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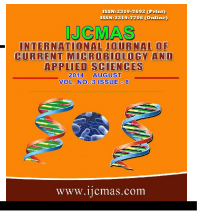

Original Research Article

A Threatening Approach of Wound Microflora to Diabetic Ulcer Foot Management

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A B S T R A C T

Foot ulcer is one of the major complications of diabetes mellitus. It can even result in morbidity and mortality due to increased infection susceptibility and impaired wound healing. In this study we have focussed on occurrence of pathogenic bacteria in diabetic foot ulcers and on their antibiotic susceptibility pattern. The infected bacteria were isolated from pus samples of 76 diabetic ulcer foot patients and identified. Antibiotic sensitivity of commonly used antibiotics like ampicillin, oxacillin, ciprofloxacin and gentamicin were done. Predominant isolates were gram-positive cocci followed by gram-negative bacilli. The organisms isolated were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Corynebacterium*, *Bacillus*, *Streptococcus*, *Lactobacillus*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli* and *Proteus vulgaris*. Even though both gram-negative and gram-positive organisms were found as β-lactam antibiotic resistant, majority of isolates were found to be sensitive to gentamicin and ciprofloxacin. The prevalence of bacterial flora in foot ulcers of this study population indicate current medical scenario of increased multi-drug resistant diabetic ulcer foot infections. Appropriate selection of antibiotics plays good role in diabetic ulcer foot management. It has been found that gentamicin and ciprofloxacin could be used for effective treatment against bacterial pathogens in foot infections under selected study population.

Introduction

People with diabetes mellitus always have an increased risk of lower limb complications like foot ulcers with increased infection susceptibility and ensuing amputations than the people without diabetes. Due to the multifactorial cause, foot ulcer is one of the major complications in the diabetic condition. The peripheral neuropathy and other micro vascular complications along with infection contributes to the development of foot ulcer in the diabetic patients (Waspadji, 2005). Diabetic foot ulcers can even result in considerable morbidity and mortality of the patient due to increased infection susceptibility and impaired wound healing. Of the total diabetic population around 15-20% will experience a foot ulcer in their life time and 2-3% of diabetic patients develop one or multiple foot ulcers every year (Ramsey et al., 1999).

A major hindrance in the controlling of diabetic foot ulcers is the colonisation of wounds by bacterial pathogens. It involves a mixture of aerobic and anaerobic organisms (Bal, 2002). Aerobic gram-positive cocci like *Staphylococcus aureus* are predominates in the infection (Lipsky et al., 2004).

Most common bacteria isolated from the diabetic wounds are *Escherichia coli, S.aureus, Enterococcus spp., Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus* spp. (Ozer et al., 2010). A variety of antibiotics is now used to treat the infected wounds. The concentration of the antibiotic and the duration of the antibiotic treatment play an important role in the wound healing process. Mechanism of antibacterial action differs from one antibiotic to another. The most common mechanism is the inhibition of cell wall synthesis and inhibition of protein synthesis. Some fight the bacteria by damaging or altering cell membranes, inhibiting nucleic acid synthesis or through anti-metabolite activity. So the antibiotic treatment has to be changed according to the microbes involved and their sensitivity to antibiotics (Wagner, 1981). The inappropriate use of antibiotics can lead to development of bacterial resistance.

This study focuses on the prevalence of different bacterial pathogens of diabetic foot ulcers and their antimicrobial susceptibility to commonly using antibiotics. Besides the bacterial pathogens, we are evaluating the current status of emergence of drug resistance in infections of diabetic foot ulcers.

Materials and Methods

Study population

The study population consisted of 76 diabetic patients with foot ulcer and healing impairment. The samples were obtained from patients of "Diabcare India", a diabetes care centre, Calicut, Kerala, India. Institutional ethical committee approval of "Diabcare India", a diabetes care centre, Calicut, Kerala, India was obtained for the study and an informed written consent was obtained from the patients before including them in this study. The patients having ulcer foot infection were selected from age group of 35 to 65. The patients with type 1 diabetes, gestational diabetes, secondary diabetes, patients with acute organ dysfunction, treatment for chronic infections like tuberculosis, leprosy etc., any organ failure like liver, cardiac or renal failure, patients with myocardial infarction, stroke etc were excluded from the study. The severity of the wound was decided with help of Wagner classification (Wagner, 1987) and the wounds coming under grade 2 - grade 5 were included in the study.

All the samples were handled and processed in aseptic conditions. The Pus sample was taken using a sterile curette from deep portion of the ulcer or by scrapping the base of ulcer and kept in sterile collection bottle in ice (Shankar et al., 2005). These specimens were immediately transported to the microbiology laboratory for further testing.

