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Prevalence of Bacterial Infection in Patients with Diabetic Foot Lesions

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ABSTRACT- Diabetic foot infections are the most common problems in persons with diabetes. Among the 50 samples, 43 (86%) showed positive results of bacterial infection. Diabetic foot lesions are divided into six grades based on the depth of the wound and extent of the tissue necrosis. Incidences of bacteria were recorded as *Staphylococcus aureus (*31.37%) followed by *Proteus mirabilis* (21.05%), *Pseudomonas aeruginosa* (15.79%), *Streptococcus pyogenes* (14.04%), *Escherichia coli* (7.02%), *Clostridium botulinum* (5.26%), *Peptococcus spp*. (3.50%) and *Salmonella typhimurium* (1.75). The prevalence of diabetic foot infections varies according to sex, age, sugar level and economic status. Males were more susceptible to infection than females because of higher outdoor activities. Age groups of 40-50 years and fasting sugar levels of 100-150 mg/dl showed maximum incidence of bacterial infection in diabetic foot lesions. Maximum incidences of bacterial infection were found in patients of poor economic status followed by those of middle and high economic status respectively, due to lack of education about the disease and unhygienic surroundings. Except *Peptococcus* spp. the remaining isolates exhibited Multiple Drug Resistance (MDR). The selection of empiric antibiotic therapy depends on various factors such as infection severity, over all patient condition, medication allergies, previous antibiotic treatment, antibiotic activity, toxicity, excretion and glycemic control. Proper identification of causative agents, appropriate antibiotic therapy and management of complications of diabetic foot infections remain essential to the achievement of a successful outcome.

Key words: Diabetic foot infection and Multiple Drug Resistance

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INTRODUCTION

Diabetes mellitus is a chronic disorder and affects large segment of population and is a major public health problem. Diabetes and foot problems are almost synchronous. [1-4] The group of three problem leading on to the diabetic foot is neuropathy, vascular changes and Infections, which constitute the diabetic foot syndrome. [5-6] Foot infections in the diabetic constitute a tremendous clinical and financial burden to the patients involved, the clinicians caring for these patients, and the community as a whole. Approximately 20% of diabetics admitted to the hospital are seen primarily for their foot problems. [7] Fifty to seventy per cent of all non-traumatic amputations are performed on these diabetic patients. [8]

Foot ulceration and infections are one of the leading causes of mortality and morbidity, especially in developing countries. The numbers of cases and problems associated with diabetic foot infections (DFI) have dramatically increased in recent years. [9-10]

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The main reason for this increase is the growing diabetic population in younger groups. Ulceration of the foot in diabetics is common and disabling and frequently leads to amputation of the leg. Mortality is high and healed ulcers often recur. The pathogenesis of foot ulceration is complex, clinical presentation is variable, and its management requires early expert assessment. [11] Foot ulcers are a significant complication of diabetes which is the most common cause of no traumatic lower extremity amputations in the industrialized world. The risk of lower extremity amputation is 15 to 46 times higher in diabetics than in persons who do not have diabetes mellitus. [12-13] *Diabetes mellitus* is recognized as an epidemic in the Asian sub-continent affecting nearly 25 millions in India alone. Diabetic foot ulcers are estimated to affect 15%of all diabetics during their lifetime and precede almost 85% of all foot amputations. [14-15] Defects in host defense mechanisms have been described in the diabetic patient. Such defects include reduced leukocyte mobilization and chemotaxis and reduced phagocytic and bactericidal capacity. [16-17] Diabetes by virtues of its other complications like neuropathy and vasculopathy and other factors alters the musculoskeletal and soft tissue mechanics in a manner that elevates planter pressure and makes tissue damage more likely, causing nonresolving neuro-ischemic ulcers at the weight bearing sites. This is why most of the skin injuries in

diabetics are seen on the planter surface, frequently at the site of highest pressure under the foot. [18-19]

Infection complicates the pathological picture of diabetic foot and plays a main role in the development of moist gangrene. [4-6] *Pseudomonas* spp., *Enterococcus* spp. and *Proteus* spp. carry a special role and are responsible for continuing and extensive tissue destruction with the poor blood circulation of the foot. A high frequency of anaerobic Infection has also been reported. [20-22] Patients with diabetes also can have a combined infection involving bone and soft tissue called fetid foot. This extensive soft tissue and bone infection causes a foul exudate, is chronic, and usually requires extensive surgical debridement and/or amputation. In general, people with diabetes have infections that are more severe and take longer to cure than equivalent infections in other people.

