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"MICROBILOGICAL PROFILE OF DIABETIC FOOT: A PROSPECTIVE CROSS SECTIONAL STUDY FROM A TERTIARY HOSPITAL IN SOUTH INDIA."

KEYWORDS: Diabetes mellitus, MRSA, diabetic ulcer.

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ABSTRACT

Background: *Although infection is rarely implicated in the etiology of diabetic foot ulcers, the ulcers are susceptible to infection once the wound is present. It is essential to assess the magnitude of bacterial infection of these lesions to avoid further complications.*

Methods: *a prospective cross sectional study of 150 patients conducted in the Department of Surgery at Govt. Medical College, Kozhikode.*

Results: *In our study 56.7% (85) of patients had polymicrobial infections and 41.3% (62) had mono microbial infections. 62% of cases yielded only gram negatives and 9.3% yielded only gram positive organisms. 62.6% of all the isolates were multidrug resistant.*

Conclusions: *To prevent emergence of multidrug resistant organisms, clinically uninfected ulcers need not be given antibiotics. Tissue specimens should be sent for culture before starting antibiotic therapy.*

INTRODUCTION

Although infection is rarely implicated in the etiology of diabetic foot ulcers, the ulcers are susceptible to infection once the wound is present. It is essential to assess the magnitude of bacterial infection of these lesions to avoid further complications. Early diagnosis of microbial infection is aimed to institute the appropriate antibacterial therapy and to avoid further complications1. Foot infections are among the most common causes of hospitalisation in the diabetic population, accounting for 20% of all diabetes-related admissions2.

METHODS

STUDY DESIGN : Prospective Study
SAMPLE SIZE : 150
STUDY SETTING : Department of Microbiology and Surgery, Govt. Medical College, Kozhikode.
STUDY GROUP : Patients admitted in surgery wards
INCLUSION CRITERIA : Diabetic foot ulcers ≥ Wagner's grade 2
EXCLUSION CRITERIA : Diabetic foot ulcers of Wagner's grade 0 and 1
PERIOD OF STUDY : One year [1.4.2015 to 31.3. 2016]

SPECIMENS COLLECTED

Tissue specimen or curettings from ulcer base
Aspirates in cases of abscesses

PROCESSING

Specimens were homogenised with a sterile mortar and pestle.
Specimens were subjected to
1.Gram staining
2.Culture & sensitivity

CULTURE

Specimens were inoculated to
(1) Blood agar (BA)
(2) MacConkey agar
(3) Salt agar (SA)
(4) Glucose broth (GB)
(5) Robertson's cooked meat medium (RCM)

Antibiotic susceptibility testing was done by standard disc diffusion method. Antibiotic sensitivity of staphylococci was done using Kirby-Bauer disc diffusion method on Mueller-Hinton Agar. DETECTION OF MRSA: Cefoxitin disc diffusin method was used as per CLSI Guide lines. ESBL screening was done for all gram negative isolates which were resistant to 3rd generation cephalosporin (except Acinetobacter spp and Pseudomonas aeruginosa). Inducible clindamycin resistance was detected by approximation of clindamycin and erythromycin discs

RESULTS: 241 organisms were isolated from 150 patients. 50.7% of patients had infection due to two organisms whereas 6% had infection due to three organisms. 2% patients had sterile culture.[table1]

Table 1: Distribution of cases according to number of isolates

Number of isolates	Number of cases	%
0	3	2
1	62	41.3
2	76	50.7
3	9	6
Total	150	100

As shown in table 2, 14 (9.3%) patients had infection due to gram positive organisms whereas 93 (62%) had infection with gram negative organisms only. 40 (26.7%) patients had mixed gram positive and gram negative infections.

Table 2: Distribution of gram positive and gram negative isolates

Distribution	Number	%
Sterile	3	2
Gram positive	14	9.3
Gram negative	93	62
Gram positive and negative	40	26.7
Total	150	100

Total 241 organisms were isolated from 150 samples in which Gram negative organisms were predominant (77.6%). 25.3% of isolates were *Pseudomonas aeruginosa*. Other common organisms isolated were *Escherichia coli* (19.9%), *Klebsiella pneumoniae* (11.2%), MRSA (11.2%).[table3] **graph1.**

