5. Discussion

This study presents a comprehensive Clinico-microbial profile of infections in diabetic foot ulcers of hospitalized patients in a tertiary care hospital with special reference to Extended-spectrum β-Lactamases in *Enterobacteriaceae* isolates.

With the rise in the prevalence of diabetes mellitus globally, more so in developing countries, there is increasing problem of infections among patients especially diabetic foot ulcers which according to some studies account for 20% of hospital admission (Lavin et al., 1998). As multidrug resistance is a growing problem because of frequent use of antibiotics by primary care physicians, efforts were made to study the profile of infection in diabetic foot ulcer patients and to see the associations of different study characteristics with the presence of MDR organisms.

In this study, data obtained from all the patients were evaluated and various risk factors for diabetic foot ulcer assessed *(Table 23, Table 41)*. **Male gender** predominance is consistent with many earlier studies like that of Edgar et al. [2005], Abdulrazak et al. [2005], Gadepalli et al.[2006], Ako-Nai et al., [2006], Raja et al., [2007], Kiziltan et al. [2007], Ghanassia et al. [2008], Bansal et al [2008], Hokkam, [2009], Aziz [2010], Xiang Li et al. [2011]. It is possible to suggest that males are more liable to get foot trauma because of outdoor activities [Norris, 1992, Gadepalli et al., 2006, Ako-Nai et al., 2006, Raja et al., 2007, Kiziltan et al., 2007]. The average age in this study was in a range from 41-80 years in majority of cases, which is in accordance with studies reported by many workers [Apelqvist and Agardh, 1992, Boyko et al., 1999, Abdulrazak et al., 2005, Gadepalli et al., 2006, Ako-Nai et al., 2006, Raja et al., 2007, Kiziltan et al., 2007, Ghanassia et al., 2008, Bansal et al., 2008, Hokkam, 2009]; it may be due to higher life span in this region. The reported average duration of the diabetes was higher than the figures reported by Littzelman et al. [1993] but consistent with Oyibo et al. [2001], Ako-Nai et al., [2006], Gadepalli et al., [2006], Ghanassia et al., [2008], Bansal et al., [2008], Hokkam et al., [2009] and Xiang Li et al., [2011]. In the present study risk factor for foot ulcer was 1.28 in those whose duration of diabetes was > 10 years *(Table 41)*, which is similar to the studies conducted by Leymarie et al., (2005), Edger et al., (2005), El-Shazly et al., (2002).
The size of ulcer (>4 cm²) was found to be a significant risk factor with an OR 2.61 (Table 41). The ulcer size >4 cm² was associated with factors like multidrug resistance infection, neuropathy [Gadepalli et al., 2006]. The greater the area, the longer the time taken by the ulcer for healing (Oyibo et al., 2001). In accordance with our study results, Winskley et al., [2007] also found patients who underwent longer duration of healing had increased ulcer size.

The presence of chronic sensory/motor peripheral neuropathy was found to be a predictive risk factor (Table 41, Table 42, Table 43) in the present study. Neuropathy and ischaemia, two important complications of diabetes mellitus were found to be underlying risk factors for the development of foot ulcers. Patients living in the rural areas and those with poor socio-economic status often walk bare-foot, have got poor knowledge of foot care and lack of foot examination and care by the physicians put them at risk for development of foot ulceration. The observations are in accordance with the reports of Nelson et al., (1998); Chowdhary et al., (2000) and Leymarie and Richard (2005). Further, it has also been observed that poor glycemic control and anaemia increases the risk for development of foot complications similar to the observations of Edger et al., (2005); Al-shazly et al., (2002); Boyko et al., (1999) and Bresater et al., (1996). Poor glycemic control was significant risk factors for poor outcome in the present study as reported by other worker [Adlere et al. 1983, Lehto et al., 1996, Resnick et al., 2004, Gadepalli et al., 2006].

A significant risk of association was also observed with prior history of foot ulcer as reported by Abbott CA et al., (2002) with an OR 2.57 and Kumar et al., (1994) with an OR 12.7. In the present study, greater bodyweight and past history of foot ulcer were not a significant risk factor for diabetic foot ulceration which is in similar to the reports of Oyibo et al., (2001) and Bresater et al., (1996), however, a positive association between these factors was reported by Boyko et al., (1999).

