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DIABETIC FOOT ULCERS;
COMMON ISOLATED PATHOGENS & IN VITRO ANTIMICROBIAL ACTIVITY.

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ABSTRACT... (SUMMARY) Aim: To determine common pathogens isolated in diabetic foot ulcers & in vitro antimicrobial activity. **Setting:** Muhammad Medical College Hospital, Mirpurkhas and Liaquat University of Medical & Health Sciences Jamshoro. **Period:** 1st November 2007 to 30th June 2008. **Research Methodology:** Pus samples for bacterial culture were collected from 60 patients admitted with diabetic foot infections. Anti-microbial susceptibility testing of aerobic isolates was performed by the standard disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards. Microbroth dilution test was arranged for susceptibility of anaerobic organisms to metronidazole and amoxicillin/clavulanate. A vancomycin screen agar (6 µg/ml) was used to detect vancomycin intermediate isolates of Staphylococci. **Results:** Clinical grading and bacteriological study of 60 patients revealed, 44 (73.33%) patients had gram-negative organisms, & Pseudomonas aeruginosa was the most common. While 16 (26.66%) patients had gram-positive organisms & Staphylococci was the most common organism. Infection with anaerobes was observed in one patient (1.66%). Both gram-positive and -negative organisms were seen in 8 (13.33%). Pseudomonas aeruginosa & S.aureus exhibited a high frequency of resistance to the antibiotics tested. All the isolates were uniformly susceptible to fosfomycin, levofloxacin, amikacin and vancomycin. **Conclusions:** Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis and *Proteus* were the most common causes of diabetic foot infections in this study. And the rate of antibiotic resistance was 66.66% among the isolates. All the isolates were uniformly susceptible to fosfomycin, levofloxacin, amikacin and vancomycin.

Key words: Diabetic foot, common pathogens, antibiotic resistance.

INTRODUCTION

Foot ulcerations and infections are one of the leading causes of mortality and morbidity, especially in developing countries. The number of cases and problems associated with diabetic foot infections (DFI) have dramatically increased in the recent years. The main reason for this increase is the growing diabetic population in younger groups. Ulceration of the foot in diabetes is common and disabling and frequently leads to the amputation of the leg[1]. The risk of lower leg amputation is 15 to 46 times higher in diabetics than in persons who do not have the diabetes mellitus. Furthermore, foot complications are the most frequent reason for hospitalization in patients with diabetes[2].

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. Interventions should be directed towards infections control, peripheral ischemia

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management, and abnormal pressure loading management caused by peripheral neuropathy and limited joint mobility.

Despite treatment, ulcers readily become chronic wounds. Diabetic foot ulcers have been neglected in health care research and planning, and clinical practice is based more on opinions than the scientific figures and facts. Furthermore, the pathological processes are poorly understood and poorly taught. Communication between the many specialties involved is disjointed and is insensitive to the needs of the patients. Ischemia, neuropathy, and infection in patient with DM combined to produce tissue necrosis and ulcers. Early recognition of lesions and prompt initiation of appropriate antibiotic therapy, as well as aggressive surgical debridement of necrotic soft tissues and bones, and a modification of host factors i.e., hyperglycemia, and concomitant arterial insufficiency are all equally important for successful outcome. Initial therapy of diabetic foot infections is frequently empiric because reliable culture data is lacking. There is variability in prevalence of common bacterial pathogens isolated, as shown in different studies. The choice of empirical antimicrobial therapy is influenced by various factors. These include the severity of the illness (Wagner grading), the most likely type of causative organism, and coexisting complications, such as underlying osteomyelitis. Host factors, for example comorbid conditions, good glycaemic control, concomitant renal and cardiovascular diseases can affect the need for hospital admission and the choice of specific agents or their dosing intervals[1]. In terms of the infecting microorganisms and the likelihood of successful treatment with antimicrobial therapy, acute osteomyelitis in people with diabetes is essentially the same as in those without diabetes. Chronic osteomyelitis in patients with diabetes mellitus is the most difficult infection to cure. Adequate surgical debridement, in addition to antimicrobial therapy, is necessary to cure chronic osteomyelitis[3]. The aim of this study was to evaluate relative frequency of bacterial isolates cultured from diabetic foot infections presenting at the Muhammad Medical College Hospital Mirpurkhas and Liaquat University of Medical & Health Sciences Jamshoro and assess their in vitro susceptibility to the commonly used antibacterial agents.

