not clinically infected
Grade 2: Ulcer of the skin extending through the subcutis with exposed tendon or bone and without osteomyelitis or abscess formation
Grade 3: Deep ulcer, abscess formation, and bone involvement
Grade 4: Localized gangrene
Grade 5: Gangrene of whole foot

Inclusion criteria was all clinically diagnosed diabetes patients above

18 years of age with type 2 diabetic mellitus and affected with diabetic foot ulcers according to Wagner's Grade.

Exclusion criteria were non-diabetic patients with ulcers, diabetic foot caused by fungi, post-operative patients develop a wound infection and diabetic foot Patients less than 18 years of age.

The clinical details of the patients such as age, sex, types of diabetes, duration of diabetes and ulcer were recorded. The ulcers were graded according to the Wagner's grade classification.

Pus & Tissue sample were collected from the patients under strict aseptic precautions and transported immediately to the laboratory in appropriate settings and sample processing done. Pus samples were taken from the site of wound, ulcer, or abscess of diabetic foot patients. Ulcer surfaces were rinsed with sterile normal saline and pus was collected using a sterile cotton swab from the base of the ulcer. In case of an abscess, pus aspirated with sterile syringe for the identification of aerobic and anaerobic isolates. Patients having Wagner's grade 1-2 were processed for aerobic culture, Patients had grade 3-5 were processed for both aerobic and anaerobic culture sensitivity. Gram's staining was done followed by culture on blood agar, MacConkey's agar. After incubation, bacterial growth was identified by colony morphology, biochemical reactions as per the standard operative procedure (SOP) of the laboratory.

Pus samples for anaerobic culture were immediately inoculated on anaerobic blood agar and MacConkey agar and kept in McIntosh and Fildes' anaerobic jar and the gas pack is used to maintain an anaerobic environment. Pseudomonas aeruginosa (ATCC 27853) is used as a control of an anaerobic environment. Robertson cooked meat medium (RCM) is also used for anaerobic culture for suspecting patients for gas gangrene, anaerobic isolates were identified by following all standard guidelines. Antibacterial susceptibility test was done by Kirby Bauer's method for appropriate antibiotics.

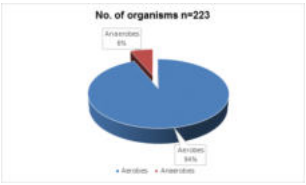
RESULTS

Total 144 patients assessed in this study, 107 (74%) were male and 37(26%) were females, The maximum number of patients were from the age group 51 - 60 years is 51 (35.41%) followed by 61 to 70 years age group 32(22.2%).

Table No.1 Patients Distribution Of Diabetic Foot Ulcer According To WAGNER'S Grading.

Wagner's grading	Number of patients	Percentage %
Grade 1	23	15.90%
Grade 2	64	44.44%
Grade 3	43	29.80%
Grade 4	9	6.25%
Grade 5	5	3.45%
Total	144	100%

Majority of patients in present study belong to Grade-2 ulcers were 64 (44.4%), followed by Grade-3 (44.44%) and Grade-2 (15.90%).



Pie Chart 01: Distribution Of Aerobic And Anaerobic Organisms In Diabetic Foot-infected Patients.

In present study, out of 144 patient's aerobic organisms isolated were 209 (93.7%), while 14 (6.3%) were anaerobes. Maximum aerobic isolates in the form of gram-negative and gram-positive bacteria. Anaerobic organisms are most commonly isolated from deep wounds Anaerobe's isolation in less percentage because of a smaller number of patients admitted of grade 4 and 5 diabetic foot ulcer.

The present study shows predominant monomicrobial flora isolated in patients of diabetic foot infection 80(55.55%), followed by 64 (44.44%) patients belongs to Polymicrobial flora. In this study maximum patients shows monomicrobial flora have less duration of ulcers and Wagner's grading of 1 and 2.

Table No.02- Distribution Of Bacterial Isolates In Diabetic Foot Infections.

Bacterial isolates	Percentage (%)
Total number of isolates	223
Aerobes	209(93.7%)
Gram-positive organisms	63(28.3%)
1)Staphylococcus aureus	45(20.2%)
2)Enterococcus sp	12(5.4%)
3)Streptococcus sp	3(1.34%)
4)Coagulase-negative staphylococcus	3(1.34%)
Gram-negative organisms	146(65.5%)
1)Pseudomonas aeruginosa	48(21.5%)
2)Klebsiella pneumoniae	43(19.3%)
3)Escherichia coli	25(11.2%)
4)Acinetobacter baumannii	14(6.3%
5)Proteus mirabilis	12(5.4%)
6)Enterobacter species	4(1.8%)
Anaerobes	14(6.3%)
1)Peptostreptococcus species	6(2.7%)
2)Clostridium species	4(1.8%)
3)Bacteroids species	2(0.9%)

In present study total 223 organisms isolated, majority were gram negative 146(65.5%) followed by Gram-positive 63(28.3%) and 14(6.3%) isolates were anaerobes.