Increased Leukocyte Count (WBC) count was found to be an independent risk factor for foot ulceration in the present study by OR & RR (Table 41), pearson correlation (Table 42) and multistep linear regression analysis (Table 43). In accordance with present observation, Yesil et al., [2009] also found increased WBC counts at baseline to be an independent risk factor for foot ulceration. Recently WBC count >11,000 cells/μl
in a severe diabetic foot ulcer patients was reported to be single most important predictive marker for poor clinical response [Fleischer et al., 2011].

The finding of a positive association between smoking (Table 41) and risk is consistent with the findings of Gadepalli et al., (2006) and inconsistent with findings from a study of Pima Indians [Nelson et al., 1998] and Gurlek et al., [1998]. Smoking was an independent risk factor for increased risk of amputation also in diabetic patients (Moss et al., 1999). A likely explanation for our finding is that smokers in this part of India (North), smoke higher number cigarettes per day than the national average.

Nephropathy was found to be significant factors by OR & RR (Table 41), pearson correlation (Table 42) and multistep linear regression analysis (Table 43). Studies conducted by Resnick et al., [2004] and Nelson et al., [1998] also found nephropathy to be a significant prognostic factor. However, Gurlek et al., [1998] did not find nephropathy to be a significant predictive factor. We also observed that reduced Creatinine Clearance (CCre) was associated with impaired wound healing in diabetic foot ulcers (Table 46 & 47). Diabetic nephropathy has been proposed as a risk factor for diabetic foot ulcer development [Griffith and Wieman, 1990]. The frequency of foot ulcers increases with the progression of nephropathy [Alebiosu et al., 2003; Guerrero and Rodriguez, 1998; Schleiffer et al., 1998]. Renal failure has been associated with poor outcome in patients with diabetic foot ulcers [Papanas et al., 2007]. Aulivola et al. [2005] reported that wound healing was inferior in patients with renal failure who underwent infra-popliteal angioplasty. Johnson et al. [1995] reported that failure of foot salvage in patients with end stage renal disease and critical ischemia after surgical revascularization was due to wound healing problems rather than graft thrombosis. Another study showed a significant association between end stage renal disease and the failure of trans-metatarsal amputations to heal [Pollard et al., 2006]. Although majority of evidence has pointed out the ischemia as the underlying problem which led to ulcer development and poor healing in the presence of the ulcer in diabetic patients with renal failure, neuropathy is particularly common, possibly aggravated by a component of uraemic neuropathy [Yasuhara et al., 2008; Margolis et al., 2008; Yasuhara et al., 2002]. The present study clearly showed that wound healing was associated with the decrease in CCre in patients with diabetic foot ulcers. Experimental studies have
demonstrated that uraemia impairs wound healing [Papanas et al., 2007; Yue et al., 1986; Colin et al., 1979; Kursh et al., 1977].

Similarly, retinopathy was also found to be a significant factor in our study (Table 41), similar to findings by Gadepalli et al., (2006) and whereas Gurlek et al.,[1998] didn’t report this. In Pearson correlation analysis (Table 42) retinopathy was found to a significant factor. No significant relationship was found between the amputation and presence of retinopathy (Table 45) whereas, a positive significant association was observed by Nelson et al., (1988), Reiber et al., (1992), Lee et al., (1993), Selby and Zhang, (1995), Mayfield et al., (1996), Lehto et al., (1996), Moss et al., (1999) and Hamalainen et al., (1999).

