

The Clinicobacterial study of diabetic ulcers with specific reference to antibiotic resistance

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Abstract

Diabetic ulcers are the most feared complications of Diabetes mellitus. Once in a lifetime people with diabetes will develop an ulcer which can increase the risk of lower extremity amputations. The present study was aimed to isolate the bacterial etiological agents from diabetic ulcers and to study susceptibility towards commonly used antimicrobials. Looking to present scenario of increasing prevalence of drug resistance, MDR and ESBL producers present study was carried out. During the study period, 53 clinical samples were collected and out of which 73 isolates were obtained. Polymicrobial nature of samples was found with predominance of Gram positive bacteria as leading causative agent, *Staphylococcus aureus* (68.7%). Antimicrobial susceptibility testing results indicated Linezolid and Gentamicin as the most sensitive towards Gram positive isolates while Gram negative shown most sensitivity towards Amikacin. High prevalence of MDR was found among Gram positive isolates (77%) compare to Gram negative (16%) isolates. 76% of isolates were identified as ESBL producers. Many of them were identified as biofilm producers when detected by Tube method and Micro titer plate Technique.

Keywords: Diabetic ulcers, Antimicrobial susceptibility, Biofilm, MDR

Introduction

Diabetes is a chronic metabolic disorder characterized by relative or complete insulin deficiency when pancreas does not able to produce enough insulin or when the body does not able to effectively use the insulin. This leads to defects in glucose, fat and protein metabolism. Diabetes of all types can lead to many types of complications which include heart attack, stroke, kidney failure, vision loss, nerve damage and ulcers. Out of all these, ulcers are the most worrisome problem in diabetic patients which results from neuropathy and trauma.

Diabetes patients have impaired microvascular circulation which limits the access of phagocytes which favours the development of an infection. Infection is caused due to any bacteria that came in contact with the ulcer; it may be skin flora or environmental flora. These infections constitute a major barrier to healing and can cause adverse effect on patient's quality of life. An ulcer becomes major cause for hospitalization and lower extremity amputations. About 60% infected ulcers are reported during presentation and which can increase the risk of amputations by 50% as

compared to nondiabetic ulcers (Anvarinejad M et al., 2015)

Materials and methods

Sample collection

We have studied 53 clinical samples collected from patients of diabetic ulceration from various hospitals of Surat. Samples were collected by sterile swab using Levine's technique as follows: (Alavi et al., 2014)

Exclusion Criteria

Non-diabetic patients having ulcers were excluded from the study. Obligate anaerobes and fungi were also excluded.

Sample Processing

Swab samples were carried to the laboratory as soon as possible and then were streaked on Nutrient agar, MacConkey's agar and incubated at 37°C for 24 hours. All plates were examined after 24 hours for bacterial growth. The isolated bacteria were identified by morphological, colonial and biochemical characterization using standard references (John G. Holt, Bergey's manual of Determinative bacteriology, 9th edition, and Jean F. Macfaddin, Biochemical Tests for Identification of Medical Bacteria, 3rd Edition).

Storage of isolate

Isolates were stored on Nutrient agar Slant at 4°C as working culture and as glycerol stock as stock culture. .

Antimicrobial Susceptibility Test: (CLSI & WHO, 2011).

The isolates were further tested for their antimicrobial susceptibility towards commonly used antibiotics with known concentration (commercially available) was used. Antibiotic susceptibility tests were performed by Kirby-Bauer disk diffusion method.

Determination of Prevalence of MDR

Multidrug resistance is antimicrobial resistance shown by microorganism to multiple antimicrobial drugs. Such microorganism mostly result into therapeutic failure an even spread the resistance among other species of bacteria by horizontal gene transfer. The isolate that show resistance against more than two group of antimicrobials were considered as MDR.

Determination of frequency of ESBLs producers (Jacob & Prince 2005 & CLSI 2007)

The worldwide prevalence of extended spectrum beta lactamase producing *Enterobacteriaceae* is increasing and knocking the necessity for optimization detection technique. For screening of ESBL producer, cephalosporin alone (ceftriaxone, cefoperaxone) and in combination with clavulanic acid were applied. The inhibition zone around the cephalosporin disc combined with clavulanic acid was compared with the zone around the disc with the cephalosporin alone. The test was considered positive if the inhibition zone diameter was >5mm larger with clavulanic acid than without.

Detection of biofilm formation

Bio film formation capabilities amongst all clinical isolates were detected by two methods: Tube Method(TM) (Christensen et al., 1982) and Microtiter Plate (MTP) method (Christensen et al., 1985)

Results

A total non-repetitive, 73 clinical isolates has been isolated from 53 diabetic ulcers samples (many of them were polymicrobial) that have collected from the various hospitals in Surat, Gujarat. All isolates studied for their morphological, colonial and biochemical characteristics using standard references (John G. Holt, Bergey's manual

of Determinative bacteriology, 9th edition, and Jean F. Macfaddin, Biochemical Tests for Identification of Medical Bacteria, 3rd Edition).