The infection leads to the early development of complication even after a trivial trauma, the disease progresses and becomes refractory to antibacterial therapy. [23-24] It is essential to assess the magnitude of bacterial infection of the lesions to avoid further complications and save the diabetic foot. Early diagnosis of micorbial infections is aimed to institute the appropriate antibacterial therapy and to avoid further complications. [1, 20] However, these infections are difficult to treat because these patients have impaired micro-vascular circulation, which limits the access of phagocytic cells to the infected area and results in a poor concentration of antibiotics in the infected tissues. For this reason, cellulitis is the most easily treatable and reversible form of foot infections in patients with diabetes. Deep skin and soft tissue infections also usually are curable, but they can be life threatening and result in substantial long term morbidity. [25]

Diabetic soft-tissue infections result in significant morbidity in this population of patients. The spectrum of disease ranges from infected foot ulcers, cellulitis to chronic osteomyelitis. Infections in diabetes are often polymicrobial, involving a mixture of aerobic and anaerobic flora. [26]Antibiotic therapy is often empirical and an antibiotic with anaerobic cover is often recommended. [26]

Antibiotic resistance in aerobic bacteria is of global concern; however, antibiotic resistance in anaerobes is often overlooked. With reports of resistance to anaerobic antimicrobials, [27-28] and variable antimicrobial resistance amongst anaerobic genera [29] continued surveillance of anaerobic susceptibility patterns is vital to determine current and future trends. [30]

The present study assumes significance in the Indian context where the disease is itself detected late, there is little awareness for foot care in patients and there is a significant delay in seeking the treatment. Further, a significant population is rural and work in the fields barefoot, thus increasing the chances of further infection. In such a situation, the treating physician is left with the option of treating empirically till the culture reports are available. A rough idea of the antibiotic pattern would be a useful aid for him.

MATERIALS AND METHODS

Place of work

The present study was conducted in the Department of Microbiology and Fermentation Technology, Sam Higginbotom Institute of Agriculture, Technology and Sciences, Deemed to be- University, Allahabad.

Study Material

Foot lesions samples were collected from 50 patients, suffering from Diabetic Foot infection and treated in different hospital (Swaroop Rani Hospital, Pooja hospital, The Leprosy Mission Hospital and Toshi Pathology) of Allahabad. These patients were clinically assessed and the foot lesions are classified and graded according to Wagner grading system. In the Wagner classification system, foot lesions are divided into six grades based on the depth of the wound and extent of tissue necrosis.

Grade 0- Preulcer. No open lesions skin intact; may have deformities, erythematous areas of pressure or hyperkeratosis.

Grade 1 - Superficial ulcer clinically not infected.

Grade 2 - Deep ulcer often infected but no bone involvement.

Grade 3 - Deep ulcer, abscess formation and bone involvement.

Grade 4 - Localized gangrene.

Grade 5 - Gangrene of whole foot.

Collection of samples

Discharge from the incised lesions or ulcer was collected with sterile swabs. Pus aspirated from the abscesses and debrided necrotic materials were collected for aerobic and anaerobic culture. During the sample collection the patient Performa containing details of the patient was collected.

Isolation

Gram stained direct smear of the specimen was examined. The specimen was inoculated on to Blood agar, MacConkey's Agar and Thioglycollate broth (App. 2.4, 2.1 and 2.3) for aerobic and anaerobic culture and incubated at 37°C for 48 hrs.

Identification of isolates

The bacterial isolates were identified by cultural and physiological, morphological and biochemical tests according to Bergey's manual of determinative bacteriology. [31]

Cultural and physiological characteristics

The isolates were identified on the basis of different colony characteristics like colour, texture, opaque *etc.* on culture plate (Fig.1).