Bacterial isolation and identification

The isolation of bacteria was done by streaking on nutrient agar plates and incubating at 37^0C for overnight. The individual colonies were isolated and the identification was done according to standard medical microbiology laboratory procedures (Baron et al., 1999). Identification was done by gram staining, biochemical tests like catalase test, oxidase test, coagulase tests, novobiocin sensitivity

test, urease, citrate utilization test, triple sugar iron test, etc. (Winn and Koneman, 2006).

Antibiotic susceptibility

All the bacterial isolates were subjected to antibiotic susceptibility tests. The antibiotics tested in this susceptibility study were ampicillin, oxacillin, ciprofloxacin and gentamicin which are commonly using to treat foot ulcer infections. Ampicillin and oxacillin are β-lactam antibiotics which can act on the cell wall of gram positive and gram negative bacteria. Ciprofloxacin comes under the category of fluoroquinolone antibiotic and gentamicin is an amino glycoside antibiotic. All the antibiotic discs used were obtained from Hi-Media labs, Mumbai, India.

The in vitro susceptibility test to antimicrobial agents of each isolate was conducted by Kirby–bauer method using broth culture of bacterial isolates having turbidity of McFarland turbidity Standard No.0.5 (Wayne, 2006). For this purpose, Dehydrated Mueller Hinton Broth (MHB) and Mueller Hinton agar (MHA) (Difco Laboratories, Detroit, MI) was purchased from local market. The Mueller Hinton agar plates were inoculated with 50 µl of the inoculum. The inoculated agar plates were then incubated at 37^0C overnight. After incubation the diameter of the zone of inhibition was measured in millimetre and the results interpreted according to the criteria given by the Clinical and Laboratory Standards Institute (Wayne, 2006).

Results and Discussion

Out of 76 patients involved in the study, 114 bacterial isolates were obtained giving an average of 1.5 organisms per wound isolate. Similarly, an average of 1.47 organisms per sample was reported in a study by Raja et al. (Raja, 2007). The samples showed both monomicrobial and polymicrobial growth pattern while previous reports showed the polymicrobial nature of foot infections (Suresh et al., 2011), (Turhan et al., 2013).Only 19.30% of total isolates were comprised of gram-negative bacilli while 50.88% and 30.70% were gram-positive cocci and gram positive bacilli respectively. No gram negative cocci were obtained in this study.

Among the gram-positive bacteria, the most isolated organisms from diabetic foot ulcers were *S. aureus* (31.52%). The degree of occurrence of other gram positive isolates was *Staphylococcus epidermidis* (25%) > *Corynebacterium* spp. (19.57%) > *Bacillus* spp. (16.3%) > *Streptococcus* spp. (6.52%) > *Lactobacillus* spp. (1.09%). The order of occurrence of gram negative isolates was *Proteus mirabilis* (22.73%) > *Enterobacter aerogenosa* (18.18%) > *Klebsiella pneumonia* (18.18%) > *Pseudomonas aeruginosa* (13.64%) > *Salmonella typhi* (13.64%) > *Escherichia coli* (9.09%) > *Proteus vulgaris* (4.55%). Graph 1 shows the prevalence of different bacterial infections in diabetic foot ulcers under this study.

The antibiogram study was conducted against commonly using antibiotics namely, ampicillin, ciprofloxacin, gentamicin and oxacillin. The overall results of this study were showed that the isolates under this study were highly sensitive to Gentamicin (93.9%) and ciprofloxacin (86.8%). Sensitivity of the isolates towards ampicillin and oxacillin was only 12.3% and 14.9% respectively. Gram positive organisms showed highest sensitivity to gentamicin (92.4%), followed by the sensitivity to ciprofloxacin (87.0%), then to oxacillin (10.9%) and to ampicillin (10.9%). Almost

similarly, for gram negative organisms, the highest sensitivity was shown against gentamicin (100%), followed by ciprofloxacin (86.4%), oxacillin (31.8%) and ampicillin (18.2%). i.e., both gram positive and negative bacteria showed similar decreasing order of sensitivity against the antibiotics. The sensitivity against antibiotics had the following order: gentamicin>ciprofloxacin> oxacillin> ampicillin. Mean values of zone of inhibition of both gram positive and negative isolates are given in table 1.

The moderate sensitivity against each antibiotic was considered in this study as the intermediate status of antibiogram of study population. A percentage of 2.2% gram positive organisms showed moderate sensitivity to ampicillin and 5.4% of organisms showed moderate sensitivity to ciprofloxacin. None of them showed moderate sensitivity to gentamicin and only one gave moderate sensitivity to oxacillin. Among the gram negative organisms, only 13.6% showed moderate sensitivity to ampicillin and none of them showed moderate sensitivity to other 4 antibiotics. This small percentage or 0% of moderate sensitivity might turn to resistant condition due to prolonged and ineffective use of such antibiotics.