The rate of amputation varies significantly in different regions of the world. In USA, the Strong Heart Study has reported only 4.4% amputation in their 8 years follow-up of 1,974 DFU patients [Resnick et al., 2004]. In India, Vishwanathan and Kumpatla in their multicentric study have reported high prevalence of amputation rate of 65.2%, which include 3, 4, 3 and 21 study centres from North, East, West and South India respectively [Vishwanathan and Kumpatla, 2011]. In the present study, the overall amputation rate was 28.4% which is much less compared to report of Viswanathan and Kumpatla from South India [2004] which was probably achieved by well-organized multidisciplinary foot care teams, good glycemic control, offloading, debridement and proper selection of antibiotics and by educating patients on foot care in the present study. The risk of amputation was associated with male sex, a consistent finding in at least two previous prospective studies in American Indians. In the Oklahoma Indian Diabetes Study [Lee et al., 1993] (n= 875), risk of incident in men was twice than in women, and in a study of 4,399 Pima Indians, the rate of amputation in men was 2.6 times higher than in women, adjusted for age and diabetes duration [Nelson et al., 1998]. However, the observation that men are at higher risk is not a universal finding. In a national study of 20-year risk of amputation, sex did not predict amputation [Resnick et al., 2004]. One reason for the absence of a sex effect in the latter study may be due to the fact that it included a mix of individuals with and without diabetes, potentially masking an important sex effect in studies exclusively of individuals with diabetes. Several factors might explain the persistent observation that men with diabetes experience more amputation(s) than women with diabetes. It is possible that men experience more minor trauma to the foot
that ultimately results in amputation in view of their outdoor activities, walking barefoot especially among rural subjects in India.

Several risk factors for amputation among the patients with diabetes have been cited in the literature including age [Resnick et al., 2004], male sex [Lee et al., 1993, Nelson et al., 1988], size of ulcer [Oyibo et al., 2001], hypertension [Lee et al., 1993, Nelson et al., 1988], neuropathy [Nelson et al., 1988], nephropathy [Boulton et al., 2005, Resnick et al., 2004, Nelson et al., 1988], poor glycemic control [Resnick et al., 2004], white blood cells [Yesil et al., 2009, Fleischer et al., 2011] and lipid abnormalities [Nordesrgaard et al., 2007, Chaturvedi et al., 2001]. Although there are inconsistencies among studies regarding all the various risk factors for amputation, PVD was identified by different studies as an independent risk factor. In our analysis, presence of PVD (Table 44) also led to a significant higher rate of amputations [P=0.02, OR 6.95; RR 2.5], which was comparable with previous studies. The development of a foot ulcer was considered to be the result of multiple factors including peripheral neuropathy, foot deformity, external trauma, PVD and peripheral oedema [Boulton et al., 2005]. Most important risk factor for amputation reported from India was infection, glycemic control, duration of diabetes, vascular complications [Vishwanathan and Kumpatla, 2011, Chaturvedi et al., 2001]. Adequate blood supply was vital for ulcer healing and combating the ulcer infections. However, patients with diabetes had a higher prevalence of PVD than patients without diabetes [Brasil, 1993]. Thus, it was not surprising that PVD was associated to be one of the important risk factors with amputation.

Diabetes typically exhibited mixed dyslipidemia characterized by elevated triglycerides and low level of HDL-C. These lipid abnormalities and high serum cholesterol level have proven to be a major risk factor for cardiovascular risk and have commonly been linked with worst outcome [Nordesrgaard et al., 2007]. In the present study (Table 41), the levels of triglycerides (>150 mg/dl), cholesterol (>150 mg/dl), LDL-C (>100mmol/l), HDL-C (<40mg/gl) were associated with the risk of risk of diabetic foot ulcer as also reported by Chaturvedi et al [2001].

Other significant risk factors were type of infection in foot. In the present study, subcutaneous and osteomyelitis infection were significantly associated with the risk of amputation (Table 44). However osteomyelitis was found to be a significant prognostic
factor [Gurlek et al., 1998]. Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In the present study polymicrobial etiology was found in 68.5% and monomicrobial in 31.4% patients (Table 25) with the rate of isolation of about 1.82 bacteria per patient which is lower than the previous studies [Gadepalli et al., 2006, Gerding, 1995] which showed rate of isolation between 2.3% -5.8%. The major infective organisms in diabetic foot ulcers in our series of patients appear to be different. We found gram negative aerobic bacteria as most frequently isolated organisms (Table 27) which is in accordance with previous reports [Ramakant et al., 2011, Gadepalli et al., 2006]. However, the studies from western countries show that gram positive aerobes are the predominant organisms isolated from DFU [Dang et al., 2003; Citron et al., 2007; Lipsky et al., 1990; Grayson, 1995; Joseph and Axler, 1990]. The prevalence gram negative bacilli in this series of DFU unlike western countries may be because of the chronicity of ulcers also due to prior use of antibiotic therapy where gram negative bacilli especially members of Enterobacteriaceae predominates (bailey and scot). The environmental factors such as sanitary habits, e.g. use of water for peri-anal wash (ablution) after defaecation leading to contamination of hands with faecal flora, may also be responsible for the increase in the prevalence of gram negative organisms in the developing World as compared with the Western countries [Ramakant et al., 2011; Prasad et al., 2004]. In this study, the gram positive to gram negative ratio was 1:1.8 which is in similar to the findings reported earlier [Gadepalli et al., 2006]. Gadepalli et al., [2006] also reported gram negative aerobes to be most frequently isolated pathogens (28.7%), followed by 13.8% gram positive aerobes. Similar results were also reported by Ramakant et al., (2011) and Shankar et al., [2006]. Studies from Malaysia have also reported a predominance of gram negative bacteria (52%) in patients with DFU, the most common pathogens isolated being Proteus sp., Klebsiella pneumonia, E coli and Enterobacter cloacae [Raja et al., 2007].

