1. INTRODUCTION

India has a diabetic population of about 50.8 million, which is expected to increase to 87 million by 2030 [Shaw et al., 2011]. Studies report that 15% of all diabetic patients develop a foot ulcer at some point in their lifetime and around 28% of them may require some form of amputation [Boulton et al., 2005, Reiber et. al., 1998]. Foot ulceration is common and occurs in both type 1 and type 2 diabetes (Boulton & Vileikyte, 2000). Thus, Joslin, who wrote in 1934 that “diabetic gangrene” is not heaven-sent, but earth born, was correct. The development of foot ulceration results from the way we care for patients or the way in which patients care for themselves. Foot infections in persons with diabetes are common, complex and costly problem. In addition to causing severe morbidities, they now account for the largest number of diabetes–related hospital beds–days (Reiber, 1996) and are the most common proximate, non–traumatic cause of amputations (Reiber et. al., 1999; Crane & Warber, 1999). The presence of peripheral neuropathy, peripheral vascular disease, and poor glycemic control in conjunction with minor foot trauma increases the likelihood that patients with diabetes will develop foot ulcers. Ulcers, in turn, often progress to infections of the surrounding tissue, osteomyelitis, and amputation (Reiber et al., 1992, Abbott et al., 2005).

The etiology of diabetic foot ulcers usually has many components. (Frykberg et al 2000; Boyko et al., 1999). A multicentric study (Reiber et al 1999) attributed 63 percent of diabetic foot ulcers to the critical triad of peripheral sensory neuropathy, trauma, and deformity. Other factors in ulceration are ischemia, callus formation, and edema. Although infection is rarely implicated in the etiology of diabetic foot ulcers, the ulcers are susceptible to infection once the wound is present. Many of the risk factors for foot ulcer are also predisposing factors for amputation, because ulcers are primary cause leading to amputation (Armstrong DG et al 1998; Boyko EJ et al 1999; Pecoraro RE et al 1990).

The three components of neuropathy–sensory, motor, and autonomic – may contribute to ulceration in the foot. Sensory neuropathy reduces the perception of painful, thermal and vibration mobilities, and neuropathic injury results mainly from loss of pain sensation. Damage is occasionally inflicted by acute trauma, more often by foreign bodies inside the shoe or the daily repetitive trauma of walking, especially if the posture of the foot is
abnormal or if the shoes fit badly. Loss of joint – position sense may also disturb foot posture (Dyck et al., 1987, Sharma et al., 2002). Moreover, damage to the motor innervations causes weakness and wasting of the small (intrinsic) muscles of the foot, and the resulting imbalance between the flexor and the extensor muscles leads to clawing of the toes, prominence of the metatarsal heads and flattering of the arch. This concentrated the body’s weight on to smaller areas – typically the metatarsal heads and the heat and extensive pressure loading at these points stimulate the callus formation, which is a precursor of ulceration (Robinson et al., 1992, Sosenko et al., 1986). Overall, therefore, diabetic neuropathy plays an important role in the foot ulceration, but it must be emphasized that the neuropathic foot does not ulcerate spontaneously and the trauma in the various forms is required to produce tissue breakdown (Young, 1993, DCCT-1993).

In the pathogenesis of ulceration, peripheral vascular disease itself in isolation rarely causes ulceration (Colwell, 1986, Gadepalli et al., 2006). However the common combination of vascular disease with minor trauma may lead to ulceration. Thus, minor injury and subsequent infection increase the demand for blood supply beyond the circulation capacity, ischaemic ulceration and risk of amputation. Doppler derived ankle pressure can be misleadingly high in long–standing diabetes, but the presence or absence of a dorsalis pedis or posterior tibial pulse is the simplest and most reliable indicator of significant ischaemia.