Antibiotic Susceptibility Testing

The gram-positive aerobic cocci response, in accordance with antibiogram from table no.03 showing 100% sensitivity to Linezolid and Vancomycin. Maximum resistance to Penicillin and cefoxitin found in Staphylococcus aureus. Table no.04 shows that in aerobic gram-negative isolates Amikacin and Gentamicin have majority sensitivity followed by Meropenem and Imipenem. Among Enterobacteriaceae family Klebsiella pneumoniae showing Maximum resistance pattern. Acinetobacter baumannii had maximum resistance belongs to non-fermenter group. Ciprofloxacin, Trimethoprim/ Sulfamethoxazole ole and Cefazolin showing increased resistance among gram-negative isolates. Antibiotic susceptibility pattern in anaerobes shows majority of sensitive to Vancomycin, Piperacillin/ Tazobactam, Kanamycin and Metronidazole. Anaerobes showing resistance to Penicillin, Gentamycin and Clindamycin.

Table No.03: Antimicrobial Susceptibility Pattern In Aerobic Gram-positive Isolates

Antibiotic disc	Staphylococcus aureus (n=45)		Enterococcus species (n=12)		Streptococcus sp n=(03)	
	S%	R %	S%	R %	S%	R %
Linezolid	100%	0%	100%	0%	100%	0%
Vancomycin	100%	0%	100%	0%	NA	NA
Doxycycline	84.44%	8.88%	50%	50%	0%	100%
Clindamycin	73.33%	22.22%	NA	NA	33.33%	66.66%
Trimethoprim/ Sulfamethoxazole	51.11%	44.44%	NA	NA	NA	NA
Gentamicin	48.88%	48.88%	NA	NA	0%	100%
Cefoxitin	28.88%	71.11%	NA	NA	NA	NA
Ciprofloxacin	22.22%	57.77%	8.33%	91.66%	NA	NA
Erythromycin	11.11%	57.77%	10%	80%	0%	100%
Penicillin G	8.88%	91.11%	50%	50%	0%	100%
Gentamicin-High	NA	NA	50%	50%	NA	NA
Levofloxacin	NA	NA	100%	0%	100%	0%

Ampicillin	NA	NA	33.33%	66.66%	NA	NA
Cefotaxime	NA	NA	NA	NA	0%	100%
Bacitracin	NA	NA	NA	NA	0%	100%
S- sensitive, R- Resistance, NA-Not applicable						

Table No.04: Antimicrobial Susceptibility Pattern In Aerobic Gram-negative Isolates

Antibiotics disc	Pseudomonas aeruginosa (n=48)		Klebsiella pneumoniae (n=43)		Escherichia coli (n=25)		Acinetobacter baumannii (n=14)		Proteus species (n=12)	
	S%	R%	S%	R%	S%	R%	S%	R%	S%	R%
Amikacin	56.25%	39.58%	16.27%	76.74%	68%	24%	21.42%	78.57%	83.33%	8.33%
Tobramycin	54.34%	45.65%	NA	NA	NA	NA	42.85%	57.14%	NA	NA
Piperacillin/Tazobactam	52.17%	41.30%	9.30%	88.37%	64%	32%	35.71%	57.14%	100%	0%
Imipenem	45.83%	50%	9.30%	83.72%	48%	36%	21.42%	78.57%	75%	16.66%
Meropenem	43.75%	52.08%	11.62%	81.39%	64%	32%	21.42%	71.42%	83.33%	8.33%
Ceftazidime	43.75%	56.25%	NA	NA	NA	NA	28.57%	71.42%	NA	NA
Aztreonam	41.66%	45.83%	NA	NA	NA	NA	NA	NA	NA	NA
Gentamicin	41.30%	54.34%	16.27%	83.72%	68%	24%	14.28%	78.57%	66.66%	33.33%
Levofloxacin	39.13%	45.65%	0%	90.69%	28%	68%	NA	NA	50%	41.66%
Cefepime	37.50%	52.08%	4.65%	93.02%	28%	64%	21.42%	78.57%	41.66%	25%
Ciprofloxacin	33.33%	60.41%	0%	97.67%	16%	72%	21.42%	78.57%	16.66%	58.33%
Trimethoprim/Sulfamethoxazole ole	NA	NA	20.93%	76.74%	36%	60%	21.42%	78.57%	66.66%	25%
Cefuroxime	NA	NA	0%	95.34%	4%	88%	NA	NA	16.66%	58.33%
Cefotaxime	NA	NA	0%	97.67%	12%	80%	NA	NA	41.66%	50%
Cefazolin	NA	NA	0%	100%	0%	100%	NA	NA	25%	75%
Ampicillin	NA	NA	NA	NA	8.69%	91.30%	NA	NA	41.66%	58.33%
Minocycline	NA	NA	NA	NA	NA	NA	42.85%	42.85%	NA	NA
Ampicillin/Sulbactam	NA	NA	NA	NA	NA	NA	28.57%	50%	NA	NA
Tetracycline	NA	NA	NA	NA	NA	NA	28.57%	64.28%	NA	NA
S- sensitive, R- Resistance NA-Not applicable										