Poor blood supply also predispose to anaerobic infections. In the present study, Peptostreptococcus sp was the most predominant one which is in accordance to the previous studies [Scher and Steele, 1988, Louie et al., 1976]. Other anaerobes isolated in their study were Bacteroides fragilis, Clostridium sp, Eggerthella lenta and Propionibacterium. Clostridium sp. was the most commonly isolated anaerobe, followed
by *Bacteroides* [Raymundo and Mendoza, 2002], we recovered fewer anaerobic species compared with earlier culture reports [Goldstein *et al.*, 1996, Viswanathan *et al.*, 2002]. However, 12.3% patients had gangrene associated with their infections. This may be an indication of fewer anaerobic species among non-threatening lower-extremity infections, which is also reported earlier [Lipsky and Berendt, 2000].

Many studies have been done on the prevalence and spectrum of bacterial infections, the role of systemic/local antibiotics, and their effect on wound healing. However, the magnitude of fungal infections in diabetic foot wounds is an area which has received very little attention. Studies have shown that toe web dermatophyte infection provides a hospitable niche for subsequent colonization by bacteria. Exacerbation of a mild dermatophyte infection (dermatophytosis simplex) can arise in the occlusive environment of the toe web space (Chellan *et al.*, 2010). Fungal infection induces damage to the stratum corneum, which allows overgrowth of resident bacteria and maceration, itching, and often malodor at the site (Kates *et al.*, 1990, Leyden, 1993). Mlinaric Missoni *et al.*, (2005) from Croatia, had reported the fungal incidence in tissue biopsy specimens of 22 diabetic patients who had clinical evidence of fungal infections. In their study, predominant isolates were *C. parapsilosis* (45.5%), *C. tropicalis* (22.7%), *C. albicans* (9.1%), and *C. glabrata* (9.1%). Bansal *et al.*, (2008) from India had reported 9% isolation of fungi from superficial swabs taken from 103 patients with diabetic foot wounds, and the predominant species were *C. tropicalis* (29%), *C. albicans* (14%), and *C. guilliermondii* (7%), followed by *Aspergillus flavus* (21%), *Aspergillus niger* (14%), and *Fusarium* species (14%). The same spectrum of fungi was isolated from immunocompromised patients’ blood by Pfaller *et al.*, (2008) and Bedini *et al.* (2006). Though these studies confirm pathogenic fungal infections in DFU, the spectrum of fungi and their prevalence in wounds have not been explored properly. In this study, the prevalence of fungi was 6.25%, among these, 75% were yeasts and 25% were filamentous fungi (Table 27). The isolates obtained in this study were similar to the spectrum of species isolated from bloodstream samples by Abdulrazak *et al.*, (2005), Raja, (2007), Gonzalez *et al.* (2008), Bansal *et al.*, (2008), and Tascini *et al.*, (2011).

There is a strong association between heavy antimicrobial consumption within a population and the frequency of recovery of resistant bacteria [Enne *et al.*, 2001]. Cars
et al. [2001], compared the non-hospital use of antibiotics in the 15 member states of the European Union in 1997 and found that in 11 countries, the most commonly used antibiotic was broad-spectrum penicillin. Data on antibiotic sales are not publicly available in many countries, including India. The overall resistance in our patients was high (Figure 15). The Gram-negative isolates showed high rates of antibacterial resistance. The reason may be the indiscriminate use of antibiotics in the community (Ramakant et al., 2011), another possible reason could be that most of our patients received some antimicrobial treatment before presenting at our centre from the referring hospitals using a combination of different antimicrobials empirically. And also the variations in the infection control policies, different climatic conditions, and other unrecognized factors [Hart and Kariuki, 1998] play a significant role.

