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SPECTRUM OF MICROBIAL FLORA IN DIABETIC FOOT ULCER AND ITS ANTIBIOTIC SENSITIVITY PATTERN IN TERTIARY CARE HOSPITAL IN AHMEDABAD, GUJARAT

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ABSTRACT

Introduction: A Prospective study "Spectrum of Microbial flora in diabetic foot ulcer and its antibiotic sensitivity pattern" was carried out in a tertiary care hospital, Ahmedabad on 125 patients in which 85 were male patients and 40 were female patients.

Material and Methods: Swabs samples were collected from the edge and margins of ulcers and organism were identified by gram staining culture and biochemical reactions.

Results: Out of 125 specimens 108 specimens showed growth of organisms. Total 157 aerobic organisms were isolated from culture positive specimens. It represents an average of 1.25 organisms per case. Among these organisms, 130 gram negative and 27 gram positive organisms were isolated. Pseudomonas aeruginosa (30.57%) was predominant organism followed by Klebsiella spp. (22.29%). Staphylococcus aureus were 12.74% in which Methicillin resistant S. aureus (MRSA) was 55%.

Conclusion: incidence of growth was 86.4% in which Pseudomonas aeruginosa (30.57%) is most common isolate. Organisms in mixed infections showed multidrug resistance as compared to single isolated strain. Diabetic foot infections are polymicrobial in nature. As the Wagner's grade increased, the prevalence of isolates also increased.

Key words – Diabetic Foot Ulcer, Polymicrobial Ulcer, Wagner's grade

INTRODUCTION

Diabetes mellitus is a disorder that shares the phenotype of hyperglycaemia. The prevalence of diabetes depends on many aetiological factors such as age, sex, heredity, diet, socio-economic conditions and physical activity, environmental factors, life style choices etc. Diabetes is multi factorial disease in which various factors act in complex manner. [1]

Among persons with diabetes mellitus, the risk of developing a foot ulcer is estimated to be 15%. Based on recent studies, the annual population based incidence ranges from 1.0% to 4.1% and the prevalence range from 4% to 10%, suggesting the life time incidence as high as 25%. [2]

This study was performed to determine the relative frequency of aerobic microbial isolates from diabetic foot ulcers and to compare in vitro antibiotic susceptibility patterns of organisms isolated from diabetic foot ulcers and to study the incidence of emergence of multidrug-resistant organisms. As not enough data and studies are available in Gujarat, this study will give a reference for further studies and will behave as a baseline incidence.

MATERIAL & METHOD

The study was conducted on the 125 patients admitted in the surgical department of a tertiary care hospital, Ahmedabad from February 2009 to April 2010. All patients were included consecutively after the initial clinical diagnosis of diabetic foot ulcer was made and foot lesions graded depending on the severity of lesions.

Performa includes age, sex, registration no, unit, occupation, history of trauma, ulcer is healing or not healing, habits of bare foot walking, alcohol, smoking, socio-economic status, past history of the disease and duration of the disease, treatment taken and any complications, existing co-morbidities e.g. hypertension, tuberculosis, malnutrition, anaemia, peripheral vascular disease.

Criteria for inclusion of patients:

On the basis of MAGGIT WAGNER's classification,[3] diabetic foot ulcers of grade 1-5 were included and grade 0 and patient with limb amputation were excluded. Diabetes mellitus Type -2 pts were included.

Collection of specimen:

Discharge from margins and edges of ulcer was collected with help of two sterile swabs, one for gram stain and one for culture before antiseptic dressing was applied. Then swabs were immediately transported to the laboratory for culture.

Laboratory Procedures:

All laboratory methods followed standard protocols. The specific identification of bacterial pathogens was based on microscopic morphology, staining characteristics, culture and biochemical properties using standard laboratory criteria. Antimicrobial sensitivity of the bacterial isolates was done on Mueller Hinton agar using Kirby Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI).[4]

RESULTS

Table 1 shows the pattern of isolation of microbial growth from Diabetic Foot Ulcer. 125 patients with the clinical diagnosis of Diabetic Foot ulcer were enrolled for this study. Out of 125 specimens, 108 (86.4%) specimens showed bacterial growth in which 157 organisms were isolated while 17 (13.65%) specimens did not show any growth. It represents an average of 1.25 organisms per case.