Table No.05: Antimicrobial Susceptibility Pattern In Anaerobic Isolates

Antibiotics	Peptostreptococcus (n=06)		Clostridium sp (n=04)		Bacteroides sp (n=02)	
	Sensitivity %	Resistance %	Sensitivity %	Resistance %	Sensitivity %	Resistance %
Metronidazole	67%	33%	100%	0%	0%	100%
Kanamycin	100%	0%	100%	0%	50%	50%
Vancomycin	100%	0%	100%	0%	100%	0%
Piperacillin/ Tazobactam	100%	0%	100%	0%	100%	0%
Gentamycin	67%	33%	0%	100%	0%	100%
Penicillin	33%	67%	0%	100%	0%	100%
Cefoxitin	83%	17%	50%	50%	50%	50%
Imipenem	83%	17%	75%	25%	100%	0%
Clindamycin	67%	33%	50%	50%	0%	100%
S- sensitive, R- Resistance						

DISCUSSION

Diabetic foot is one of the most feared complications of diabetes and leading cause of hospitalization in diabetic patients. Diabetic foot characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration, and infection with or without osteomyelitis, Diabetic foot infections in the form of foot ulcers are a major complication of diabetes mellitus leading to the development of gangrene and even leads limb amputation[14].

Various bacteria are responsible for causing diabetic foot infections. These diabetic foot infections are treated with empirical treatment in nearby hospitals. when patients come to our hospital proper culturing of samples and identifying the causative microorganism with its antibiotic sensitivity pattern may help patient to cure infections. This study presents a detailed bacteriological profile of organisms and their antibiotic sensitivity pattern of the patient admitted to tertiary care hospital.

In Present study out of 144 patients, 107 (74%) were males and 37 (26%) were females. Male predominance was seen with a male to female ratio of 2.84: 1. The present study, showed male predominance which is concurrence with Mishra et al[15], Sasikumar et al[16.Al] Benwan et al[17.] Males are more at risk of developing diabetic foot infections could be due to more outdoor activities so more chances of getting trivial injuries leads to chronic nonhealing ulcers.[15]

The present study showed that Diabetes mellitus Type-II diabetes patients were included, most of the patients with diabetic foot infection in the age group 51-60 years was 35.41%. It may be because Diabetes mellitus Type II is classically seen in elderly patients, a large number of type II patients remain asymptomatic and develop complications due to prolonged hyperglycemia.[18] Similar age group affected in Gadepalli et al[19] and Ramakant et al[20].

As per Wagner's grading system present study shows a maximum number of patients with infected diabetic foot ulcers belonging to Wagner's grade-2 were 63(44.40%) Similar occurrence of diabetic foot ulcers in maximum cases belonging to Wagner's grade 2 noted by Chaudhry et al[21] and Uivaraseanu et al [22], Priti shah et al[18], study. In the present study, among total 223 organisms isolated 93% aerobes and 7% anaerobes. A similar finding noted in Otta S et al[23] study. Aerobic predominance concurrence with Shah P et al [24] study. In this study, the most common isolates were monomicrobial 80 (56%) and polymicrobial 64 (44%). A possible reason for such a high incidence of monomicrobial infections might be due to the role of low severity of infections[22]. A decrease of polymicrobial flora in our study could be because of widespread use of higher broad-spectrum antibiotics without doing pus culture before initiating antibiotics[25]. A similar predominance of monomicrobial isolate was also noted in studies done by Otta et al[23], Noor et al[25]. The maximum predominance of Gram-negative bacteria 146 (65.47%) was seen. Gram-positive cocci 63 (28.25%) was noted. Among the most common aerobic gram-negative bacteria Pseudomonas aeruginosa 48(21%) and among the gram-positive cocci Staphylococcus aureus 45 (20%) were isolated. Similar predominant isolates as Pseudomonas aeruginosa and Staphylococcus aureus were found in studies, Such as Goh et al[26], Ogba et al [27].