RESEARCH DESIGN AND METHODS

Sixty diabetic patients with clinically infected foot ulcers admitted in the Department of Medicine, Muhammad Medical College Hospital Mirpurkhas and Liaquat University of Medical & Health Sciences Jamshoro from 1st November 2007 to 30th June 2008 were studied. Ulcers were graded using the Wagner classification. Age, sex, type and duration of diabetes, glycaemic control during the hospital stay, presence of retinopathy, nephropathy (creatinine ≥150 µmol/l or presence of micro- or macroalbuminuria), neuropathy (absence of perception of the Semmes-Weinstein monofilament at 2 of 10 standardized plantar sites on either foot), peripheral vascular disease (ischemic symptoms and intermittent claudication or rest pain, with or without absence of pedal pulses), duration and size of ulcer, clinical outcome, and duration of hospital stay were noted on each patient. Clinical assessment for signs of infection (swelling, exudates, surrounding cellulitis, odor, tissue necrosis, crepitation, and pyrexia) was made. Ulcer size was determined by multiplying the longest and widest diameters and expressed in centimeters squared. Osteomyelitis was diagnosed on suggestive changes in the radiographs. All cases were monitored until discharge from the hospital. Written consent was obtained from all subjects, and clearance was obtained from the institute's ethics committee.

Specimen-collection

Culture specimens were obtained at the time of admission, after the surface of the wound had been washed vigorously by saline, and followed by debridement of superficial exudates. Specimens were obtained by scraping the ulcer base or the deep portion of the wound edge with a sterile curette. The soft tissue specimens were promptly sent to the laboratory and processed for aerobic and anaerobic bacteria.

Anti-microbial susceptibility testing

Anti-microbial susceptibility testing of aerobic isolates was performed by the standard disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards. All anaerobic isolates were tested

for susceptibility to metronidazole and amoxicillin/clavulanate by microbroth dilution test. A vancomycin screen agar (6 µg/ml) was also used to detect vancomycin intermediate isolates of Staphylococci.

Antibiotic-treatment

Intravenous empirical antibiotic therapy of amoxicillin-clavulanate combination (1.2 g i.v. every 8 h) was started at the time of admission. This was switched to oral administration (625 mg p.o. every 8 h). Metronidazole (500 mg i.v. every 8 h) was added to the drug regimen if cellulitis or gangrene was also present. Antibiotics were adapted based on the results of anti-microbial studies to target the most likely pathogenic organisms.

Statistical-methods

Quantitative variables were expressed as means ± SD while qualitative variables were expressed as percentages. A P-value of <0.05 was taken as significant. All statistical data was analyzed on Stata SPSS 10.

RESULTS

The general & clinical characteristics of 60 patients with diabetic foot are shown in Table I. The mean age of the subjects was 59 ± 3.7years. The mean duration of diabetes was 19.8 ± 5.7 years, and nearly two-thirds (66.33%) had the condition for >19 years. Nearly 34 (56.66%) had diabetic foot lesions for >1 month before presentation at the hospital. In general the patients were of old age and had been on oral hypoglycemic agents. The recommended glycaemic control was not seen in any of the sixty patients. The majority of subjects had type 2 diabetes (88.8%). Males were predominant (76.66%) in the study subjects. All diabetic foots 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. It's a simplified system which only attaches modifiers for ischemia (A) and infection (B), shown in Table II. It's recognized that grades 3 through 5 usually have some degree of infection inherent within these lesions. In our study all patients had ulcers graded 3–5 in the Wagner classification. The details of the patients according to the Wagner classification are shown in Table III.

