In Vitro Bectriologic Study and Empiric Antibiotic Regimens for Diabetic Foot Ulcers

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Abstract— To determine common pathogens isolated in diabetic foot ulcers and in vitro antimicrobial activity. The University of Leicester, University Hospitals of Leicester NHS Trust, The University of Cambridge, University of Lahore, Pakistan. Period: 1st January 2017 to 30th June 2018.

Research Methodology: Pus samples of bacterial culture were collected from 100 patients admitted with diabetic foot infection. Antimicrobial susceptibility testing of aerobic isolates was performed by the standard disc diffusion method as recommended by National Committee for the Clinical Laboratory Standards. Micro broth dilution test was arranged for susceptibility of anaerobic organisms to metronidazole and amoxicillin/clavulanate. A vencomycine screen agar (6 µg/ ml) was used to detect vencomycine intermediate isolates of Staphylococci.

Results: Clinical grading and bacteriological study of 100 patients revealed, 69 (69.0%) patients had gram-negative organisms and pseudomonas aeruginosa was the most common, while 21 (21.0%) patients had gram-positive organisms and Staphylococci was the most common. Infection with anaerobic was found in one patient (1.0%). Both gram-positive and –negative organisms were seen in 9 patients (9.0%). Pseudomonas aeruginosa and S.aureus exhibited a high frequency of resistance to the antibiotics tested. All the isolates were uniformly susceptible to fosfomycine, levofloxacin, amikacin and vencomycine.

Conclusion: In this study Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Staphylococcus epidermidis and proteus were the most common causes of diabetic foot infections. The rate of antibiotic resistance was 61.86% among the isolates. All the isolates were uniformly susceptible to fosfomycine, levofloxacine, amikacin and vencomycine.

Index Terms— Diabetes Mellitus, foot Ulcer, Staph aureus, common pathogens, antibiotic resistance, bacteria, regimens, empiric

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1 INTRODUCTION

THE one of the main causes of mortality in developing countries is the Foot ulceration and infection. The number and cases of with diabetic foot infection (DFI) associated problems have noticeably augmented in the current years. The budding reason of this increase is the growing diabetic population in younger groups. The Foot Ulceration in diabetes is very familiar and frequently leads to the confiscation of the leg1. The lower leg confiscation risk is 17 to 41 times higher in the diabetics than in a normal healthy person. Moreover, foot complications are the most common reason for hospitalization in patients with diabetes 2.

In diabetic foot ulcer patients mortality is elevated and ulcers often recur after healing. The pathogenesis of foot ulceration is intricate and clinical presentation is variable. The management of foot ulceration often requires early expert diagnosis. Interventions should be aimed at infections control, irregular pressure loading management and peripheral ischemia management caused by partial joint mobility and peripheral neuropathy.

Regardless of treatment, ulcers readily develop into chronic wounds. Diabetic foot ulcers have been neglected in health care research and planning. Clinical practice is often made on opinions than the scientific facts and figures. In addition, pathological processes are poorly understood and taught. Communication between various specialists involved is incoherent to the needs of the patients. Infections in patients with diabetes mellitus often have neuropathy and Ischemia, which combined to produce modification of bones, tissues and host factors i.e. hyperglycemia, and concomitant arterial insufficiency is all equally essential for thriving results. Primary treatment of diabetic foot infections is commonly empiric because consistent culture data is missing. There is unpredictability in prevalence of common bacterial pathogens isolated, as described in different studies 3, 4, 5 . The selection of empirical antimicrobial remedy is influenced by various factors including the probability of the causative organism type, severity of the illness (Wagner grading), and concomitant complications i.e. underlying osteomyelitis. Host factors, for example good glycemic control, co-morbid conditions, associated cardiovascular and renal diseases can influence the need for hospital admission and choice of specific agents of their dosing intervals 1. In requisites of affecting microorganisms and the possibility of successful therapy with anti-microbial treatment, acute osteomyelitis in people with diabetes is fundamentally the same as in normal healthy persons. The most complicated infection in patients with diabetes mellitus is chronic osteomyelitis which is very intricate to treat. Ample surgical debridement with antimicrobial therapy is nedeed to alleviate chronic osteomyelitis 5. The aim of this study was to assess comparative occurence of bacterial isolates cultured from diabetic foot infections presenting at the Leicester General Hospital, Leicester and Department of Microbiology, University of Cambridge, to evaluate their in vitro susceptibility to the commonly used antibacterial agents.2 Procedure for Paper Submission

2. RESEARCH DESIGN AND METHODS

Hundred diabetic patients were admitted with clinically infected foot ulcers and were studied during the period of 1st January 2017 to 30th June 2018. Ulcers were graded using the

Wegner classification. Age, sex, type and duration of diabetes, glycemic control during the hospital stay, presence of retinopathy, nephropathy (creatinine > 150 μmol/l or presence of micro- or macro albuminuria), neuropathy (absence of perception of the Semmes-Weinstein monofilament at 2 of 10 standardized planter 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 cellulites, odor, tissue, necrosis, crepitation, and pyrexia) was made. Ulcer size was determined by multiplying the longest and widest diameter and expressed in centimeter 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.

