ABSTRACT: A diabetic foot is one of the leading causes of the hospitalization among diabetic patients. The present study revealed the bacteriological profile of diabetic foot with special reference to the antibiogram. 150 Samples from established cases of diabetic foot ulcers were collected over a period of six months by using sterile swabs and they were processed according to CLSI 2013 guidelines. Bacterial etiology could be identified among 67 cases out of 150 (38%); single organism was isolated in 58 (87%) among which Pseudomonas aeruginosa was the commonest (31.34%), followed by Escherichia coli (23.8%) and Staphylococcus aureus (22.4%). Enterococcus faecium, Proteus vulgaris, Klebsiella pneumoniae were isolated in 2 cases each. Among Polymicrobial cases, Staphylococcus aureus along with Klebsiella oxytoca were isolated in 4 and in rest 5 cases, Pseudomonas aeruginosa along with Escherichia coli were isolated. Gram negative (72.36%) isolates significantly outnumbered the Gram positive ones. Out of total 19 isolated Staphylococcus aureus, 7 were methicillin resistant (36.84%) but all were sensitive to both Vancomycin and Linezolid. One isolated Enterococcus faecium was Vancomycin resistant (van-A type), [MIC Value>64μg/ml]; according to VITEK 2 AES. Among 50 isolated gram negative bacteria, 23 (46%) produced ESBL, 17 (33.33%) were AmpC beta lactamase producers and 4 (8%) were carbapenemase producers; 33 gram negative isolates were fluoroquinolone-resistant (66%). Among the Carbapenemase producer Pseudomonas spp, two isolates were resistant to both Tigecycline and Colistin and one was resistant to Colistin but sensitive to Tigecycline; all of them were sensitive to Polymyxin-B.

INTRODUCTION: A diabetic foot is one of the most feared complications of diabetes and is the leading cause of the hospitalization among diabetic patients. Major increase in mortality among diabetic patients, observed over the past 20 years is considered to be due to the development of macro and micro vascular complications, including failure of the wound healing process. Wound healing is an innate mechanism of action that works reliably most of the time. A key feature of wound healing is stepwise repair of lost extracellular matrix (ECM) that forms the largest component of the dermal skin layer. Controlled and accurate rebuilding is essential to avoid under- or over-healing that may lead to various abnormalities. But in some cases, certain disorders or physiological insult disturbs the wound healing process. Diabetes mellitus is one such metabolic disorder that impedes the normal steps of the wound healing process. Many histopathological studies show a prolonged inflammatory phase in diabetic wounds, which causes a delay in the formation of mature granulation tissue and a parallel reduction in wound tensile strength.
The individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than individuals without diabetes. The Indian diabetic population is expected to increase to 57 million by the year 2025.

AIMS AND OBJECTIVES: To evaluate the bacteriological profile of diabetic foot with special reference to the antimicrobial susceptibility pattern to formulate the policy of empirical antimicrobial therapy to minimise the diabetes associated mortality & morbidity.

MATERIALS & METHODS: The present study includes 150 known diabetic patients with foot ulcers attending both IPD & OPD over the period of last six months. A clinical history was elicited with regards to the age of the patient, duration of diabetes, the type of treatment which was received and the presence of other systemic illnesses. The patients were also assessed clinically and the ulcers were graded according to Wagner's grade.

Wagner Grading System (Indication: Evaluation of Diabetic Foot Ulcer)

1. Grade 1: Superficial Diabetic Ulcer
2. Grade 2: Ulcer extension
   1. Involves ligament, tendon, joint capsule or fascia
   2. No abscess or Osteomyelitis
3. Grade 3: Deep ulcer with abscess or Osteomyelitis
4. Grade 4: Gangrene to portion of forefoot
5. Grade 5: Extensive gangrene of foot

The samples were collected after obtaining informed consents from the patients. After obtaining proper patient informed consent, samples (purulent drainage or curetted material) were collected from the deeper portion of the ulcers (ulcer base) by using 2 sterile swabs which were dipped in sterile glucose broth. Cultures are best taken from from the ulcer base. The samples were collected by making a firm, rotatory movement with the swabs. One swab was used for Gram staining and the other was used for culture. A direct Gram stained smear of the specimen was examined. The specimens were inoculated onto blood agar, chocolate agar, Mac Conkey's agar and thioglycollate medium. The inoculated plates were incubated at 37°C overnight and the plates were examined for growth, the next day. The further processing was done according to the nature of the isolate, as was determined by Gram staining and the colony morphology. The organisms were identified on the basis of their Gram staining properties and their biochemical reactions.