Graph 2 indicates the prevalence of drug resistance of the total isolates against selected antibiotics. Even though 2.63% of isolates showed resistance to all antibiotic taken into consideration, 62.28% and 14.91 % were showed resistance against two and three types of antibiotics respectively. 87.72 % of isolates is resistant to at least one type of antibiotic of this study. This result indicates the alarming status of emergence of the antibiotic among the bacterial isolates of diabetic ulcer foot. The over use or abuse of the other antibiotics may also lead to incurable stage of multidrug resistance in diabetic foot management. In this study we could see that diabetic foot ulcers show a combination of mono microbial and poly microbial pattern of infection including both gram-positive and negative organisms. Of the 114 isolates, gram-negative bacteria to gram-positive to ratio were 1:4.18. Hence the predominantly isolated microorganisms from diabetic foot infections in this study were gram-positive bacteria.

This was in accordance with the studies done by Citron et al. (Citron et al., 2007) and Dang et al.(Dang et al., 2003). But some Indian studies have shown that gram negative organisms predominate in the foot infections (Suresh et al., 2011), (Gadepalli et al., 2006). This indicates that diabetic foot ulcers never posses a specific predominance of infection susceptibility towards gram positive bacteria or towards negative ones. From the antibiogram results, an increased prevalence of antimicrobial resistance amongst both gram-positive and gram-negative isolates was found.

The result indicates that ciprofloxacin and gentamicin to be effective against broad spectrum of both gram-positive and gram-negative isolates of diabetic foot ulcer. Hence ciprofloxacin and gentamicin could be effective against mono/polymicrobial infections of diabetic foot ulcer, caused by both gram positive and gram negative organisms.

The overall results of this study points towards a dreadful condition of the multiple drug resistance to these antibiotics. Majority of isolates showed multi resistance to ampicillin and oxacillin. Ninety five (83.3%) isolates had resistance against ampicillin and 96 (84.2%) showed multi resistance to oxacillin. The organisms showing oxacillin resistance also confer resistance to methicillin (Lee, 2003).

Table.1 Mean value of zone of inhibition for different antibiogram studies

		Zone of inhibition (mm)*			
		Ampicillin	**Ciprofloxacin**	**Gentamicin**	**Oxacillin**
Gram positive	**Moderately Sensitive**	14.17±0.17	18.13±0.50	---	27.90±0
	Sensitive	28.60±3.36	27.95±0.45	23.09±0.41	12.33±2.79
	Resistant	4.86±0.62	11.14±0.85	11.05±0.66	0.35±0.20
Gram negative	**Moderately Sensitive**	14.67±0.51	---	---	---
	Sensitive	22±0.79	31.79±0.96	26.50±1.02	24.29±2.47
	Resistant	4.69±1.54	6.89±3.70	---	---
*** Mean ± SEM**					