Most of the skin injuries on the feet of diabetic patients with neuropathy occur in forefoot, with equal distribution on plantar and dorsal surface (Edmond et al., 1986), and those on plantar at the site of higher pressure areas (Veves et al., 1991, Stess et al., 1997). One of the important aspects of biomechanics study is the planter pressure examination. Defined by Armstrong et al., (Armstrong et al., 1998), a pressure of 750kPa will provide a cut-off value to differentiate between the low risk and high risk patients. The second most important aspect is foot deformity which is the key factor for injury in the foot. The high foot pressure on dorsal and plantar surface will cause most skin injury. This may lead to vulnerable areas on the foot predisposing to ulceration. Motor neuropathy, with imbalance of the flexor and extensor muscles in the foot, frequently results in foot deformity with prominent metatarsal heads and clawing of the toes. In turn, the combination of proprioceptive loss due to neuropathy and the prominence of metatarsal
heads lead to increase in the pressures and load under diabetic foot (Brownlee et. al., 2003). In the charcot process, the foot is also erythematous, hot, and swollen, but in the healed stage, these findings are absent. The third aspect is the **callus** in the foot. The **limited joint mobility also plays an important role**. The relationship of joint limitation and plantar ulceration was established in a study by Delbridge et al., (1985).

One of the strongest predictors of ulceration is a previous ulcer, or amputation of other leg. Patients with retinopathy or neuropathy are at increased risk of foot ulceration, presumably reflecting widespread microvascular damage. Several studies have shown that patients with a poor understanding of diabetes and its management are more prone to ulceration (Reiber et. al., 1992). Other risk factors include a poor social background and older age, the elderly have many-diabetes-related and other problems to contend (Gadepalli et al., 2006).

A diabetic foot infection is most simply defined as any infra-malleolar infection in a person with diabetes mellitus (Lipsky et al., 2004). Spectrum of infection includes paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis, and osteomyelitis. The most common and classical lesion, however, is the infected diabetic “mal perforans” foot ulcer. The wound results from a complex amalgam of risk factors (Caputo et al., 1994; Frykberg, 1998) because, once the protective layer of skin is breached, underlying tissues are exposed to bacterial colonization. The wound may progress to become actively infected, and, by contiguous extension, the infection can involve deeper tissues. This sequence of events can be rapid (occurring over days or even hours), especially in an ischemic limb. Various poorly characterized immunologic disturbances, especially those that involve polymorphonuclear leukocytes, may affect some diabetic patients, and these likely increase the risk and severity of foot infections (Schubert et al 1995; Gin 1993; Joshi N et al 1990; Geerlings et al., 1990). These have ranged from highlighting the occurrence of particular species (Schraibman, 1990) or groups of organisms (Wall et al., 2002), to assessing the impact of microbial populations on clinical outcomes (Madsen et al., 1996).

Knowledge of the etiologic agent(s) causing the wound infection is generally helpful in selecting definitive antibiotic therapy. Infections in patients with diabetes are difficult to treat. Cellulitis is the most easily treatable and reversible form of foot infections in
patients with diabetes. Deep skin and soft tissue infections are usually curable, but can be life threatening too and result in substantial long-term morbidity (Frykberg, 1999).

Optimal management of diabetic foot infections can potentially reduce the incidence of infection-related morbidities, the need for and duration of hospitalization, and the incidence of major limb amputation (Armstrong et al 1995; Calhoun et al 1988). Some argue that many apparently uninfected diabetic foot ulcers are actually subclinically infected—that is, they contain a high “bioburden” of bacteria (usually defined as 1105 organisms per gram of tissue) that results in “critical colonization” levels and impairs wound healing (Kingsley 2001; O’Meara et al 2001; Robson et al 1999; Bowler 2003; Edmonds et al 2004). Available published evidence does not support the use of antibiotics for the management of clinically uninfected ulcerations, either to enhance wound healing or as prophylaxis against infection (Chantelau et al 1996; Hirschl et al 1992). Antibiotic use encourages antimicrobial resistance, incurs financial cost, and may cause drug-related adverse effects. In some circumstances, it is difficult to decide whether a chronic wound is infected, such as when the foot is ischemic, has abnormal coloration or a fetid odor, has friable granulation tissue, is associated with unexpected pain or tenderness, or when an otherwise properly treated ulcer fails to show healing progress (Cutting et al 1994; Schultz et al 2003).