Antibiotic Susceptibility testing: The antibiotic susceptibility testing was done by the Modified Kirby Bauer disc diffusion method, as per the CLSI guidelines, 2013. The antimicrobial discs which were used were those of Ampicillin (20μg), Aztreonam (30μg), Gentamicin (10μg), Amikacin (30μg), Cefazolin (30 μg), Cefuroxime (30μg) Ceftazidime (30μg), Cefotaxime (30μg), Ceftriaxone (30μg), Cefepime (30μg), Cefoperazone/Sulbactam (75/10μg), Piperacillin/Tazobactam(100/10μg), Imipenem (10μg), Meropenem (10 μg), Polymyxin B (300 units) and Colistin (10μg), for the Gram negative bacilli. Penicillin, Ampicillin, Azithromycin (15μg), Cefoxitin (30μg), Cefotaxime (30μg), Chloramphenicol (30μg), Clindamycin (2μg), Erythromycin (15μg), Oxacillin (1μg), Vancomycin
(30μg), Teicoplanin (30μg), Ciprofloxacin, Ofloxacin (5μg), Linezolid (30μg) and Tetracycline (30μg) were used to study the susceptibility patterns of the Gram positive cocci MRSA, Vancomycin resistance, ESBL, Amp-C-β Lactamase and carbapenemase production were detected as per the CLSI guidelines 2013.

**MRSA detection:** The phenotypic test for the detection of MRSA was done by using a cefoxitin (30 μg) disc.

A zone of inhibition which was equal to or more than 22 mm was considered as susceptible to Cefoxitin and the organism was reported as Methicillin Sensitive Staphylococcus aureus. Those isolates which produced a zone of inhibition which was less than or equal to 21 mm were considered as Methicillin Resistant Staphylococcus aureus (MRSA).

ESBL production was confirmed by using discs of Ceftazidime (30 μg) and Ceftazidime Clavulanic acid (30/10 μg) respectively. The test organism was inoculated as a lawn on a Mueller Hinton agar plate and the above mentioned discs were placed on the plate. The plates were incubated at 37°C overnight and they were examined next day. An increase in the zone diameter, which was equal to or more than 5 mm for the antimicrobial agent which was tested in combination with clavulanic acid, in comparison to the antimicrobial which was tested alone, indicated that the strain was an ESBL producer.

Carbapenemase production was detected by using the Modified Hodge test. A 0.5 Mac Farland’s suspension of ATCC Escherichia coli 25922 was diluted 1:10 in sterile saline. This was inoculated on a Mueller Hinton agar plate, as for the routine disc diffusion testing. The plate was dried for 5 minutes and a disc of Meropenem 10 μg was placed in the centre of the agar plate. Colonies of the test organism were picked and inoculated in a straight line, from the edge of the disc, up to a distance of at least 20mm. The plates were incubated at 37°C overnight and they were examined next day. They were checked for an enhanced growth around the test organism, at the intersection of the streak and for a zone of inhibition. The presence of an enhanced growth indicated Carbapenemase production, and the absence of an enhanced growth meant that the test isolate did not produce carbapenemase.

Enterococcal species confirmation and MIC value determination were done by VITEK2 AES automated system.

**RESULTS:** Out of 150 test samples, 85 Diabetic foot ulcer cases were included in Wagner’s grade 1 and rest 65 were included in Wagner’s grade 2.

Bacterial etiology could be identified among 67 cases out of 150 (38%); single organism was isolated in 58 (87%) among which Pseudomonas aeruginosa was the commonest (in 21 cases), followed by Escherichia coli (in 16 cases) and Staphylococcus aureus (in 15 cases). Enterococcus faecium, Proteus vulgaris, Klebsiella pneumoniae were isolated in 2 cases each.

Polymicrobial association was found in 9 cases. In 4 cases, Staphylococcus aureus along with Klebsiella oxytoca were isolated and in rest 5 cases, Pseudomonas aeruginosa along with Escherichia coli were isolated.
Gramp negative (55) isolates significantly outnumbered the Gram positive ones (21).

p value <0.05 i.e. the result is statistically significant.