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 deep portion of the wound with a sterile curette. The soft tissue specimens were promptly sent to the laboratory and processed for aerobic and anaerobic bacteria.

2.1. Antimicrobial Susceptibility Testing:

Anti-microbial susceptibility testing of aerobic isolates was performed by the standard disc diffusion method as recommended by National Committee for clinical laboratory standards. All anaerobic isolates were tested for susceptibility to metronidazole and amoxicillin clavulanate combination (1.2 g i.v. every 8h) was started at the time of admission. This was switched to oral administration (625mg p.o. every 8 h). Metronidazole (500mg i.v. every 8 h) was added to the drug regimen if cellulites or gangrene were also present. Antibiotics were adapted based on the results of anti-microbial studies to target the most likely pathogenic organisms.

2.2. 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 Stat SPSS 10

3. RESULTS

The general and clinical statistics of 100 patients with diabetic foot are shown in Table 1. The mean age of the subjects was 56 4 years. The mean duration of the diabetes was 21.8 5.7 years and nearly two third (66.33%) had a condition of > 19 years. Nearly 34 (56.66%) had diabetic foot lesions for >1 month before presentation at hospital. In general the patients were of old age and had been on oral hypoglycemic agents.

The recommended glycemic control was not seen in any of the sixty patients. The majority of patients 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 group (Table 2). In the modern Wagner classification, foot lesions are divided into six groups 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 2. It's recognized that grade 3 through 5 have some degree of infection within lesions. In my study all patients had ulcer graded 3-5 in Wagner classification. The details of patients according to Wagner classification are shown in table 2.

The diabetic foot lesions were gangrenous in 74 patients (74.00%) cases. Twenty six (26.00%) patients had neuropathy, 82 (82.00%) had peripheral vascular disease, 13 (13.00%) had nephropathy, 15 (15.00%) had retinopathy and 72 (72.00%) were hypertensive. Osteomyelitis was present in 17 (17.00%) subjects table 1.

Seventy four (74.00%) patients had gram-negative organisms with pseudomonas aeruginosa being the most common. While gram-positive were found in 26 (26.00%) with staphylococci being the most common organism. Infection with anaerobes was found in only one patient (1.00%). Both gram-positive and –negative were seen in 14patients (14.00%). The profile of the isolated organisms is detailed in table 3. Pseudomonas aeruginosa exhibited a high frequency of resistance to the antibiotics tested. High levels of resistance to ampicillin, co-amoxiclav, ciprofloxacin, ofloxacin, cefotaxime, cefoparazone, cefazoline, cefuroxime were noted. All the isolates were uniformly susceptible to fosfomycine, levofloxine, gentamycin and amikacin. B. pyocyneus (Pseudomonas pyocyneus) was found in only one patient and it was sensitive only to fosfomycine & doxycycline. B. pyocyneus showed resistance to aminoglycosides. The results of susceptibility studies for gram-negative organism are shown in table 4. While the results of susceptibility studies for gram-positive organism are shown in table 5. S aureus exhibited a high frequency of resistance to antibiotics tested. High levels of resistance to ampicillin, ciprofloxacin, ofloxacin and cafazoline was noted. However, no high level aminoglycosides resistance was

observed. All the isolates were uniformly susceptible to fosfomycine, levofloxacin and vancomycin.

4. DISCUSSION

This study represents the clinical and microbiological assessment 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 disease5. Although infection is rarely implicated in the etiology of diabetic foot ulcer, the ulcers are susceptible to infection once the wound is present.Most of the 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 conducted1. While foot infections in the 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 result has varied and often has contradictory. A number of studies have found that Staphylococcus aureus is the main causative pathogen, but other investigations have reported a predominance of gram-negative aerobes, which is also evident in our studies6. The ratio of gram- positive aerobes to gram-negative aerobes was 1:2.75 which is in reversal to the reported7.The difference in the age, sex, ulcer grades, study-settings etc. in our study population and those earlier studies might be a reason of difference.