Table-I. Clinical Data of 60 diabetic patients with infected foot ulcers (n=60)

Features	No. of Patients	%age
Age (Years)	**Mean age 59 ± 3.7**	**Range 30-72 yrs**
< 40 years	28	46.66%
> 40 years	32	53.33%
Sex		
Male	24	83.33%
Female	19	16.66%
Co morbidities		
Hypertension	43	71.66%
Diabetic neuropathy	22	51.1%
Ischemic heart disease	11	25.5%
Diabetic retinopathy	9	20.9%
Diabetic nephropathy	7	16.2%
PVD	49	81.66%
Osteomyelitis	19	16.66%
Time Duration of Infection		
< 10 days	11	6.66%
< 29 days	15	25%
> 30 days	34	56.66%

Table-II. Modified wagner Classification System

Grade	Characteristic Lesion
0	**No open lesions: may have deformity or cellulitis** A. Ischemic B. Infected
1	**Superficial ulcer** A. Ischemic B. Infected
2	**Deep ulcers to tendon, or joint capsule** A. Ischemic B. Infected
3.	**Deep ulcers with abscess, osteomyelitis, or joint sepsis** A. Ischemic B. Infected
4	**Localized gangrene-forefoot or heel** A. Ischemic B. Infected
5	**Gangrene of entire foot** A. Ischemic B. Infected

Table-III. Number and percentage of patients according to Wagner grade (n=60)		
Wagner Grades	**Number**	**%age**
0	0	0
1	1	1.66%
2	5	8.33%
3	8	13.33%
4	41	68.33%
5	5	8.33%

The diabetic foot lesions were gangrenous in 45 (73.33%) cases. Twenty two (36.66%) patients had neuropathy, 49 (81.66%) had peripheral vascular disease, 7 (11.6%) had nephropathy, 9 (15%) had retinopathy, and 43 (71.66%) were hypertensive. Osteomyelitis was present in 19 (16.66%) subjects. (Table I).

Forty four (73.33%) patients had gram-negative organisms, with Pseudomonas aeruginosa being the most common. While gram-positive organisms were found in 16 (26.66%) patients with Staphylococci being the most common organism. Infection with anaerobes alone was observed in one patient (1.66%). Both gram-positive and -negative organisms were seen in 8 (13.33%). The profile of the isolated organisms is detailed in Table IV.

Pseudomonas aeruginosa exhibited a high frequency of resistance to the antibiotics tested. High levels of resistance to ampicillin, co-amoxiclav, ciprofloxacin, ofloxacin, cefotaxime, cefoperazone, cefazolin, cefuroxime were noted. All the isolates were uniformly susceptible to fosfomycin, levofloxacin, gentamicin and amikacin. B.pyocyneus (Pseudomonas pyocyaneus) was found in one patient and it was sensitive only to fosfomycin & doxycycline. B. pyocyaneus showed resistance to the aminoglycosides.

Table-IV. Bacteria Isolated from the diabetic foot infection of 60 patients (n=60)		
Bacteria Isolated	**Number**	**%age**
Staph aureus	12	20%
Staph: epidermidis	2	3%
Streptococci	2	3.33%
Pseudomonas aeruginosa	27	48%
B. Pyocyaneus (Pseudomonas pyocyaneus)	1	1.66%
Proteus mirabilis	1	1.66%
Proteus vulgaris	2	3.33%
E.Coli	6	10%
Klebsiella	2	3%
Citrobacter	3	5%
Enterobacter spp.	1	1.66%
Morganella morganii	1	1.66%

The results of susceptibility studies for the Gram negative organism are shown in Table V.