The frequent or unnecessary uses of antibiotics result in a selection favouring resistant bacteria. In this study, history and discharge summaries showed that overwhelming majority (40.1%) of the diabetic foot ulcer patients who were referred to our centre had received an antibiotic treatment before (Table 23). However, in rest of the cases, the antibiotic treatment history could not be retrieved because most of our patients were illiterate, and did not know which antibiotic was prescribed and in what doses.

Resistance to antibiotics is seen when they are used for a prolonged period of time. This resistance is an acquired form rather than an intrinsic one. The former develops following a mutation in the DNA of a microorganism or by acquisition of a new DNA. Acquisition of new DNA is accomplished by genetic elements such as plasmids or transposons. Resistance plasmids may have approximately 10 resistance genes for various antibiotics. Bacteria can transmit these characteristics to other bacteria [Gold and Mollering, 1996, Murray, 1991]. Infections of these foot ulcers require a longer duration of treatment with antibiotics and the use of an appropriate antibiotic in an appropriate dosage [Raymakers et al., 2001]. Insufficient blood flow which causes ischemia further hinder penetration of antibiotics into the wound and therefore, a delay in wound healing occurs [Parkhouse and LeQuesne, 1998].

In our study, 78.3% of isolated S aureus were methicillin resistant by using 1µg oxacillin disk and 68.3 % of isolated S aureus was resistant to 30µg cefoxitin disk. Cefoxitin disc shows high percentage of sensitivity over oxacillin disc. The prevalence of MRSA production were
higher (Table 31) in our population as compared with previous studies (Boyko et al., 1999, Hartemann Heurtier et al., 2004, Gadepalli et al., 2006, Ako-Nai et al., 2006, Raja et al., 2007, Kiziltan et al., 2007, Ghanassia et al., 2008, Bansal et al., 2008). None of the gram positive isolates were resistant to vancomycin (VRSA). These observations are important, especially for patients’ management and deciding the antibiotic treatment policies.

Members of family Enterobacteriaceae are an important group in community and hospital-acquired infections. They are common precipitants of sepsis by virtue of the inflammatory response, activated by endotoxins present in the Gram-negative cell wall. Patients with diabetes mellitus and dialysis patients are at high risk for enterobacterial infection. Unfortunately, resistance has become increasingly common among gram-negative bacteria, making empirical therapy decisions more difficult. The most serious resistance patterns now emerging among Gram-negative organisms include resistance to extended-spectrum cephalosporins and penicillins [Diekema et al., 1999]. This resistance is commonly mediated by ESBLs in Escherichia coli and Klebsiella species, or by the hyper production of chromosomally mediated cephalosporinases (Bush group I AmpC enzymes) in Serratia and Citrobacter species [Cars et al., 2001]. The ESBL genes generally result from point mutations in the genes of broad-spectrum b-lactamase Ambler class A enzymes, such as TEM-1, TEM-2 or SHV-1. They are usually located in conjugative megaplasmids, which often carry genes responsible for resistance to other antibacterial drugs, making it extremely difficult to treat infections caused by bacteria that produce these enzymes [Livermore, 1995].

Along with ESBLs, plasmid-mediated Ambler class C cephalosporinases (or Bush group 1 cephalosporinases) have also been found in clinical isolates of the Enterobacteriaceae. These enzymes can produce resistance to cephemycins, extended spectrum cephalosporins and aztreonam, and unlike class A ESBLs, β-lactamase inhibitors do not inhibit these bacteria [Bush et al., 1995]. The resistance pattern in our study was similar to the recent studies done in India and outside [Ramakant et al., 2011, Raja, 2007, Gadepalli et al., 2006, Shankar et al., 2006]. In the present study, on screening for ESBL, 74.2% of gram negative DFU isolates were ESBL positive and 67.8% ESBL producers tested positive with β lactam inhibitors in a confirmatory test (Table 35 & 36), similar to the reports of Mathur et al., from India [Mathur et al., 2002]. Babypadmini et al [2004] also showed that 40% of K. pneumoniae isolates and 41% of E. coli isolates were
ESBL producers in their study cohort. Gadepalli et al., [2006] have reported 54.5% E. coli isolates to be ESBL producers in diabetic foot infections. A study in Brazil reported, the prevalence of ESBL only in 6% among E. coli isolates [Armstrong et al., 2004]. In a recent study Shobha et al., (2009) have reported 27.3% Klebsiella pneumonia, 25.2% Escherichia coli, 21.42% Pseudomonas sp, 25% Enterobacter sp and 17% Acinetobacter sp to be ESBL producer.