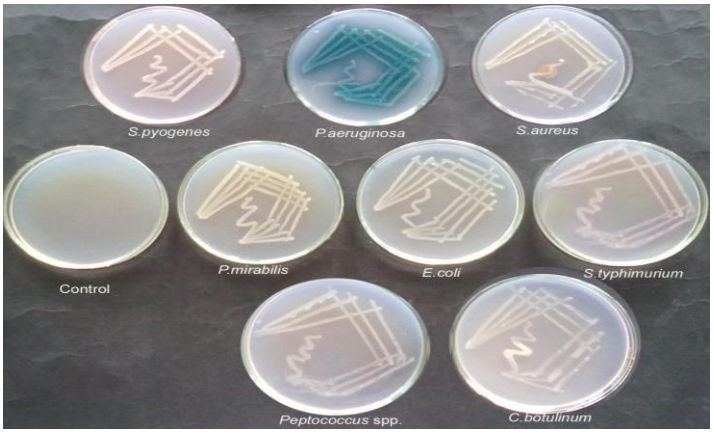

Fig. 1: Cultural characteristics of diabetic foot lesion isolates

Morphological characteristics

A gram staining of isolated bacteria was done and observation of shape and arrangement under 100x objective microscope.

Biochemical characteristics

Different biochemical tests were performed for the identification of the microorganism. [32]

Antibiotic Susceptibility Test

Antibacterial susceptibility testing was performed by Kirby Bauer's disc diffusion method according to National Committee for Clinical Laboratory Standards guidelines. [33]

Statistical analysis

The data recorded during the course of investigation were statistically analysed by using chi square (χ^2) test, correlation, t-test and conclusion was drawn. [34]

RESULTS AND DISCUSSION

Prevalence of different lesions in diabetic foot lesions

In the present study total 50 patients of diabetic foot lesion were studied for the presence of bacteria in their pus samples. Of them 50 pus samples, 43 (86%) showed positive results of bacterial infection. The polymicrobial infection rate was low (20.93%) in this study. There were more monomicrobial cultures than polymicrobial cultures (34 vs. 9) in this study with an average 1.33 pathogen isolated from diabetic foot lesion. This rate of isolated pathogen per lesion was low compare to the studies of [35] the low prevalence of polymicrobial infection and low rate of isolated pathogen per lesion may be attributable to lack of severity of most infection and low virulence of isolated organism in this study.

All diabetic foot were classified and grouped according to Wagner grading system. In the modified Wagner classification system, foot lesions are divided into six grades based on the depth of the wound and extent of the tissue necrosis. In the present study all patients had ulcer graded 0-3 in the Wagner classification. 23(53.49%) of our patients presented with preulcer (Grade 0), 15(34.88%) with superficial ulcers (Grade I), 3(6.98%) with deep ulcer but no bone involvement (Grade II) and 2(4.64%) deep ulcer with bone involvement (Grade III), whereas Grade IV, Grade V were absent.

While considering the bacterial infection in the diabetic patients studied under Wagner grade maximum incidence was recorded in grade 0. Gram positive tend to occurs higher as compare to Gram negative bacteria. Subsequently, grade I patients were found to be colonized with higher incidental rate of Gram negative as compare Gram positive. Further grade II and III also found to be infected by different bacterial pathogens. Since none of the samples studied included grade IV and V of Wagner grade, the bacterial incidence in these criteria could not be recorded (Table 1 and Fig. 2). Similarly higher incidence of gram positive organism in grade I and gram negative in grade II was also recorded in the study of those authors. [36]

Among the different organisms isolated from diabetic foot lesions, maximum incidence were recorded against *Staphylococcus aureus (*31.37%) followed by *Proteus mirabilis* (21.05%), *Pseudomonas aeruginosa* (15.79%), *Streptococcus pyogenes* (14.04%), *Escherichia coli* (7.02%), *Clostridium botulinum* (5.26%), *Peptococcus spp.* (3.50%) and *Salmonella typhimurium* (1.75) (fig. 4.1).On analyzing the data the difference was found to be statistically significant. However slitly higher incidence of *S. aureus* was reported in. [37, 38] The incidence of *Streptococcus* (14.3%) observed in the present study is comparable with the findings of. [36] [39] reported that low-virulence organisms such as *S.aureus*, *Streptococcus viridans*, *Staphylococcus epidermidis*, enterococci and certain Gram-negative bacteria caused two-thirds of mild diabetic foot infections. Most of the patients stuudied (53.49%) had Grade I ulcers which are usually uncomplicated. This may be the reason for our low isolation of anaerobes. Anaerobic organisms flourish in deep seated infections. This indicates that with an increasing grade of ulcer, the anaerobic conditions are produced as a result of increase in the depth of the wound and decrease in peripheral blood flow, leading to higher rate of infections by anaerobes. Low isolation rates of anaerobes could be due to improper sampling and unnecessary delay in transportation of samples to the microbiology laboratory as well as previous treatment of patients with multiple antibiotics. The predominance of gram positive in cases that require major amputation may be due to the high proportion of *Staphylococcus aureus*. Such bacteria have high pathogenicity and cause severe tissue damage because of the production of extracellular enzymes and toxins.