While the results of susceptibility studies for the Gram posistive organism are shown in Table VI. S.aureus exhibited a high frequency of resistance to the antibiotics tested. High levels of resistance to ampicillin, ciprofloxacin, ofloxacin, and cefazolin was noted. However, no high-level aminoglycoside resistance was observed. All the isolates were uniformly susceptible to fosfomycin, levofloxacin, and vancomycin.

Table-V. Antimicrobial Sensitivity/ resistance of Common gram-negative bacilli (n=60)						
Antimicrobial agent	**Pseudomonas aeruginosa (n=28)**		**E.coli (n=6)**		**Proteus (n=3)**	
	Sensitive (%)	**Resistant (%)**	**Sensitive (%)**	**Resistant (%)**	**Sensitive (%)**	**Resistant (%)**
Ampicillin	0(0%)	28(100%)	0(0%)	6(100%)	0(0%)	3(100%)
Co-amoxiclav	13(46.42%)	15(53.57%)	3(50%)	3(50%)	1(33.3%)	2(66.6%)
Ciprofloxacin	12(42.85%)	16(57.14%)	6(100%)	0(0%)	3(100%)	0(0%)
Ofloxacin	11(39.28%)	17(60.71%)	6(100%)	0(0%)	3(100%)	0(0%)
Cefotaxime	14(50%)	14(50%)	5(66.6%)	2(33.3%)	3(100%)	0(0%)
Gentamicin	14(50%)	14(50%)	6(100%)	0(0%)	3(100%)	0(0%)
Amikacin	27(96.42%)	1(3.75%)	6(100%)	0(0%)	3(100%)	0(0%)
Ceftazidime	20(71.42%)	8(28.57%)	3(50%)	3(50%)	1(33.3%)	2(66.6%)
Cefoperazone	12(42.85%)	16(57.14%)	3(50%)	3(50%)	2(66.6%)	1(33.3%)
Cefazolin	12(42.85%)	16(57.14%)	3(50%)	3(50%)	2(66.6%)	1(33.3%)
Fosfomycin	28(100%)	0(0%)	6(100%)	0(0%)	3(100%)	0(0%)
Cefuroxime	4(14.28%)	24(85.71%)	2(33.3%)	5(66.6%)	1(33.3%)	2(66.6%)
Levofloxacin	27(96.42%)	1(3.57%)	6(100%)	0(0%)	3(100%)	0(0%)
Doxycycline	1(3.75%)	-	-	-	-	-

Table-VI. Antimicrobial resistance pattern of gram-positive cocci (n=60)						
Antimicrobial agent	Staph: aureus (n=12)		Staph: epidermidis (n=2)		Streptococci (n=2)	
	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)	Sensitive (%)	Resistant (%)
Ampicillin	0(0%)	12(100%)	0(0%)	2(100%)0	2(100%)	0(0%)
Co-amoxiclav	6(50%)	6(50%)	2(100%)	(0%)	2(100%)	0(0%)
Ciprofloxacin	9(75%)	3(25%)	2(100%)	0(0%)	2(100%)	0(0%)
Ofloxacin	10(83.33%)	2(16.66%)	2(100%)	0(0%)	2(100%)	0(0%)
Cefotaxime	7(58.33%)	5(41.66%)	1(50%)	1(50%)	2(100%)	0(0%)
Gentamicin	9(75%)	3(25%)	2(100%)	0(0%)	2(100%)	0(0%)
Amikacin	12(100%)	0(0%)	2(100%)	0(0%)	2(100%)	0(0%)
Ceftazidime	6(50%)	6(50%)	2(100%)	0(0%)	2(100%)	0(0%)
Cefoperazone	8(66.66%)	4(33.3%)	2(100%)	0(0%)	2(100%)	0(0%)
Cefazolin	4(33.3%)	8(66.66%)	1(50%)	1(50%)	2(100%)	0(0%)
Fosfomycin	12(100%)	0(0%)	2(100%)	2(100%)	2(100%)	0(0%)
Cefuroxime	9(75%)	3(25%)	0(0%)	2(100%)	2(100%)	0(0%)
Levofloxacin	12(100%)	0(0%)	2(100%)	0(0%)	2(100%)	0(0%)
Vancomycin	12(100%)	0(0%)	2(100%)	0(0%)	2(100%)	0(0%)