Table 1: Distribution of Isolates as per Gram Reaction.

Isolates	Frequency
Gram positive isolates	48
Gram negative isolates	25

As represented, Gram positive isolates predominated (65.75%) in compare to Gram negative (34.25%)

Table 2: Prevalence of Isolates.

Microorganisms	Number
<i>Staphylococcus aureus</i>	33
<i>Streptococcus pyogenes</i>	11
<i>Streptococcus pneumoniae</i>	4
<i>Pseudomonas aeruginosa</i>	11
<i>Escherichia coli</i>	8
<i>Klebseilla pneumoniae</i>	4
<i>Proteus vulgaris</i>	2

In present study, *Staphylococcus aureus* found with higher frequency of 68.7% while *Proteus vulgaris* found as least with prevalence of 8%.

Antibiotic susceptibility pattern of Gram Positive Isolates: Linezolid and Gentamycin were found most effective while Lincomycin and Roxythromycin as resistant antimicrobial against most Gram positive isolates (Fig:1).

Antibiotic Susceptibility Pattern of Gram Negative Isolates: As represented in figure 2, Amikacin found most sensitive while, Gatifloxacin as most resistant towards gram negative isolates.

Prevalence of MDR

Multi Drug Resistance is one of the prime causes of therapeutic failure. We found higher prevalence of MDR among Gram positive isolates (77%) compare to Gram negative (16%).

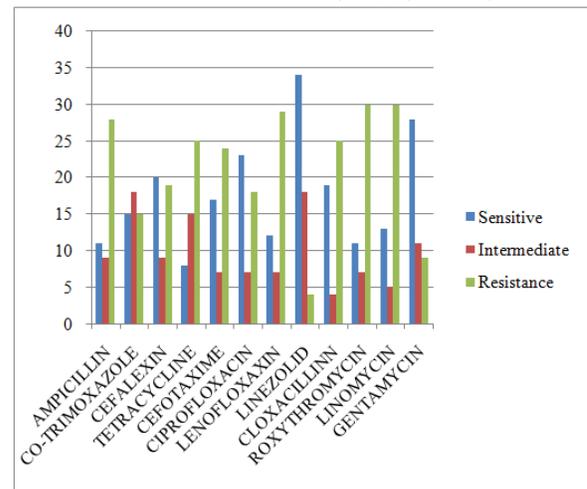


Figure 1: Antibiogram of Gram Positive isolates.

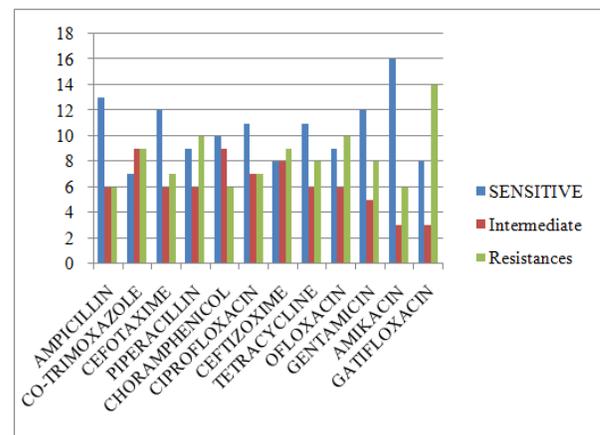


Figure 2: Antibiogram of Gram negative isolates.

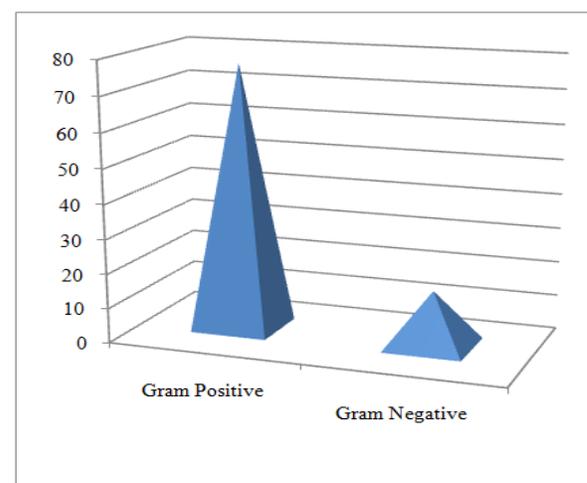


Figure 3: Graphical Representation of MDR.

Frequency of ESBLs producers: Extended-spectrum β -lactamases (ESBLs) producers are posing a major therapeutic challenge today in the treatment.