Table 1: Distribution of bacterial infection in diabetic patients of different Wagner grade

Total Samples	Positive Patients	Wagner Grade	Diabetes (%)	Bacterial Incidence (%)							
				Gram Positive			Gram Negative				
				Staphylococcus	*Streptococcus pyogenes*	*Peptococcus*	*Clostridium botulinum*	*Proteus mirabilis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Salmonella typhimurium*
50	43(86%)	0	23(53.49)	12(35.29%)	6(17.65%)	0(0%)	0(0%)	9(26.47%)	4(11.76%)	2(5.88%)	1(2.94%)
		1	15(34.88)	4(26.67%)	2(13.33%)	0(0%)	0(0%)	3(20%)	5(33.33%)	1(6.67%)	0(0%)
		2	3(6.98%)	1(20%)	0(0%)	1(20%)	2(40%)	0(0%)	0(0%)	1(20%)	0(0%)
		3	2(4.64%)	1(33.33%)	0(0%)	1(33.33%)	1(33.33%)	0(0%)	0(0%)	0(0%)	0(0%)
		4	0	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
		5	0	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Total=57(100%)			**18(31.5**	**8(14.04%)**	**2(3.50**	**3(5.26**	**12(21.05%)**	**9(15.79**	**4(7.02**	**1(1.7**	

Correlation Co-efficient(r) = - 0.898, $t_{(cal)5\%} = 9.25 > t_{(tab)5\%} = 8.61$ (P=0.0004), S= Significant

Grade 0 - Preulcer. No open lesions skin intact; may have deformities, **Grade 1** - Superficial ulcer clinically not infected. **Grade 2** - Deep ulcer often infected but no bone involvement.

Grade 3 - Deep ulcer, abscess formation and bone involvement. **Grade 4** - Localized gangrene. **Grade 5** - Gangrene of whole foot.

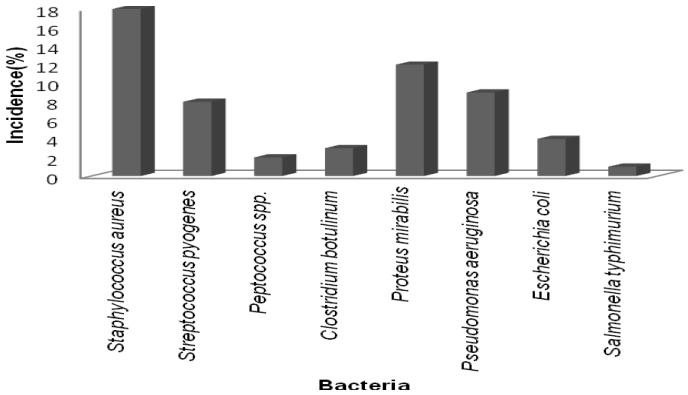

Fig. 2: Incidence of different bacterial flora in diabetic foot lesions

Antibiotic susceptibility of the isolates

The antimicrobial susceptibility patterns of the Gram positive bacteria isolated from Diabetic Foot Lesions against some antimicrobial agents were shown in Table 2. In the present study maximum organisms were sensitive to Ciprofloxacin, Ofloxacin, Gentamycin, Imipenem and Chloramphenicol and resistant to Cefuroxime, Erythromycin and Piperacillin. Except *Peptococcus* spp. other Gram positive bacteria were Multiple Drug Resistant (MDR).