We observed a recovery of multidrug resistance pseudomonas aeruginosa, which is similar as reported earlier4. This raises concern as P. aeruginosa is an aggressive gram-negative Bacillus. 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 patients4, 5. Compared with earlier reports, we recovered fewer a species. Our patients had chronic draining wounds, and 73 (73.00%) 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. 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 Hertamann9. Almost 67 (67.00%) of the 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 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 patients. Results indicate that higher mortality rates were reported in patients with diabetic foot syndrome whose blood glucose level were poorly controlled10. 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 cost11, 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. Empiric antibiotic regimen for diabetic foot ulcers is given as under Antibiotic coverage should subsequently be tailored according to the clinical response of the patient, culture results, and sensitivity testing. Surgical drainage, deep debridement, or local partial foot amputations are necessary adjuncts to antibiotic therapy of infections that are deep or limb-threatening3 (Frykberg et al. 2000) (Reference—Evidence level B: uncontrolled study)

5. 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. The rate of antibiotic resistance was 67% among the isolates. All the isolates were uniformly susceptible to fosfomycine, levofloxacin, amikacin and vancomycin.

6. ACKNOWLEDGMENT

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Features	No. of Patients	Feature	No. of Patients
Age (Years)	Range	**Co-morbidities**	
< 4o	(30-75yrs)	Hypertension	63
>40		Diabetic neuropathy	32
Sex		IHD	15
Male	32	Diabetic nephropathy	9
Female	68	Diabetic retinopathy	11
		PVD	37
	71	Osteomyelitis	19
	29		
Co-morbidities		**Time of duration of infection**	
Hypertension	63	<10 days	11
Diabetic neuropathy	32	<29 day	27
IHD	15	>30 days	62
Diabetic nephropathy	9		
Diabetic retinopathy	11		
PVD	37		
Osteomyelitis	19		

TABLE1. CLINICAL DATA OF 100 DIABETIC PATIENTS WITH INFECTED FOOT ULCERS (N=100)

Table 2. Wagnner s Classification and Number of patients according to Wegner's Grade (n=100)

Modified Wagner Classification System					
Grade 0	No open Leision. May have deformity or cellulitis a)Ischemic b) Infected	1	Grade 3	Deep Ulcers with abcess, Osteomyelitis, joint sepsis a)Ischemic b) Infected	21
Grade 1	Superficial Ulcers a)Ischemic b) Infected	7	Grade 4	Localized gangarene forefoot or heel a)Ischemic b) Infected	53
Grade 2	Deep Ulcers to tendons/joint capsules a)Ischemic b) Infected	11	Grade 5	Gangarene of entire foot a)Ischemic b) Infected	7

Table 3. Bacteria isolated from diabetic foot infection of 100 patients (n=100)

Bacteria isolated	Number
Staph. Aureus	21
Staph. Epidermidis	03
Streptococci	01
Pseudomonas Aeruginosa	48
B. pyocyaneus	01
Proteus mirabilis	01
Proteus vulgaris	03
E. coli	07
Klebseilla	04
Citrobacter	06
Entrobacter Spp.	02
Morganella morgana	03

Table 4. Antimicrobial Sensitivity/Resistance of Common Gram Negative Bacteria (n=100)

Antimicrobial Agents	Pseudomonas Aeruginosa 74 (n=58)		E Coli (n=12)		Proteus (n=4)	
	Sensitive	Resistance	Sensitive	Resistance	Sensitive	Resistance
Ampicilin	0 (0%)	58(100%)	0 (0%)	12 (100%)	0 (0%)	4 (100%)
Coamoxiclave	22 (37.93%)	36 (62.06%)	7(58.34%)	5(41.66%)	1 (25.0%)	3 (75.0%)
Ciprofloxacin	25 (43.11%)	33(56.89%)	8(66.66%)	4 (33.34%)	4 (100%)	0 (0%)
Ofloxacin	10(17.25%)	48(82.75%)	12 (100%)	0 (0%)	4 (100%)	0 (0%)
Cefotaxime	28(48.27%)	30 (51.73%)	8(66.66%)	4 (33.34%)	4 (100%)	0 (0%)
Gentamicin	26 (44.83%)	32 (55.17%)	6 (50%)	6 (50%)	3 (75.0%)	1(25.0%)
Amikacin	45(77.59%)	13(22.41%)	7(58.34%)	5 (41.66%)	2(50%)	2 (50%)
Ceftazidime	35(60.35%)	23 (39.65%)	7 (58.34%)	5 (41.66%)	0 (0%)	4(100%)
Cefoperazone	23 (39.65%)	35(60.35%)	5 (41.66%)	7 (58.34%)	3 (75.0%)	1 (25.0%)
Cefazolin	27 (46.55%)	31(53.45%)	6 (50%)	6 (50%)	2(50%)	2(50%)
Fosfomycin	37 (63.79%)	21(36.21%)	0 (0%)	12(100%)	3(75.0%)	1 (25.0%)
Cefuroxime	16 (27.59%)	42 (72.41%)	4(33.34%)	8(66.66%)	4(100%)	0 (0%)
Levofloxacin	49(84.48%)	9 (15.52%)	5(41.66%)	7(58.34%)	4(100%)	0 (0%)
Doxycycline	9(15.52%)	-	-	-	-	-