The identifications of the genes involved in ESBL mediated resistance are necessary for the surveillance and epidemiological studies for their transmission in hospitals. Recently, a number of molecular methods have been proposed for the identification of TEM, SHV and CTX-M derivatives. Multiplex PCR can be used to screen blaCTX-M, blaTEM and blaSHV. Edelstein et al (2003) detected 15.8% E.coli and 60.8% K. pneumoniae isolates as CTX-M ESBL positive; of which 93% were CTX-M-1 and 7% were CTX-M-2 type enzymes. In India, a variant of CTX-M-3 enzyme, designated CTX-M-15, was reported from six unrelated members of family Enterobacteriaceae [Bonnet, 2004]. In South India, Babypadmini et al., (2004) studied a total of 411 isolates (353 E. coli and 58 K. pneumoniae) and it was found that 41% of E. coli and 40% of K. pneumoniae were ESBL positive. In another study, 14 of 39 selected isolates were positive for blaCTX-M gene by Sekar et al., [2006]. The prevalence of blaCTX-M was found in 77.4% of the E. coli isolates which were found to be CTX-M group-1 positive by PCR in a North Indian study [Shahid et al., 2006]. Another study from north India reported the prevalence of ESBL as 63.6 per cent in E. coli and 66.7% in K. pneumoniae isolates [Goyal et al., 2006]. Overall CTX-M was the commonest (54.3%) genotype, 97.2 percent of them belonged to CTX-M-1 cluster. On the sequence analysis all 20 CTX-M-1 cluster matched with CTXM-3 subtype [Goyal et al., 2006].

In the present study, occurrence of ESBL in the bacterial isolates was substantiated by blaCTX-M, blaTEM and blaSHV in DFU isolates using PCR to find out true resistance to antibiotics. The bla gene positivity was 89.3% in which, blaCTX-M positivity was higher (81.8%) followed by blaTEM(50.0%) and blaSHV (46.9%). We also found higher blaCTX-M, blaTEM and blaSHV positivity in bacteria isolated from DFU patients. The most prevalent ESBL gene was blaCTX-M (81.8%), followed by 50% blaTEM and 46.9% blaSHV in the cefotaxime resistant Enterobacteriaceae member (E coli & Klebsiella sp).
isolated from DFU patients (Table 38). The genotypic methods helped us to confirm the genes responsible for the production of ESBLs in a single isolate.

Univariate analysis for the factors which were associated with the bla gene (CTX-M, TEM, SHV) positivity in cefotaxime resistant Enterobacteriaceae member (E coli and Klebsiella sp) isolated from DFU patients was summarized in table 48. The duration of infection more than 1 month, hospital stay (>1 month), nature of ulcer (necrotic), T2DM, history of previous antibiotic use, dyslipidemia [LDL-C (>100mg/dl), HDL-C(<40 mg/dl), triglycerides (>200mg/dl), and total cholesterol (>150mg/dl)] were the independent predictors of bla gene positivity (Table 48).

Presence of vascular disease characterized by disrupted micro- and macro-circulations cause a delay in wound healing in diabetic patients [Defronzo et al., 2004; Calvet and Yoshikawa, 2001]. Disruption of wound healing results from a decreased blood flow into the ulceration and an aberrant expression of growth factors and cytokines as well. These factors, which delay wound healing, cause foot ulcers. Infections of these foot ulcers require a longer duration of treatment with antibiotics and the use of an appropriate antibiotic in an appropriate dosage [Raymakers et al., 2001]. In fact, we found that ESBL positive bacteria were more frequently isolated in our cases. Although this increase was statistically not so significant, we believe that a multicentric study with large number of cases would positively affect the significance.