In this study, among the total 14 (7%) anaerobic organisms predominantly 6 (2.8%) isolates were Peptostreptococcus sp followed by Clostridium species 4(1.7%) in a fewer number of Bacteroids species were isolated. Similarly In Anandi et al [28]study, predominant anaerobes were Clostridium species .

As regards to Antibiotic sensitivity pattern of Gram-positive cocci, observed that the predominant multidrug resistance pattern. Cephalosporin was (90%) resistant in gram-positive cocci, followed by Penicillin derivative (85%), Macrolide (84%), Aminoglycosides also shows 74% resistance, and other class of drugs such as Tetracycline, Lincosamide shows more than 50% resistance pattern. Among all Gram-positive cocci, isolate Staphylococcus aureus were isolated showing 100% sensitivity to Linezolid and Vancomycin,84% sensitive to Doxycycline, Clindamycin is sensitive in 73% patients,51%to Trimethoprim/sulfamethoxazole, 48% to Gentamycin. Murghan et al[29] and Mehta et al [30] partly concurrence with. present study

In this study Enterococcus species was 100% sensitivity to Linezolid, Levofloxacin, Vancomycin and more than 50% resistance to Ciprofloxacin Doxycycline, Penicillin G, Gentamicin-High, Ampicillin, Erythromycin, Ciprofloxacin. Majority of resistance was shown to Ciprofloxacin. Narwade et al[31] and Anyim et al[32] study had concurrence with present study.

Enterobacteriaceae family have most resistance pattern to Cephalosporin, Penicillin derivatives, Fluroquinolones as 78%, 74%, 65% respectively. Among all non- fermenters isolates more than 50% resistance pattern seen to Cotrimoxazole, Fluoroquinolones, Cephalosporin, Carbapenem, Aminoglycoside and Penicillin derivative is seen.

This study shows Pseudomonas aeruginosa in majority sensitive to Amikacin (56.25%) followed by Tobramycin, Piperacillin/ Tazobactam, Imipenem Meropenem and Ceftazidime. Jain et al[33] and Goh et al[26]studysimilar sensitivity pattern.

In this study, Klebsiella pneumoniae predominantly multidrug resistance pattern showing maximum resistance to cefazolin 100%, other antibiotics such as Ciprofloxacin, Cefotaxime, Cefuroxime, Cefepime also show resistance pattern more than 93%. Meropenem, Imipenem, Gentamycin shows 80-83% resistance. Alhubail et al [34] study and Chaudhry et al[21] study partly concurrence with similar resistance pattern. Escherichia coli isolates were 68% sensitive to Aminoglycosides,64% to Piperacillin/Tazobactam and Meropenem whereas Cefazolin and Cefuroxime were predominant resistance 100% and 88% respectively, 91% resistance to Penicillin derivatives, more than 60% resistance to Cotrimoxazole, Fluroquinoles. In Shanmugam et al[35] Escherichia coli shows multidrug resistance pattern 100% resistance to penicillin derivatives, Cefazolin and cotrimoxazole 82% resistance, Cephalosporins also shows more than 70% resistance. Present study partially correlates with Shanmugam et al study for similar multidrug resistance pattern.

The antibiotic susceptibility pattern of anaerobes was reported by very few literatures. In the present study, Anaerobes were 100% sensitive to Piperacillin/Tazobactam and Vancomycin 83% sensitivity of cefoxitin in Peptostreptococcus. The resistance patterns of anaerobes were 66%,89%, and 61% Metronidazole, Penicillin & Clindamycin respectively. n Goh et al[26] 100% sensitivity to Piperacillin/Tazobactam, Metronidazole, Cefoxitin for Peptostreptococcus, Clostridium, and Bacteroids sp. Higher rates of antibiotic resistance observed in the present study may be due to our hospital is a tertiary care hospital. Referred patients from other hospital and had received empirical antibiotics so that failing to control infection. Microbiological laboratory facilities for identification of organisms and antibiotic sensitivity pattern are not available in district hospital. Indiscriminate use of antibiotic therapy results in resistant to organisms.

CONCLUSION

Diabetic foot ulcer infection should be treated according to culture and antibiotic sensitivity report to avoid unnecessary usage of antibiotic which may result in development of Multi Drug Resistant strains. The high rates of antibiotic resistance shown in the present study may be due to such factors including hospitalization, recent use of broad-spectrum antibiotics, history chronic wounds, irrational use of antibiotics, and the transfer of resistance genes by transport means. Empirical treatment of diabetic foot infection should be based on recent report of articles of same geographical region. This study could help clinician to know the sensitivity pattern of organism. It is health providers responsibility to enlighten the foot care in diabetes and consequences of foot infection and use of proper foot wear which could decrease development of foot ulcer.

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