Table 3: Profile of bacteria isolated from diabetic foot ulcers

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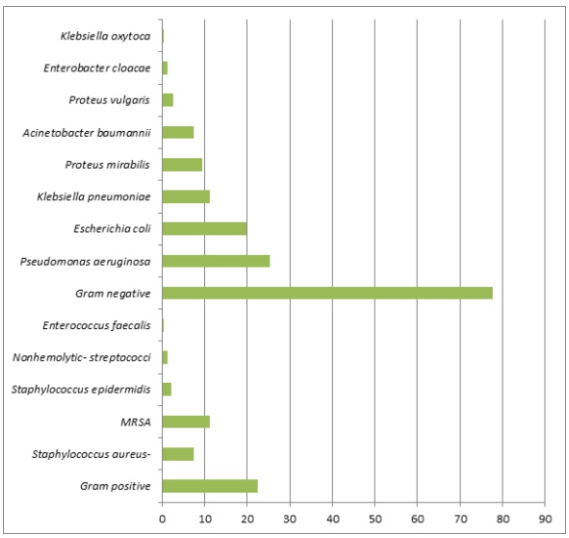
Bacteria isolated	Number	%
Gram positive	54	22.4
Staphylococcus aureus-		
MSSA	18	7.5
MRSA	27	11.2
Staphylococcus epidermidis	5	2.1
Nonhemolytic- streptococci	3	1.2
Enterococcus faecalis	1	0.4
Gram negative	187	77.6
Pseudomonas aeruginosa	61	25.3
Escherichia coli	48	19.9
Klebsiella pneumoniae	27	11.2
Proteus mirabilis	23	9.5
Acinetobacter baumannii	18	7.5
Proteus vulgaris	6	2.5
Enterobacter cloacae	3	1.3
Klebsiella oxytoca	1	0.4

graph1: Distribution of organisms isolated from specimens



Among 45 isolates of *Staphylococcus aureus* 27 (60%) were resistant to cefoxitin. Of the Methicillin Sensitive S*taphylococcus aureus* isolates 22% of isolates were sensitive to penicillin, 39% of isolates were sensitive to erythromycin, 72% of isolates were sensitive to gentamicin. 33% of isolates were sensitive to co-trimoxazole, 83% were sensitive to clindamycin and amikacin.

Out of the 27 isolates of MRSA, 100% of them showed resistance to penicillin and first generation cephalosporin. 82% of the isolates were sensitive to clindamycin and amikacin. 4% of the isolates were sensitive to erythromycin, 7.4% of isolates were sensitive to gentamicin and 19% were sensitive to cotrimoxazole.

There were 5 isolates of *Staphylococcus epidermidis* of which 3 (60%) were resistant to cefoxitin. Sensitivity to co-trimoxazole and first generation cephalosporin was 20% and 40% respectively. 80% of the isolates showed sensitivity to clindamycin.

There were 3 isolates of nonhemolytic streptococci, out of which 33% were sensitive to penicillin and erythromycin. 33% of isolates were sensitive to 1st generation cephalosporin. 100% of isolates were sensitive to vancomycin.

There was only one isolate of *Enterococcus faecalis,* which was sensitive only to vancomycin.

Among 61 isolates of *P.aeruginosa,* all were sensitive to imipenem (100%). 57 (93%) isolates were sensitive to piperacillin+tazobactam and 42 (69%) isolates were sensitive to piperacillin. 34 (56%) isolates were ceftazidime sensitive, 54 (88.5%) isolates were sensitive to ceftazidime+clavulanic acid. 28 (46%) isolates were amikacin sensitive and 20 isolates (33%) were ciprofloxacin sensitive. Gentamicin and Netilmicin sensitivity of isolates were 25% and 18% respectively.

Out of 48 *E.coli* isolates, 100% showed sensitivity to imipenem. 46 isolates (95.8%) showed sensitivity to piperacillin+tazobactam and 42 (87.5%) isolates were sensitive to cefoperazone+sulbactam. 29 (60.4%) isolates were piperacillin sensitive, 28 (58%) were amikacin sensitive and 27 (56.25%) were amoxicillin + clavulanic acid sensitive. Only 12 (25%) isolates were 3rd generation cephalosporin sensitive. Ampicillin sensitivity was only 4%.

Among the 27 isolates of *Klebsiella pneumoniae,* 100% showed sensitivity to imipenem. 26 (96.3%) isolates showed sensitivity to piperacillin+tazobactam, 24 (88.8%) isolates were sensitive to cefoperazone+sulbactam and 21 (77.7%) were piperacillin sensitive.16 (59.3%) isolates were amikacin sensitive. There was one *Klebsiella oxytoca* isolate which was sensitive to imipenem, 3rd generation cephalosporin piperacillin+tazobactam, cefoperazone+ sulbactam and imipenem.

