RESEARCH ARTICLE

A CLINICAL STUDY OF PREVALENCE OF BACTERIA ISOLATED FROM TYPE 2 DIABETIC FOOT ULCERS AND ITS ANTIBIOTIC SUSCEPTIBILITY

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ABSTRACT

Background and Objectives: The annual incidence of diabetic foot ulcer in diabetic patients is known to be about 2% to 5% and the lifetime risk ranges from 15% to 20%. The main objective of this studyis to determine the most prevalent pathogens involved in diabetic foot ulcers and its antibiotic susceptibility. The predominant organism related to the different Wagner grades were also assessed as part of this study. **Methods:** The present study was a hospital-based descriptive study conducted on patients admitted with diabetic foot in the department of General Surgery at Government Medical College, Kozhikode, Kerala during the period from August 2019 to July 2020. Tissue or swab culture was taken from the ulcer and the antibiotic sensitivity was tested by Kirby-Bauer Disk Diffusion Susceptibility Test. **Results:** At the time of presentation more than 50% of ulcers were of Wagner's grade II and III. The predominant organism isolated from diabetic foot infections were Klebsiella (32.98%) followed by Pseudomonas (24.47%). Multi-drug resistance was seen in 12 out of 62 samples that yielded Klebsiella, 2 out of 46 samples that yielded Pseudomonas, 4 out of 22 samples that yielded E. Coli and MRSA was seen in 8 out of 22 samples that yielded Staph. Aureus. **Discussion and Conclusions:** The predominant organisms isolated were Klebsiella and Pseudomonas species and were mostly sensitive to Piperacillin/Tazobactam, Meropenem, Ciprofloxacin and Amikacin. Hence it is advisable to start patients on empirical therapy with these drugs for better outcome. Proper assessment of ulcer and targeted antibiotics against these organisms can aid in early healing and avoidance of diabetic foot related complications.

INTRODUCTION

The prevalence of diabetes mellitus is expected to increase, and the number of diabetic patients worldwide is on the rise. The annual incidence of diabetic foot ulcer (DFU) or necrosis in diabetic patients is known to be about 2% to 5% and the lifetime risk ranges from 15% to 20% [1]. Diabetes is one of the high-risk factors for peripheral arterial disease [2] and PAD is associated with a 20-fold higher prevalence in patients with diabetes. It is known to be a risk factor for the highest severity of single factors in diabetic patients [3]. In addition, the probability of amputation within one year after the first ulcer or gangrene is 34.1% and the mortality rate has been reported to be approximately 5.5% [4].

Optimal outcome can be experienced by close and regular attention to details of acute infection and diabetic control.

Objectives

1.To study the most prevalent pathogens involved in diabetic foot ulcers and its antibiotic susceptibility.
2.To assess the predominant organism related to the different Wagner grades.

METHODOLOGY

STUDY DESIGN: Descriptive study. Patients admitted with diabetic foot will be evaluated in the study.

STUDY SETTING: Department of General Surgery, Calicut Medical College, Kerala.

SAMPLING: Sample size was calculated as:

$$\frac{4pq}{D2} = \frac{4 \times 21.6 \times (100-21.6)}{36} = 188$$

Inclusion criteria

- Diabetic patients admitted in our institution with foot infection of varying severity on basis of Wagner's Ulcer Classification.
- Wagner Grades 1 – 5.

Exclusion criteria

- Any patient who does not wish to participate in the study.
- Wagner Grade 0.
- Patient with previous limb amputations.

Sampling Procedure: Consecutive cases of diabetic foot admitted in our institution, which meets the inclusion criteria will be included in the study until sample size is reached.

STUDY PERIOD: August 2019 to July 2020

METHODS

Method of data collection

- Proper history taking and examination and blood tests.
- Tissue or swab culture after cleaning the ulcer with normal saline.
- Antibiotic sensitivity testing by Kirby-Bauer Disk Diffusion Susceptibility Test.

Results: At the time of presentation more than 50% of ulcers were of Wagner's grade II and III.

Table 1. Grade of ulcer at the time of admission

	Number	Percentage
Grade I	27	14.36%
Grade II	49	26.06%
Grade III	58	30.85%
Grade IV	32	17.02%
Grade V	22	11.70%

Culture Results: The predominant organism isolated from diabetic foot infections were Klebsiella (32.98%) followed by Pseudomonas (24.47%). The grade of the ulcer with relation to the various pathogens isolated are depicted in Chart 1.