Out of total 19 isolated Staphylococcus aureus, 7 were methicillin resistant (36.84%). All Staphylococci isolates were sensitive to both Vancomycin and Linezolid.

One isolated Enterococcus faecium was Vancomycin resistant (MIC Value was> 64μg/ml), it was sensitive to Teicoplanin, Dalfopristin/Quinupristin and Linezolid. This isolated Enterococcus faecium was resistant to Penicillin, Gentamicin high level synergy, Tetracycline and Fluoroquinolone leaving behind very restricted therapeutic options. According to VITEK 2 AES, the resistance was of van A type.

Among 50 isolated gram negative bacteria, 23 (46%) produced ESBL whereas 17 (33.33%) were AmpC beta lactamase producers and 4 (8%) were carbapenemase producers; 33 gram negative isolates were resistant to fluoroquinolones (66%).
All of the Carbapenemase producers were exclusively Pseudomonas aeruginosa whereas the Amp C β lactamase producers were Pseudomonas aeruginosa followed by Klebsiella pneumoniae and Klebsiella oxytoca. On the other hand, the ESBL (Extended spectrum beta lactamase) producing population comprised of Escherichia coli followed by Pseudomonas aeruginosa.

Among the isolated 4 Carbapenemase producer Pseudomonas spp, 2 were resistant to both Tigecycline and Colistin and 1 was resistant to Colistin but sensitive to Tigecycline; all of them were sensitive to Polymyxin-B.

**DISCUSSION:** Mohd Zubair et al., Anandi et al., Rama Kant et al., Pappu K et al., and Citron et al. have reported 56.6%, 19%, 23%, 92% and 16.2% monomicrobial infections and 33%, 67%, 66%, 7.7% and 83% of polymicrobial infections respectively.

In our study, we have found 87% monomicrobial infection. The findings of this study correlate with findings of Pappu et al.

Gram negative bacilli were more prevalent (55 out of 76, i.e. 72.36%) than gram positive cocci (21 out of 76, i.e. 27.63%). In our study the commonest isolate was Pseudomonas spp (34%), followed by Escherichia coli (27.63%) and Staphylococcus aureus (25%). Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa were predominant among the monobacterial isolates. These findings correlated well with those of Pappu K et al. who reported that 76% of the organisms which were isolated were gram negative bacilli, Pseudomonas being the predominant pathogen (23%), followed by Staphylococcus aureus (21%). Zubair et al. reported Escherichia coli (26.6%) and Pseudomonas aeruginosa (10.6%) as the predominant gram negative isolates. In the study of Benwan et al. which was done in Kuwait, they reported that more gram-negative pathogens (51.2%) were isolated than gram-positive pathogens (32.3%) or anaerobes (15.3%).

In contrast, Citron et al., Mohd Zubair et al. and Alavi SM et al. reported Staphylococcus aureus as the predominant pathogen, which comprised 57.2%, 28% and 26.2% of their isolates respectively.

M.B Girish et al. reported that 15% of the MRSA strains were resistant to Ampicillin, Cephalosporins and Gentamicin and that they were sensitive to Amikacin, Vancomycin, Teicoplanin and Linezolid but in our study we have found 36.84% of the isolated S. aureus was Methicillin resistant which is higher than reported by other workers whereas in accordance with the findings of Raja NS, vancomycin was effective against MRSA. Vancomycin resistant Enterococcus faecium is reported in our study which is dissimilar to the findings of Raja NS.

The worrisome fact is that, in this present study, multidrug resistant Gram negative strains have been isolated in a large number; 46% isolates were ESBL producers, mainly Pseudomonas and E. coli, 33.33% were AmpC β lactamase producers, mainly Pseudomonas and Klebsiella and 8% were Carbapenemase producers were exclusively Pseudomonas.

Multidrug resistant monomicrobial and polymicrobial infections profile of diabetic foot ulcer, in this present study recommends the use of Meropenem or Imipenem to treat the AmpC β-lactamase producers and use of Tigecycline and/or Colistin and/or Polymyxin-B to treat carbapenemase producers however, emergence of resistance to Tigecycline and/or Colistin is no doubt the footsteps of the devil.
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