DISCUSSION

This study presents a comprehensive clinical and microbiological survey of infected diabetic foot ulcers in hospitalized patients. Foot ulcers are a significant complication of diabetes and often precede lower extremity amputation. The most frequent underlying etiologies are neuropathy, trauma, deformity, high plantar pressures, and peripheral arterial disease[4]. Although infection is rarely implicated in the etiology of diabetic foot ulcers, the ulcers are susceptible to infection once the wound is present.

Most of our patients were having grade 3 through 5 foot ulcers according to Wagner grade, and grade 4 being the most common, which is similar to the study conducted[1]. While foot infections in persons with diabetes are initially treated empirically, therapy directed at known causative organisms may improve the outcome. Many studies have reported on the bacteriology of diabetic foot infections (DFIs) over the past 25 years, but the results have varied and have often been contradictory. A number of studies have found that Staphylococcus aureus is the main causative pathogen, but two recent investigations reported a predominance of gram-negative aerobes[5], & our study too reveals that gram-negative aerobic bacteria are most frequent. The ratio of gram-positive aerobes to gram-negative aerobes was 1: 2.75, which is in reversal to that reported[6]. The differences in the age-sex composition, ulcer grades, study setting, etc. between our study population and those of earlier studies might be the reason for these differences.

We observed a high recovery of multidrug resistant Pseudomonas aeruginosa, which is similar as was

reported[7]. This raises a serious concern as P.aeruginosa is an aggressive gram-negative Bacillus[7]. Staphylococcus aureus was the most frequent gram-positive pathogen, found in nearly 20% of infections. The majority of studies also noted a high frequency of these microorganisms in foot infections of diabetic patients[3, 7]. Compared with earlier reports, we recovered fewer anaerobic species. Our patients had chronic draining wounds, and 45 (73.33%) cases had gangrene associated with their infections. This may be an indication of anaerobic species among non-threatening lower-extremity infections, which is also reported earlier[8]. Clostridium species were not isolated. The present study confirms that multidrug resistant organisms (MDRO) infection is extremely common in hospitalized patients with diabetic foot ulcers. This is in accordance with the report of Heurtier et al[9]. Almost 40 (66.66%) of our patients were infected with MDROs. The high rates of antibiotic resistance observed in the present study may be due to the fact that ours is a tertiary care hospital with widespread usage of broad-spectrum antibiotics leading to selective survival advantage of pathogens. These findings are important, especially for patient management and the development of antibiotic treatment policies. The increasing prevalence of MDROs is disconcerting because infection with these organisms limits the choice of antibiotic treatment and may lead to a worse outcome.

We could not elicit the previous hospitalization details for the same wound in our study subjects. This information could have helped in explaining the reasons for the high prevalence of MDROs in our patients. Our results indicate that higher mortality rates were reported in patients with diabetic foot syndrome whose blood glucose levels were poorly controlled[10]. Thus, MDROs might lead to higher mortality among diabetic foot infections, which needs to be investigated. Though MDRO infections have been reported to increase hospital stay and cost[11], we found similar duration of hospital stay in both MDROs and non-MDROs. The duration of hospital stay may also depend on the management policy of the hospital. In our hospital, patients are discharged once the healing begins and are advised to come for follow up at the outpatient clinic every week.

CONCLUSION

In conclusion Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis and Proteus were the most common causes of diabetic foot infections in our study. And the rate of antibiotic resistance was 66.66% among the isolates. All the isolates were uniformly susceptible to fosfomycin, levofloxacin, amikacin and vancomycin.

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