Table 2. Organisms isolated

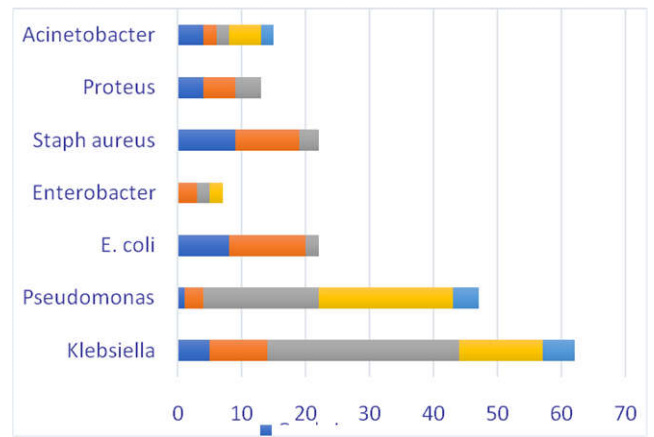| Organism Grown	Number	Percentage
Klebsiella	62	32.98%
Pseudomonas	46	24.47%
E. coli	22	11.70%
Enterobacter	8	4.26%
Staph aureus	22	11.70%
Proteus	13	6.91%
Acinetobacter	15	7.98%

Chart 1. Organisms and Grade of Ulcer

Antibiotic Sensitivity among prevalent organisms: Of the 62 samples which yielded Klebsiella as the pathogen, 12 were MDR Klebsiella. Among the antibiotics, Piperacillin/Tazobactam, Ciprofloxacin, Amikacin and Meropenem were found to be sensitive.
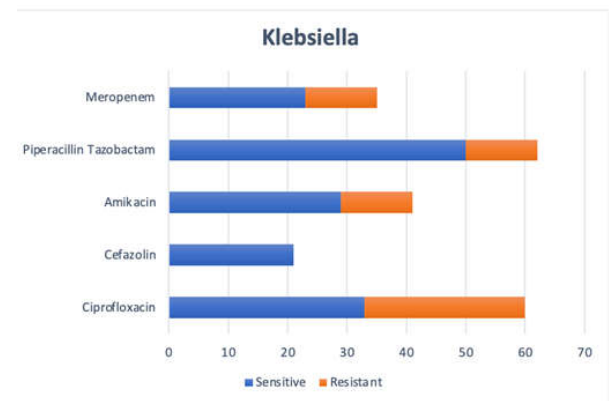

Chart 2. Antibiotic Sensitivity - Klebsiella

Of the 46 samples of Pseudomonas, 2 were MDR Pseudomonas. Among the antibiotics, Ciprofloxacin, Piperacillin/Tazobactam and Amikacin were found to be sensitive against all except MDR forms.
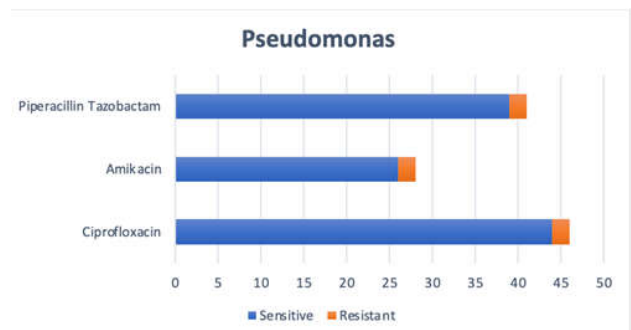

Chart 3: Antibiotic Sensitivity - Pseudomonas

Among 22 samples that yielded E.coli as the pathogen, 4 were MDR E.coli. Sensitive antibiotics were Ampicillin, Gentamicin, Ciprofloxacin, Amikacin, Piperacillin/Tazobactam and Meropenem.
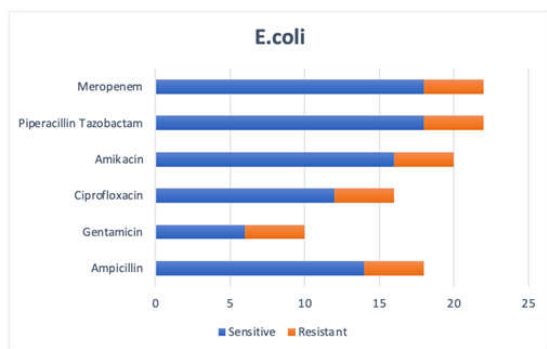
Chart 4. Antibiotic Sensitivity – E.Coli