Table 1: Pattern of isolation of microbial growth from Diabetic Foot Ulcer

Total Patients examined	125
Patients with microbial growth n (%)	108 (86.4%)
No growth n (%)	17 (13.65%)
Gram Negative Isolates	80.2%
Gram Positive Isolates	17.8%

Table 2: Bacterial Pathogens isolated from 108 Culture Positive Diabetic Foot Ulcers

Organism	Isolates (n=108) (%)
Pseudomonas aeruginosa	48 (30.57)
Klebsiella spp.	35 (22.29)
Escherischia coli	26 (16.56)
Staphylococcus aureus	20 (12.74)
Staphy. coagulase negative	7 (4.46)
Morganella morganii	6 (3.82)
Proteus mirabilis	5 (3.18)
Proteus vulgaris	3 (1.19)
Acinetobactor spp.	3 (1.19)
Providencia rettgeri	2 (1.27)
Providencia stuartii	1 (0.64)
Citrobacter spp.	1 (0.64)

Table 2 illustrates the bacterial isolates. Among the aerobic bacteria isolates, gram negative comprised of 82.80% and gram positive accounted for 17.20%. Pseudomonas aeruginosa was the most common isolate, accounting for 30.57%, followed by Klebsiella species, Escherichia coli and Staphylococcus aureus, comprising 22.29%, 16.56% and 12.74% respectively.

The Result of the test for susceptibility to the commonly used antibiotics are shown in table no. 3, 4 and 5. Antibiotic sensitivity pattern of staphylococcus aureus showed that Oxacillin resistance i.e., Methicillin resistant S. aureus (MRSA) was 55%. Combination of Amoxycillin/Clavulanic acid showed 70% sensitivity while Gentamicin and Cefuroxime showed 65% and 50% sensitivity respectively. Erythromycin and Lincomycin showed 75% resistant pattern while Cefadroxil showed 55% resistance. Almost all the isolates of Pseudomonas aeruginosa were sensitive to Cefipime + Tazobactam, Piperacillin + Tazobactam and Meropenem, 91.67%, 83.33%, 91.61% respectively (Table no. 3).

Table 3: Antimicrobial susceptibility pattern of Staphylococcal isolates from diabetic foot ulcers (n = 157)

Anti microbial agent	Proportion susceptible (%)	
	S. aureus (n = 20)	CoNS (n = 7)
Oxacillin sensitive	45	85.71
Erythromycin	25	42.86
Lincomycin	25	42.86
Teicoplanin	100	100
Minocycline	95	100
Linezolid	90	100
Gentamicin	65	71.43
Sparfloxacin	100	100
Cefadroxil	40	42.86
Cefuroxime	50	71.43
Amoxycillin/Clav.acid	70	85.72

Table 4: Antimicrobial sensitivity of isolates of Pseudomonas aeruginosa (n = 157)

Anti microbial agent	Proportion susceptible
Meropenem	91.61
Piperacilin	77.08
Piperacilin/Tazobactam	83.33
Cefotaxime	64.58
Ceftazidime	72.92
Cefixime	60
Cefpirome	75
Cefepime – Tazobactam	91.67
Amikacin	68.75
Gentamicin	62.5
Kanamycin	16.67
Netilmycin	79.17
Aztreonam	54.17
Ciprofloxacin	25
Lomefloxacin	27.08
Levofloxacin	16.67
Clarithromycin	4.17

Table 5 shows the antimicrobial susceptibility pattern of Gram negative bacilli other than Pseudomonas. In Klebsiella species majority of strains were sensitive to Imepenem (88.57%), Cefipime + Tazobactam (100%), Ceftrixone + Tazobactam (88.57%), Ampicillin + sulbactam (85.71%) and Amikacin (88.57%).