The microbiology of foot ulcers is usually polymicrobial and recent studies using molecular techniques have emphasized the complex etiology of these wounds (Davies 2003; Davies et al 2003). Using conventional techniques, the mean number of bacterial species per ulcer has been found to range from 1.6 up to 4.4 (Tentolouris et al 1999; Bowler et al 1999; Urbani-Rovan et al 1997; Kontiainen et al 1988).

Selection of the antibiotic regimen initially involves decisions about the route of therapy, the spectrum of microorganisms to be covered, and the specific drugs to be administered and involves choosing the definitive regimen and the duration of treatment. Initial therapy is usually empirical and is based on the severity of the infection and on any available microbiological data, such as recent culture and antimicrobial sensitivity. For severe infections and for more-extensive, chronic moderate infections, it is safe to commence therapy with broad-spectrum agents. These should have activity against gram-positive cocci (including MRSA in locations where this pathogen is common), as well as gram-negative and obligate anaerobic organisms. Several antibiotic trials involving
patients with complicated diabetic foot infections have been conducted. The lack of standardization among these trials makes the comparison of outcomes of different regimens inappropriate. Currently, no specific antibiotic regimen has proven superior for treating these diabetic foot infections. Clinical trials suggest that fluoroquinolones, cephalosporins, beta-lactam inhibitor penicillins, and carbapenems are effective. The differing definitions of infection severity and clinical end points used in these publications highlight the need to develop a consensus classification system for future studies. On the basis of the available studies, no single drug or combination of agents appears to be superior to others (Lipsky BA 1999), although the available data do not allow us to recommend any specific antibiotic regimen for diabetic foot infections.

ESBLs are usually plasmid-mediated β-lactamases belonging to Ambler molecular Class A and Bush-Jacoby-Medieros functional class 2be, and are capable of hydrolyzing oxyimino cephalosporins (extended spectrum cephalosporins) and monobactam antibiotics but not cephemycins or carbapenems, and are inhibited by β-lactamase inhibitors such as clavulanic acid (Bush K, et al., 1995). Introduction of third generation of cephalosporins (oxyimino cephalosporins) in clinical practice in early 1980s ushered a breakthrough to treat infections caused by β-lactamase producing Gram-negative bacteria (Knothe et al., 1983).

The β-lactam antibiotics are among the most widely prescribed antibiotics and are important components of empirical therapy. Because of its extensive use, resistance to drugs has become a major problem especially after the introduction of newer broad-spectrum cephalosporins, β-lactamase inhibitor / β-lactam antibiotics, monobactams and carbapenems (Ramkakant et al., 2011). The most common resistance mechanism to this important class of antibiotics is the production of the beta-lactamases by resistant bacteria. Currently there is paucity of data on extended spectrum beta lactamases (ESBL)-producing organisms from diabetic foot infections especially in this part of World (Karim et al. 2001, Grover et al. 2006; Lai et al. 2007). Infection with ESBLs may increase the duration of hospital stay, cost of management and may cause additional morbidity and mortality. In recent years, β-lactamase has been increasingly reported from many countries, including the Asian subcontinent. However, there is paucity of data on the current aspect from Indian hospitals on the isolates of diabetic foot origin. There are only few published reports covering this aspect. Furthermore, no molecular study
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regarding the occurrence and detection of this class of β-lactamase is published from our country in DFU infections. It is really worrying that we, from India, do not stand on the platform of international debate on these emerging bugs because of the paucity of Indian data, while whole of the world is actively involved in the scientific studies unraveling the burden and implementing the strategies to combat the problem.

After the recommendations, made by the Bio-Ethical Committee and Institutional Ethics Committee, following were the objective of this Doctoral Work.

- To study the microbial profile and antibiotic susceptibility pattern of organisms isolated from diabetic foot subjects based on conventional techniques.
- Detection of ESBLs by phenotypic and genotypic methods.
- To evaluate the etiopathogenesis of diabetic foot ulceration.
- To evaluate the outcome of these infections.