Enterobacter species were yielded from 8 samples. All of them were sensitive to Meropenem and Cefotaxime.
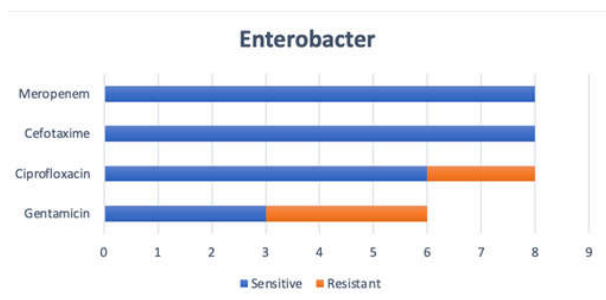

Chart 5. Antibiotic Sensitivity - Enterobacter

Among the 22 samples that yielded Staph aureus, 8 were MRSA. Staph aureus was found to be sensitive to Ampicillin, Gentamicin, Cefazolin and Cloxacillin, while MRSA was sensitive to both Vancomycin and Linezolid.
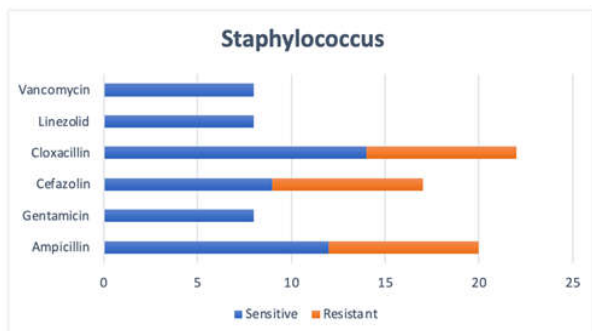

Chart 6. Antibiotic Sensitivity - Staphylococcus

Proteus was obtained in culture from 13 samples and were found to be sensitive to Ampicillin, Gentamicin, Ciprofloxacin, Cefazolin and Amikacin.
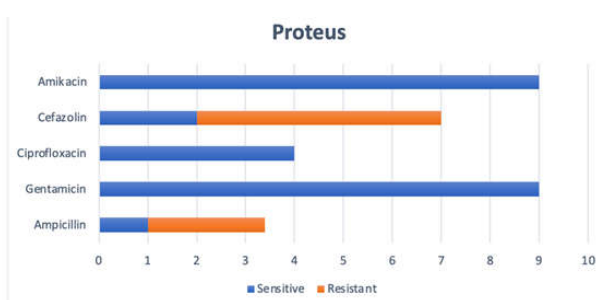

Chart 7. Antibiotic Sensitivity - Proteus

The 15 samples that yielded Acinetobacter were MDR with no documented sensitivity to any of the tested drugs.

DISCUSSION

Diabetes Mellitus represents a major public health threat worldwide. A serious complication of diabetes is the development of foot ulcers. The Diabetic Foot Syndrome is the result of complications of diabetes mellitus and may lead to amputations, restricted mobility, and even death. The syndrome includes ulcers of the lower extremities and diabetes-related Charcot foot. Charcot foot is an inflammatory process, characterized by varying degrees of bone and joint disorganization secondary to neuropathy, trauma, and alterations of bone metabolism. The knowledge of involved organisms and their antibiotic susceptibility are of primary importance not only in the core understanding of disease pathology but also for superior disease management. The study was done on 188 patients who presented to our hospital with varying grades of diabetic foot.

Grade of ulcer at time of admission: In our study, 58 patients (30.85%) had Grade III ulcer, 49 (26.06%) had Grade II ulcer and Grade IV ulcer was noted in 32 (17.02%) patients. In a similar study conducted at a tertiary care hospital on 61 patients by Edo et al for assessing the risk factors and ulcer grades, reported that grade II and III Wagner lesions were the most frequently noted grades of foot ulceration, which accounted to 8.2 % and 36.1 % respectively. They concluded that baseline ulcer grade has the strongest impact for the risk of amputations and disarticulations in diabetic population [5].