Table 5: Antimicrobial susceptibility pattern of gram negative bacteria except pseudomonas (n = 157)

Anti microbial agent	Proportion susceptible (%)								
	Klebsiella spp. (n = 35)	Escherichia coli (n = 26)	Morganella morganii (n = 6)	Proteus mirabilis (n = 5)	Proteus vulgaris (n =3)	Acinetobacter spp. (n = 3)	Providencia rettgeri (n = 2)	Providencia stuartii (n = 1)	Citrobacter sp. (n = 1)
Imipenem	88.57	88.46	100	100	100	100	100	100	100
Amikacin	88.57	88.46	100	100	100	100	100	100	100
Gentamicin	74.29	73.08	83.33	80	66.67	66.67	100	0	100
Ciprofloxacin	68.57	69.23	83.33	80	66.67	66.67	100	100	100
Moxifloxacin	91.43	80.77	83.33	80	66.67	66.67	50	100	100
Levofloxacin	80	80.77	66.67	60	66.67	66.67	50	100	100
Ofloxacin	80	88.46	100	100	66.67	66.67	100	100	100
Gatifloxacin	74.29	88.46	83.33	100	66.67	66.67	100	100	100
Cefotaxime	17.14	23.08	83.33	80	66.67	0	0	0	100
Cefuroxime	14.29	19.23	66.67	60	66.67	0	0	0	100
Ceftizoxime	11.43	19.23	66.67	60	66.67	0	0	0	100
Ceftriaxone	51.43	23.08	83.33	60	66.67	0	0	0	100
Cefipime	80	88.46	100	100	100	100	50	0	100
Tetracycline	17.14	15.38	0	40	33.33	33.33	50	0	0
Chloramphenicol	48.57	11.53	16.67	40	0	0	0	0	0
Co –trimoxazole	2.86	6.7	16.67	0	0	33.33	0	0	100
Ampicillin/ Sulbactam	85.71	88.46	100	100	100	100	100	0	100
Cefipime/Tazobactam	100	100	100	100	100	100	100	100	100
Ceftriaxone/ Tazobactam	88.57	88.46	100	100	100	100	50	100	100

DISCUSSION

Diabetic foot ulcer is the most common complication requiring hospitalization among diabetic patients. It is also the most common cause of non-traumatic lower extremity amputations. Physicians have an important role in the prevention, early diagnosis and management of diabetic foot complications. Management however entails an extensive knowledge of the major risk factors for amputation and preventive maintenance. This study allowed us to evaluate the degree of this problem in our institution.

The study is compared with studies of various researchers in India and across the world. In the present study males to females ratio was 2.12:1 which compares well with studies of Chang et al [5] 1:0.92, Llanes et al[6] 1:0.64 while a study by Benedicto et al[7] differs markedly showing an incidence of 7:1 which could be explained on the basis of geographical differences. The mean age of patients in the present study is 50.25 $\pm$ 12.5 which is on the lines of study by Llanes et al [6] 57$\pm$ 14.07 while the study of Balderas and Benedicto et al [7] showed an incidence of 68 $\pm$ 5.9 and 6 to 7 decade respectively.

In the present study, 157 organisms were isolated from 125 patients and average of 1.25 organisms per patient was found. The observations are similar with Ekta et al [8] while differ significantly from Chincholikar[9] in which the major organism are GPC which again indicated the role of geographical variations in microbial etiology.

Table no 6 summarizes the comparison of isolates from DFU. Pseudomonas aeruginosa was predominantly isolated organism 30.57% followed by Klebsiella 22.29%, E coli 16.56% and S. aureus 12.74% . Other organisms like Morganella morganii 3.82%, CONS 4.46% and Proteus mirabilis 3.18% were also isolated. Almost similar results were obtained by Ekta et al, [8] Shankar et al [10] and Prabahakar et al. [11]

Table 6: Comparison of studies on isolates in DFU

Isoletes	Chinch-olikar[9]	Ekta[8]	Present study
Avg. organism/patient	1.3	1.52	1.25
Predominant isolate	GPC	GNB	GNB
Pseudomonas	19%	22%	30.57%
S. aureus	31%	19%	12.74%
Klebsiella spp.	18%	17%	22.29%
E coli	15%	18%	16.56%
Proteus spp.	9.3%	11%	10.82%

In our study, Methicillin resistance was seen in 55% of the S. aureus which is also in concordance with findings by Gadepalli et al[12], Ekta et al[8] and Chincholikar [9] in which MRSA was seen in 56%, 55.50% and 55.56% respectively.