Table 5. Antimicrobial Sensitivity/Resistance

Antimicrobial Agents	Staph Epidermidis (n=3)		Streptococci (n=2)		S.aureus(n=21)	
	Sensitive	Resistance	Sensitive	Resistance	Sensitive	Resistance
Ampicilin	0 (0%)	3(100%)	2(100%)	0(0%)	0 (0%)	21 (100%)
Coamoxiclave	3 (100%)	0 (0%)	2(100%)	03(0%)	12(57.14%)	9(42.86%)
Ciprofloxacin	3 (100%)	0(0%)	2(100%)	0 (0%)	15(71.43%)	6 (28.57%)
Ofloxacin	3(100%)	0(0%)	2(100%)	0 (0%)	18(85.72%)	3(14.28%)
Cefotaxime	1(33.34%)	2 (66.66%)	2(100%)	0 (0%)	15 (71.43%)	6(28.57%)
Gentamicin	2(66.66%)	1 (33.34%)	2 (100%)	0 (0%)	14 (66.66%)	7(33.34%)
Amikacin	2(66.66%)	1(33.34%)	2(100%)	0 (0%)	15(71.43%)	6 (28.57%)
Ceftazidime	1(33.34%)	2 (66.66%)	2(100%)	0 (0%)	10 (47.62%)	11(52.38%)
Cefoperazone	2(66.66%)	1(33.34%)	2(100%)	0 (0%)	13 (61.91%)	8 (38.09%)
Cefazolin	2(66.66%)	1 (33.34%)	2 (100%)	0(0%)	9(42.85%)	12(57.14%)
Fosfomycin	2(66.66%)	1(33.34%)	2 (100%)	0(0%)	19(90.47%)	2(9.53%)
Cefuroxime	2(66.66%)	1 (33.34%)	2(100%)	0 (0%)	17(80.95%)	4(19.05%)
Levofloxacin	3 (0%)	0 (33.34%)	2(100%)	0(0%)	19(90.47%)	2 (9.53%)
Vancomycin	3(100%)	0 (0%)	2(100%)	0 (0%)	21(100%)	0 (0%)

of Common Gram Positive Bacteria (n=100)

IV = intravenous.

*— Persons with seri
ternative agents.

Scenario	*Drug of choice*	*Alternatives**
Mild to moderate, localized cellulitis (outpatient)	Dicloxacillin (Pathocil)	Cephalexin (Keflex); amoxicillin/clavulanate potassium (Augmentin); oral clindamycin (Cleocin)
Moderate to severe cellulitis (inpatient)	Nafcillin (Unipen) or oxacillin	Cefazolin (Ancef); ampicillin/sulbactam (Unasyn); clindamycin IV; vancomycin (Vancocin)
Moderate to severe cellulitis with ischemia or significant local necrosis	Ampicillin/sulbactam	Ticarcillin/clavulanate (Timentin); piperacillin/tazobactam (Zosyn); clindamycin plus ciprofloxacin (Cipro); ceftazidime (Fortaz) or cefepime (Maxipime) or cefotaxime (Claforan) or ceftriaxone (Rocephin) plus metronidazole (Flagyl); cefazolin (for *Staphylococcus aureus*); nafcillin (Unipen); oxacillin
Life- or limb-threatening infection	Ticarcillin/clavulanate or piperacillin/tazobactam, with or without an aminoglycoside	Clindamycin plus ciprofloxacin or tobramycin (Nebcin); clindamycin plus ceftazidime or cefepime or cefotaxime or ceftriaxone; imipenem/cilastin (Primaxin) or meropenem (Merrem); vancomycin plus aztreonam (Azactam) plus metronidazole; vancomycin plus cefepime; ceftazidime plus metronidazole

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