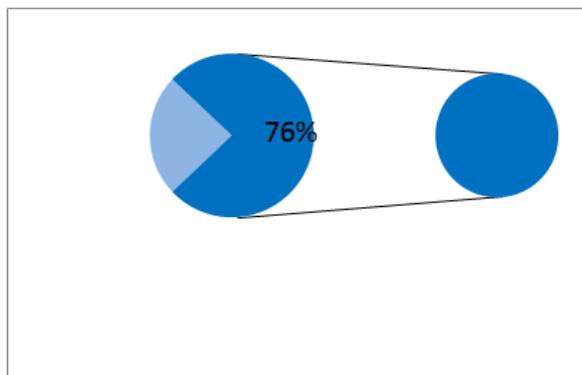


Figure 4: ESBL producer among Gram negative isolates.

Prevalence of Biofilm Producers

Biofilm producing microbial flora manifests an altered growth rate and transcribes genes that provide them with inherent resistance to antimicrobials are reported to be a major factor contributing to severity of infections.

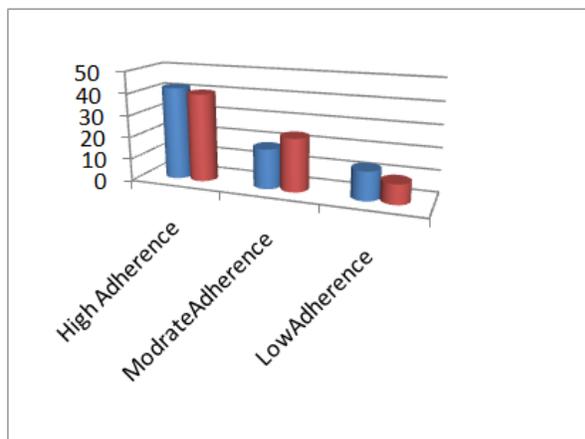


Figure 5: Graphical Representation of Biofilm formation.

In our study, the prevalence of biofilm producers were detected by Tube method and Microtitre method and high prevalence of Biofilm producers was found. By Tube method, 42 isolates while by microtiter

method, 40 isolates were found as high biofilm producers.

Discussion

Infections in diabetic ulcers worsen healing conditions in diabetic patients. Thus early diagnosis and effective treatment is necessary which requires the knowledge of etiological agents responsible for delayed healing and their sensitivity and resistance patterns.

As represented in our study 53 samples were collected from patients having ulceration which found to be 65.7% gram positive isolates among which *Staphylococcus aureus* was found to be most commonest pathogen 68.7% and 34.2% Gram negative isolates among which *Pseudomonas aeruginosa* was found 44%. Dibya Prasana et al in 2017 shows 40% Gram positive isolates and 54.1% Gram negative isolates, but predominant organisms are found to be 61.1% *Staphylococcus aureus* followed by 26.1% *Pseudomonas aeruginosa*.

Antimicrobial susceptibility test was carried out from which we found Linezolid and Gentamycin were found to be most sensitive against Gram positive bacteria while Lincomycin antibiotic resisted mostly by our gram positive isolates. Amikacin antibiotic were found to be most sensitive against Gram negative bacteria while Gatifloxacin antibiotic resisted mostly by our gram negative isolates. In contrast, Sharvari et al in 2017 reported aminoglycosides and fluoroquinolones effective against gram negative isolates and tetracycline and oxazolidinone shows effective sensitivity against gram positive isolates.

On seeing the increasing prevalence of multidrug resistance among bacteria we estimate the multidrug resistance among isolated pathogens which found highest MDR found (77%) in gram positive while among gram negative it was 16%. Study conducted by Shashikala V et al. in 2017

show multidrug resistance among 97.56% isolates which is in contrast to our study.

ESBL producers are most commonly associated with therapeutic failure and there for poor outcome of infections. And all gram negative isolates were tested for ESBL. 76% isolates found to produce ESBL. In previous study, K. Manasa Saraswathy et al., 2017 reported 68.8% emergence of ESBL among their isolates.

In the present study 57.5% of isolates show high biofilm capability, 24.6% of moderate and 17% are low biofilm formation capability. As represented high adherence 57.5% was found by Microtitre Plate method while 54.5% was found in the Tube Method. Similar study carried by Shashikala V et al., 2016 which shows 46.7% of isolates showing biofilm formation capabilities.

Conclusion

Infected ulcers in diabetic patients are the major complications of Diabetic Mellitus (DM) which leads to the patient's quality of life, hospitalization and lower extremity amputations, thus lead our interest to carry out this study. In the present study, 50 samples were collected from which 73 isolates were isolated which are the most common organisms in the diabetic patients having ulcers. They were found to be Gram positive aerobes as well as Gram negative aerobes, among which *Staphylococcus aureus* (66.7%) were found to be most common pathogen followed by *Pseudomonas aeruginosa* (44%). By performing antibiotic susceptibility tests it was found that Gram positive bacteria were most sensitive to Linezolid and Gentamycin while gram negative bacteria were sensitive to Amikacin and Gentamycin. Prevalence of MDR organisms was observed, which became an important concerning factor. On seeing emergence of ESBL producers, gram negative isolates were checked for ESBL test among which 76% isolates were found

to be ESBL producers. Biofilm formation capabilities were also detected which gives highest biofilm producers among isolated organisms.

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