Figure.1 Prevalence of different bacterial infections in diabetic foot ulcers

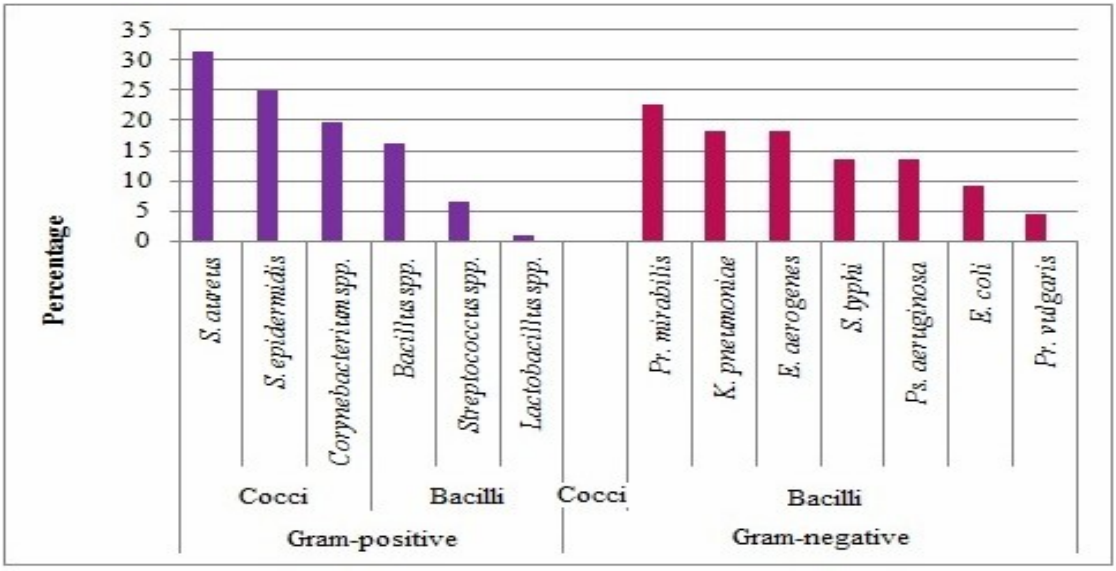
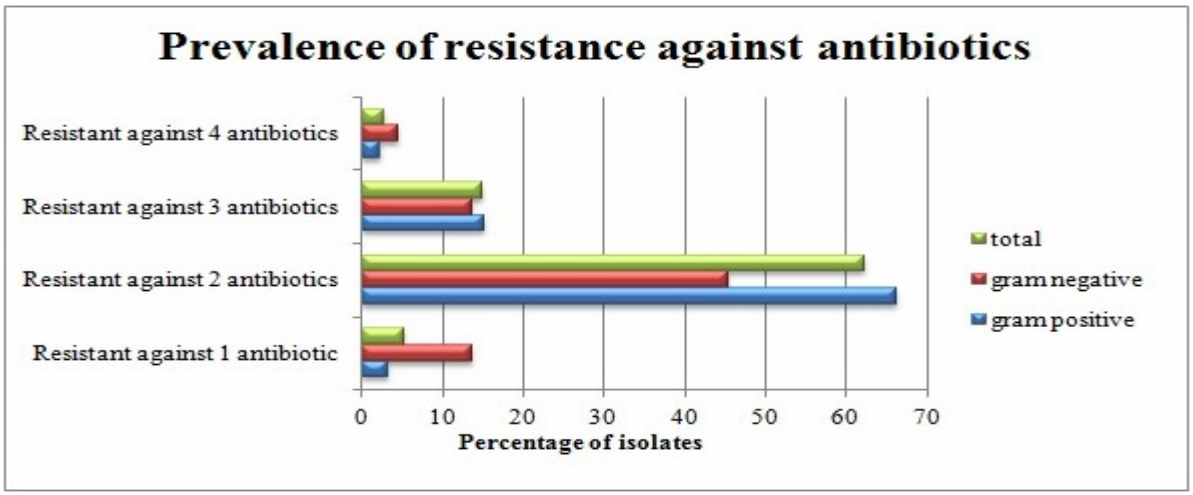


Figure.2 Prevalence of multi drug resistance of isolates of diabetic foot ulcer



The organisms exhibiting resistance to methicillin or oxacillin may also give resistance to other β-lactam antibiotics. This indicates the prevalence of resistance against β-lactam antibiotics in diabetic foot ulcers. A study from Australia illustrated the increase in susceptibility of methicillin/oxacillin- resistant organisms towards gentamicin and other non-β-lactam antibiotics like ciprofloxacin, tetracycline and erythromycin (Merlino et al., 2002). This was in accordance with our in vivo test results of antibiogram study.

This antibiotic susceptibility analysis showed that the gram positive organisms were highly resistant to oxacillin and ampicillin. Studies by BrÃŽNda et al. has showed that gram positive organisms like *S.aureus* shows resistance to oxacillin and ampicillin (BrÃŽNda, et al.).

The studies done by Suresh et al. (Suresh et al., 2011), Gadepalli et al (Gadepalli et al., 2006). and Bansel et al. (Bansal et al., 2008) reported 56%, 55.56% and 70.45% of oxacillin resistant *S. aureus* respectively. But in this study, the highest resistance was shown by 88% of the gram positive organism against oxacillin followed by resistance to ampicillin by 87% of gram positive organisms.Among the 92 gram positive coccal isolates, 31.52% of isolates were identified as *S.aureus, 2*5% as *S.epidermidis* and remaining were identified as Streptococcal species. All 29 isolates of the *S.aureus* showed oxacillin/methicillin resistance.

This indicates the prevalence of methicillin resistant *S.aureus* (MRSA) in diabetic foot ulcer infections. The rate of MRSA in the present study was found to be higher than the study conducted by Gadepalli (Gadepalli et al., 2006).

Along with methicillin resistance, *S.aureus* also exemplified resistance against ampicillin. Few isolates of *S.aureus* were sensitive to gentamicin and ciprofloxacin. Similar antibiotic resistance pattern was given by other gram positive isolates also. Among the gram negative organisms, the most prominent resistance was observed for ampicillin, and oxacillin and *Pr.mirabilis* and *S.typhi* were found the most resistant isolates.

All the *S.aureus* isolates were showed resistant to oxacillin and ampicillin. However, ciprofloxacin and gentamicin are found to be effective against broad spectrum of both gram-positive and gram-negative isolates of this study. This suggests that combinational therapy of ciprofloxacin and gentamicin could be used for treating polymicrobial infections of diabetic foot ulcer. The prevalence of drug resistant bacterial flora in the foot ulcers of this study population indicates an alarming status of diabetic ulcer foot scenario.

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