The antimicrobial susceptibility pattern of the Gram negative bacteria is shown in Table 3. Most of the organisms were sensitive to Gentamycin and Imipenem, intermediate to Erythromycin and resistant to Penicillin G, Oxacillin, Vancomycin Ampicillin and Co-trimoxazole. All Gram negative bacteria were Multiple Drug Resistant (MDR).

Similarly *Staphylococcus aureus* showed good sensitivity to ciprofloxacin as the similar results were reported previously by. [38] Finding of *Clostridium* species were highly susceptible to norfloxacin, gentamycin, erythromycin, chloramphenicol, ofloxacin, and ciprofloxacin recorded by. [40-41] observed that ciprofloxacin, gentamicin and perfloxacin were effective against Gram-positive. All the aerobes were sensitive to Amikacin and gentamicin reported in the study of. [42] [43] found that chloramphenicol was the most effective agents against Gram-positives. Ampicillin showed resistant against *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Escherichia coli* recorded.[44-45] observed that Chloramphenicol as impressive antibiotics against our anaerobic isolates (Fig. 3 – 10).

No single antimicrobial agent can cover all of the possible organisms isolated from diabetic foot infections. The present study findings illustrate that antimicrobial therapy needs to be selected based on antimicrobial sensitivity patterns of isolates.

Table 2: Antibiotic susceptibility patterns of Gram positive isolates

S.No	Name of Isolates	Antibiotics								
		Ciprofloxacin(5µg)	Ofloxacin(5µg)	Ampicillin(30µg)	Gentamycin(10µg)	Cefuroxime(30µg)	Erythromycin(15µg)	Imipenem(10µg)	Piperacillin(100µg)	Chloramphenicol(30µg)
1.	*Staphylococcus aureus*	+	+	+	+	-	-	-	-	-
2.	*Streptococcus py-*	-	-	-	+	-	+	++	-	-
3.	*Peptococcus spp.*	+	-	+	+	+	-	++	++	++
4.	*Clostridium botulinum*	+	+	-	+	+	-	++	-	++

Sensitive= +++, Intermediate= ++, Resistance= -

Table 3: Antibiotic susceptibility patterns of Gram negative isolates

S. No	Name of Isolates	Antibiotics								
		Penicillin G(10unit)	Oxacillin(5µg)	Erythromycin(15µg)	Vancomycin(30 µg)	Gentamycin(10 µg)	Cefuroxime(30µg)	Imipenem(10µg)	Ampicillin(10µg)	trimoxazole(25µg)
1.	*Proteus mirabilis*	-	-	-	-	++	-	+	-	-
2.	*Pseudomonas*	-	-	++	-	++	+	+	-	-
3.	*Escherichia coli*	-	-	++	-	++	+	+	-	-
4.	*Salmonella typhi-*	-	-	-	-	++	-	+	-	+