Out of 23 Proteus mirabilis isolates 96% showed sensitivity to piperacillin+tazobactam and cefoperazone+sulbactam and 74% showed sensitivity to piperacillin. *Acinetobacter baumannii* isolates showed 100% sensitivity to imipenem, 72.2% sensitivity to cefoperazone+sulbactam and 61% sensitivity to piperacillin+ tazobactam.

Table 4 : Distribution of mechanism of drug resistance among gram negative isolates (number of isolates and percentage)

Resistance Mechanism	*E. coli* (48)		*K.pneumoniae* (27)		*P.mirabilis* (23)		*P.vulgaris* (6)		*E.cloacae* (3)	
	No.	%	No.	%	No.	%	No.	%	No.	%
ESBL	30	62.5	20	74	9	39	2	33.3	2	66.6
Amp C	6	12.5	4	14.8	4	17.4	0	0	0	0

Out of 241 isolates, 151 (62.6%) were multidrug resistant. MRSA constituted 60% of *S.aureus* isolates.table5

Table 5: Distribution of multidrug resistant organisms among the isolates

Organism	Total isolates	MDRO-No	Percentage
Staphylococcus aureus	45	27	60
S. epidermidis	5	3	60
E.coli	48	36	75
P. aeruginosa	61	27	44.3
Klebsiella pneumoniae	27	24	88.8
Proteus mirabilis	23	13	56.5
Proteus vulgaris	6	2	33.3
A.baumannii	18	17	94
Enterobacter cloacae	3	2	66.6

DISCUSSION:

In this study, 241 organisms isolated from 150 patients with an average of 1.6 species per patient. These results were comparable with several other studies[3,4] Some studies showed higher number of isolates, this may be due to isolation of anaerobes. Due to the lack of resources our study was limited for aerobic isolates only.

In our study 56.7% (85) of patients had polymicrobial infections and 41.3% (62) had mono microbial infections.Predominance of polymicrobial culture is in accordance with studies of Umadevi et al.

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and Sankar et al.[3,5]. In our study monomicrobial involvement was seen in 41.3% of cases. This can be explained by the fact that 38.67% of patients presented within 2 weeks of illness and chances of polymicrobial infection are high when wound become chronic.

In this study 62% of cases yielded only gram negatives and 9.3% yielded only gram positive organisms. Both gram positive and gram negative organisms were isolated from 26.7% of cases. Culture was sterile in remaining 2% of cases. Out of the 241 isolates 77.6% were gram negative and 22.4% were gram positive with a ratio of 3.5:1. Similar distribution of isolates were seen in the study by S Umadevi et al. in 2009 in Pondicherry in which 52.4% had infection due to gram negative isolates and 8.6% had gram positive isolates[3]. The remaining 39% patients had both gram positive and gram negative organisms. In a study conducted by Shankar et al. in South India also showed predominant gram negative infections (57.6%) [5]. But earlier studies have documented gram positive bacteria as the predominant organisms associated with diabetic foot infections[6,7]. Therefore, there seems to be a changing trend in the organisms causing diabetic foot infections, with gram negative bacteria replacing gram positive bacteria as commonest agents.

Pseudomonas aeruginosa was the most frequent pathogen isolated (25.3%) followed by *E.coli* (19.9%) and *Staphylococcus aureus* (18.7%). Almost similar results were seen in Bansal et al. study (2007) which showed 21.67% of *Pseudomonas aeruginosa*, 18.88% of *Staphylococcus aureus* and 18.18 % Escherichia coli8. S Umadevi et al. (2009) reported a 20.5% prevalence of *Klebsiella pneumoniae* followed by *Pseudomonas aeruginosa* and Staphylococcus aureus, both 17%[3]. Our study observed an increased predominance of *Pseudomonas aeruginosa* among gram negative isolates. In a study conducted in Coimbatore by Murugan *et al.* (2008) showed a predominance of *Pseudomonas* spp[9].

In our study out of 45 Staphylococcal isolates, 27 were MRSA (60%), being the most common gram positive isolate. Almost similar results were seen in study conducted by Zubair *et al.* (57.1%)10. Several studies conducted in different parts of world also reported prevalence of MRSA varying from 4.4 to 66.6%[11,12,13]. The Manchester UK group reported a near doubling of MRSA prevalence in diabetic foot ulcer studies between 1999 and 2003[14,15]. MRSA isolates were 100% sensitive to vancomycin and linezolid. All MRSA isolates showed high degree resistance to erythromycin, gentamicin and cotrimoxazole(96%, 92.6% and 81% respectively). 82% of the isolates were sensitive to clindamycin and amikacin.