Table no 7 and 8 summarize the comparison of antibiotic sensitivity patterns of Pseudomonas and Klebsiella respectively with other studies. The antibiotic sensitivity pattern of Pseudomonas was approximately similar to study by Ekta et al. Most of the isolates were

sensitive to Imipenam. Polymicrobial resistance showed to Flouroquinolones and Cephalosporins except Ceftazidime and Cefotaxime.

Table 7: Comparative study of pseudomonas sensitivity pattern to anti microbial drugs

Antimicrobial agents	Ekta[8]	Present study
Imepenem	100%	91.61%
Piperacillin	83.33%	77.08%
Ceftazidime	94.44%	72.92%
Amikacin	78.95%	68.75%
Ciprofloxacin	62.50%	75%

Table 8: Comparative study of Klebsiella sensitivity pattern to anti microbial drugs

Anti microbial agents	Ekta[8]	Present study
Imepenem	100%	88.57%
Cefuroxime	41.67%	17.14%
Cetriaxone	41.67%	51.43%
Ciprofloxacin	47.06%	68.57%
Amikacin	56.25%	88.57%

In present study, Klebsiella and E coli were most sensitive to combinations like Ampicillin + Sulbactam, Cefipime + Tazobactam, Ceftriaxone + Tazobactam, Imepenem and these organisms were resistant to Cephalosporins, Chloramphenicol, Cotrimoxazole and Tetracycline. Similar results were found by Ekta et al[8].

CONCLUSION

In the present study "Spectrum of Microbial flora in diabetic foot ulcer and its antibiotic sensitivity pattern" 125 specimens were taken. Out of 125 specimens 108 specimens showed growth of organisms. Total 157 aerobic organisms were isolated from culture positive specimens. It represents an average of 1.25 organisms per case. Among these organisms, 130 gram negative and 27 gram positive organisms were isolated.

Pseudomonas aeruginosa (30.57%) is predominant organism followed by Klebsiella spp. (22.29%), E. coli (16.56%), Staphylococcus aureus (12.74%), Coagulase Negative Staphylococcus (4.46%), Morganella morganii (3.82%), Proteus mirabilis (3.18%), Proteus vulgaris (1.19%), Acinetobactor spp. (1.19%), Providencia rettgeri (1.27%), Providencia stuartii (0.64%) and Citrobacter spp. (0.64%).

High level of resistance to Cephalosporins, Cotrimoxazole, Macrolides were found in all isolated organisms while Imepenem, Flouroquinolones and drug combinations like Ampicillin + Sulbactam, Cefepime + Tazobactam and Ceftriaxone + Tazobactam were most effective against gram negative organisms. Gram positive organisms showed sensitivity to Teicoplanin, Minocycline, Sparfloxacin and combination of Amoxycillin + Clavulanic acid. All isolates showed intermediate sensitivity to Amikacin. Cefepime + Tazobactam, Imepenem and Amikacin would be appropriate empirical treatment.

Organisms in mixed infections showed multidrug resistance as compared to single isolated strain. Diabetic foot infections are polymicrobial in nature. As the Wagner's grade increased, the prevalence of isolates also increased. This high level of resistance observed in the present study may be due to the wide spread use of broad spectrum antibiotics leading to survival advantage of resistant pathogens.

This increasing incidence of multidrug resistant organisms is a potential risk factor in management of diabetic foot infections which may lead to devastating complications like systemic toxicity, gangrene formation and amputation of lower extremity. These multidrug resistant organisms are frequently resistant to many classes of antibiotics so it is necessary for the clinician to be completely aware of the prevalence rate of multidrug resistant organisms and their management strategies. So this study will help the clicians to choose appropriate antibiotic or combination of antibiotics for the treatment of Diabetic Foot Ulcer.

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