Sensitive= +++, Intermediate= ++, Resistance= -

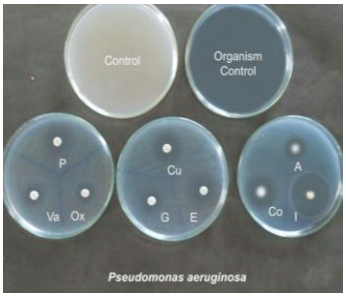

Fig. 3: Antibiotic susceptibility *Pseudomonas aeruginosa*

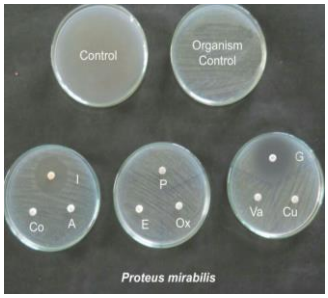

Fig. 4: Antibiotic susceptibility of of *Proteus mirabilis*

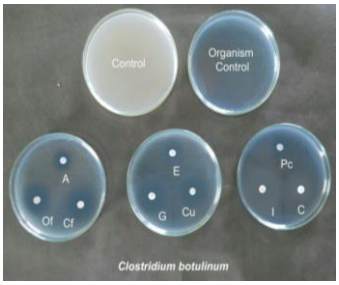

Fig. 5: Antibiotic susceptibility *Clostridium botulinum*

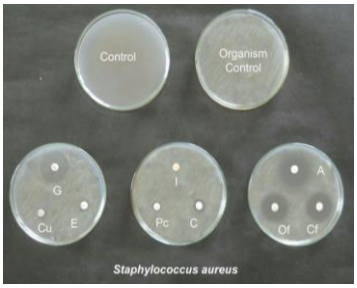

Fig. 6: Antibiotic susceptibility of of *Staphylococcus aureus*

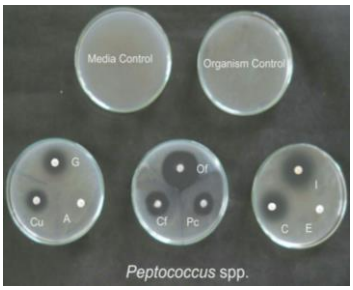

Fig. 7: Antibiotic susceptibility of *Peptococcus* spp.

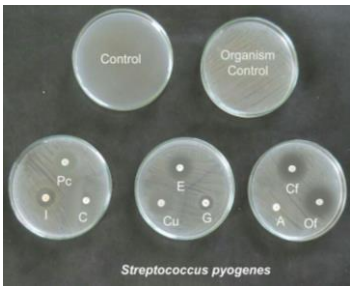

Fig. 8: Antibiotic susceptibility of of *Streptococcus pyogenes*

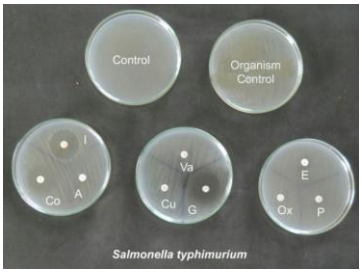

Fig. 9: Antibiotic susceptibility *Salmonella typhimurium*

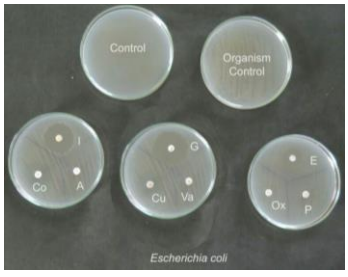

Fig. 10: Antibiotic susceptibility of of *Escherichia coli*

CONCLUSIONS

In the study we concluded that the Maximum incidence of foot lesions were observed in the category of Wagner grade 0 (53.49%) followed by grade 1 (34.88%), grade 2 (6.98%) and grade 3 (4.64%). Significant difference in incidence of *Saphylococcus aureus* were found to occur (31.54%) followed by *Proteus mirabilis* (21.05%), *Pseudomonas aeruginosa* (15.79%), *Streptococcus pyogenes* (14.04%), *Escherichia coli* (7.02%), *Clostridium botulinum* (5.26%), *Peptococcus spp.* (3.50%) and *Salmonella typhimurium* (1.75%) ($P<0.05$).

Age groups of 40-50 years tend to show maximum incidence of bacterial infection in diabetic foot lesions followed by the diabetic patients within the age group of 50-60 years. Male patients were found to be more susceptible to bacterial infection as compare to female.

Patients with sugar level of 100-150 mg/dl at fasting were found to show more bacterial infection followed by patients, who fill in the category of 150-200 mg/dl and 200-250 mg/dl. Least evidence was observed in patients with fasting sugar level 250-300 or 300-350 mg/dl and the difference was found to be statistically significant ($P<0.05$) .

Lower economic status patients (53.49%) were found to suffer more followed by medium economic status (39.53%). However less evidence was recorded among high economic status (0.70%) patients ($p<0.05$). However non-significant difference of diabetic foot on the bacterial infection was recorded.

The isolates were found to possess Multiple Drug Resistance (MDR) except *Peptococcus* species.

Diabetic foot infections are generally polymicrobial. Hence a higher incidence of different pathogenic microbes was observed in patients suffering from diabetic foot lesions. Further different factors like sex (male), sugar level (100-150 mg/dl) and economic status were found to have significant effect on the incidence of bacterial infection. Proper education regarding foot wear and foot care is strongly recommended in such patients. The selection of empiric antibiotic therapy depends on various factors such as infection severity, over all patient condition, medication allergies, previous antibiotic treatment, antibiotic activity, toxicity, excretion and glycemic control. Proper identification of causative agents, appropriate antibiotic therapy and management of complication of diabetic foot infections remain essential to the achievement of a successful outcome.

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