Other gram positive isolates include Staphylococcus aureus 7.5% (18) *Staphylococcus epidermidis* 2.1% (5), Non hemolytic streptococci 1.2% (3) and *Enterococcus faecalis* 0.4% (1). Methicillin sensitive staphylococcal isolates were sensitive to penicillin (22%), erythromycin (39%) and cotrimoxazole (33%), amikacin (83%), clindamycin (83%), gentamicin (72%). Out of the five *S.epidermidis* isolates, three were monomicrobial. Of the five isolates three were methicillin resistant and two were methicillin sensitive. Two methicillin resistant isolates were from grade 4 ulcers and these patients underwent mid tarsal amputation. In other three patients wound was healing well with appropriate antibiotic therapy. All the three methicillin resistant isolates were resistant to gentamicin and erythromycin and sensitive to vancomycin, linezolid and amikacin. 20% of the isolates were sensitive to co-trimoxazole. *Enterococcus faecalis* was isolated from one sample which is low as compared to other studies[1,16,4]

Next to Pseudomonas aeruginosa, other gram negative isolates were predominantly from Enterobacteriaceae family, which includes *E.coli* (19.9%), *K.pneumoniae* (11.2%), *Proteus* spp (12%) and *Enterobacter cloacae* (1.3%). *Acinetobacter baumannii* constituted 7.5 % of gram negative isolates. There are studies showing Enterobacteriaceae and *Pseudomonas* spp as predominant pathogens[1,16,4].

Sensitivity pattern of gram negative isolates are as follows. All gram negative isolates were 100% sensitive to imipenem. Among gram negative bacteria, *P.aeruginosa* showed maximum sensitivity to piperacillin+ tazobactam (93%) and ceftazidime+clavulanic acid (88.5%). Almost similar results were reported by Gadepalli et al. in their study[16]. *E.coli, K. pneumoniae and P. mirabilis* isolates also showed maximum sensitivity to piperacillin+ tazobactam (95.8%, 96.3% and 96% respectively) and cefoperazone+ sulbactam (87.5%, 88.8% and 96% respectively). *Acinetobacter baumannii* showed maximum sensitivity to cefoperazone+sulbactam (72.2%) and piperacillin+tazobactam (61%).

In this study multidrug resistant gram positive organisms isolated were MRSA and methicillin resistant coagulase negative staphylococci. In our study majority of gram positive and gram negative isolates were multidrug resistant. These results were in accordance with findings by Vinod Kumar et al.[17].

In gram negative bacteria, extended spectrum β lactamases (ESBL) have emerged as an important mechanism of resistance.Phenotypic confirmation was carried out using combined disc diffusion method for ESBL and screening for AmpC β-lactamase production was performed by cefoxitin disc test. Among gram negative isolates, maximum ESBL production was found in *K. pneumoniae* (74%), followed by *Enterobacter cloacae* (66.6%). AmpC production was maximum in *P. mirabilis* (17.4%) and minimum in E. coli (12.5%). These results are comparable to study by Zubair et al.80. In a study conducted in Brazil in 2001 only 6% of *E.coli* isolates were ESBL producers whereas Shobha et al. and Varaiya et al. reported a high prevalence of ESBL in their study in 2009 and 2006 respectively1[8,19,20]. Out of 150 patients, 115 (76.6%) were infected with multidrug resistant organisms, which is comparable to study conducted in Aligarh by Shakil et al. 71.42% of patients were infected with MDRO in that study. The present study confirms that MDRO infection is extremely common in hospitalised patients with diabetic foot ulcers. The high rate of antibiotic resistance observed in this study may be due to the fact that our study was conducted in a tertiary care hospital with widespread use of broad spectrum antibiotics leading to selective survival advantage of pathogen. Most of the patients coming here are getting prior antibiotic treatment from peripheral health centers and so clinicians are forced to start these patients on higher antibiotics after admission.

CONCLUSION:

To prevent emergence of multidrug resistant organisms, clinically uninfected ulcers need not be given antibiotics. Tissue specimens should be sent for culture before starting antibiotic therapy. As majority of the isolates in our study were multidrug resistant gram negative bacteria, patients may be empirically treated with a combination of piperacillin + tazobactam, cloxacillin and metronidazole. In MRSA infections, patients have to be isolated, barrier method of nursing and hand washing measures in between handling patients should be followed. In case of mild MRSA infection, vancomycin alone and in life threatening infection, a combination of vancomycin and amikacin is useful. A change of antibiotic if necessary, is advisable as per the sensitivity pattern after obtaining culture report.

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