There are some limitations in this study. Information on neuropathic assessment, degree of blood pressure control, lipid controls and assessment of chronic glycemia in the past was not available for all the subjects. To what extent estimates of these risk factors assessment have influenced the risk factors is difficult to estimate. The observation in the present study also suggests that CCre an important factor affection wound healing in patients with DFUs. DFUs healing was worst in patients with decreased CCre than in those who had normal CCre. Other factors associated with poor outcome were higher grade of foot ulcer, infection type (subcutaneous and osteomyelitis. Amputation rate were also found to be higher in those patients with poor renal functions.

Further the high rate of antibiotic resistance may be due to the fact that being a tertiary care hospital referring hospitals/physicians have already tried various antibiotics to
control infection using combinations of different antimicrobials. The facilities for microbiological studies at first contact physicians/ surgeons are usually not available at district hospitals/ smaller cities in India. Furthermore, the indiscriminate use of antimicrobial therapy without establishing the etiology is a common practice. All these factors are disconcerting because infection with these organisms limits the choice of antibiotic treatments and may lead to worst outcome. These observations are important for patients management and underscore the need for institutional infectious control committee to developed antibiotics treatment policies.

The strong points and conclusion of this study.

The observations of the present study are important because it shows the profile of amputation in a north Indian tertiary care hospital. Infection was found to be the major cause of amputation. The risk factors for the amputation include presence of peripheral neuropathy, co-morbid conditions like nephropathy and dyslipidemia, elevated WBC, high grade of ulcer and bacterial infection type (subcutaneous and osteomyelitis). An association between moderate CCre (30-90 ml/min/1.73m²) and severe CCre (≤30 ml/min/1.73m²), and an increased risk for the onset of diabetic foot ulcer, poor healing and amputation were established in this study. It is important to note that demonstrating an association is not the same as showing causation, which often requires an experimental design such as a randomized clinical trial and the demonstration of a common mechanism that causes chronic kidney disease (CKD) and failure of the skin to heal. It is likely that CKD and DFU among those with diabetes are associated more tightly than was recognized previously. Clinicians should be aware of the fact that diabetic patients with foot ulcers who have impaired kidney functions are at increased risk for poor wound healing and amputation even in absence of limb ischemia. Noted that, introduction of automatic reporting of eGFR each time a test for serum creatinine concentration is requested will increase the awareness of significance of kidney dysfunction in clinical practice and appropriate measures will help in improving the prognosis. The present study, unlike in West, concludes that gram-negative bacteria dominated in DFU patients suggesting thereby that all DFU patients admitted to a tertiary care hospital in India require empirical therapy for gram positive as well as gram negative also. The treatment modes can be modified depending on the severity of infection, microbiological culture and the antimicrobial sensitivity reports. A
detailed knowledge of the susceptibility to antimicrobial agents is necessary to facilitate the development of effective strategies to combat the growing problem of resistance especially in the ESBL producing strains. The prevalence of MDR in *Enterobacteriaceae* organisms is alarmingly high in the diabetic foot patients. In India because of indiscriminate use of antibiotics, there is an urgent need for periodic antibiotic resistance surveys to help and orient the physicians and the local population on the best treatment strategies. However, chloramphenicol and Carbapenems can be used as a reserve drugs in infections refractory to DFU with conventional drugs. For ESBLs, phenotypic methods are only screening methods for detection in a routine laboratory. The genotypic methods help to confirm the genes responsible for ESBL production. Sometimes multiple genes are responsible for production of ESBLs in a single isolates. Multiplex PCR for the detection of *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> gene in ESBL producing bacteria provides an efficient, rapid differentiation of ESBLs in selected species of *Enterobacteriaceae* and can be used as a rapid tool for epidemiological studies among ESBL isolates.

A multicentric study is needed to study the prevalence of other Class of ESBLs (Class B & Class D) from Enterobacteriaceae member isolated from Diabetic Foot patients. A policy should be framed in every hospital to decide the antibiotic therapy depending upon the sensitivity pattern and emphasis should be given to follow a guideline in this regard. It will also be desirable to educate the primary care physicians regarding the type of bacteria isolated and sensitivity pattern of the antibiotics and the same should be revisied periodically. Data regarding the use of antibiotics by primary care physicians before referring to the tertiary care hospital if available will also be helpful in the management of diabetic foot patients.