Culture Results and Antibiotic sensitivity: In our study on 188 patients, predominant organism was Klebsiella in 62 (32.98%) patients followed by Pseudomonas in 46 (24.47%) patients. There were an equal number of E. coli and Staph aureus isolates in the study group which accounted to 22 (11.70%) patients. Unlike in many other studies our study had 7.98% (n=15) Acinetobacter species isolates, which were all multidrug resistant. Other isolates were Proteus and Enterobacter which were seen in 13 (6.91%) and 8 (4.26%) respectively. Among the 62 samples that yielded Klebsiella, 12 were MDR species. The sensitive antibiotics were Piperacillin Tazobactam (n=50), Ciprofloxacin (n=33), Amikacin (n = 29), Meropenem (n=23), Cefazolin (n=21), while 27 samples showed resistance to Ciprofloxacin. Only MDR samples showed resistance to Piperacillin, Amikacin and Meropenem in the studied cultures. In the 46 samples that yielded Pseudomonas, 2 were MDR forms. Ciprofloxacin (n=44), Piperacillin Tazobactam (n=39) and Amikacin (n=26) were the sensitive antibiotics with MDR forms showing resistance to all these three antibiotics. Among 22 samples that yielded E. coli as the pathogen, 4 were MDR E. coli. Sensitive antibiotics were Piperacillin Tazobactam (n=18), Meropenem (n=18), Ampicillin (n=14), Amikacin (n=16), Ciprofloxacin (n=12) and Gentamicin (n=6) among which most strains (n=18) were sensitive to Piperacillin Tazobactam and Meropenem with MDR forms resistant to all tested antibiotics. Enterobacter species were yielded from 8 samples. All of them were sensitive to Meropenem and Cefotaxime. Other susceptible antibiotics were Gentamycin (n=3) and Ciprofloxacin (n=6). Among the 22 samples that yielded Staph aureus, 8 were MRSA. Staph aureus was found sensitive to Ampicillin (n=12), Gentamicin (n=11), Cefazolin (n=9) and Cloxacillin

(n=14), while MRSA was sensitive to both Vancomycin (n=8) and Linezolid (n=8). Proteus was obtained in culture for 13 samples. 4 of them were MDR species. They were found sensitive for Ampicillin (n=1), Gentamicin (n=9), Ciprofloxacin (n=4), Cefazolin (n=2) and Amikacin (n=9). Those 15 samples that yielded Acinetobacter were MDR with no documented sensitivity to any of the tested drugs. Hence a repeat sample was sought for. In a study conducted by Singh et al on 105 diabetic foot ulcer patients, it was found that 73.7% were Gram-negative bacteria, and 27.3% were Gram-positive. Pseudomonas was the predominant isolate (27.3%) and it was found to be sensitive to imipenem (90%), amikacin (86.6%), gentamicin (83.3%), and cefotaxime (80%). Staphylococcus aureus found in 19.1% of patients was sensitive to amikacin and gentamicin (100%), and ofloxacin (90%). They suggested that Amikacin and Gentamicin can be used as empiric antibiotics for treatment of infected diabetic foot ulcers [6].

Another study was conducted by Sugandhi et al in a sample population of 51 patients, Gram negative organisms (51%) were more prevalent than Gram positive (49%) organisms. Pseudomonas aeruginosa (35%), Enterococcus spp. (4%), Escherichia coli (4%) were the common species isolated from these samples. The antibiotic sensitivity pattern showed Meropenem, Piperacillin, Cefoperazone/Sulbactam, Piperacillin/Tazobactam and Amikacin as the most effective antimicrobial agents for the gram positive and Gram-negative bacterial species [7]. In a prospective study by Saseedharan et al on 261 patients it was observed that Gram negative pathogens were predominant (58.5%). The most frequently isolated bacteria were Staphylococcus aureus (26.9%), followed by Pseudomonas aeruginosa (20.9%) [8].

Prevalent infection and the grade of foot lesion: Among the total 188 samples studied, most Grade III and Grade IV infections were caused by Klebsiella and Pseudomonas. 30 and 18 of Grade III ulcers; 13 and 21 of Grade IV ulcers were caused by Klebsiella and Pseudomonas respectively. 8 and 9 of Grade I ulcers; 12 and 10 Grade II ulcers were caused by E. coli and staph aureus respectively. Grade V ulcers were caused by Klebsiella (5), Pseudomonas (4) and Acinetobacter (2) species. In a similar study on 104 patients by Yerat et al has observed that Gram negative bacteria are more common in Grade I (68.18%) and Grade II (84.21%) foot ulcers, while Grade III, Grade IV and Grade V ulcers had Gram negative bacteria in the proportion of 66.66 % (for both Grade III and IV), 36.36 % for Grade V. In relation to Gram positive cocci, Grade III (19.44%), Grade IV (14.66%) and Grade V (9.09%) was on the minimal side of the total sample studied [9].

Conclusion

In our study on 188 patients Klebsiella species was predominantly isolated, with almost similar proportion of Pseudomonas. Those organisms isolated were mostly sensitive to Piperacillin/Tazobactam, Meropenem, Ciprofloxacin and Amikacin. Hence it is advisable to start patients on empirical therapy with these drugs for better outcome. Many of the isolates and all the isolated Acinetobacter were multidrug resistant.

Majority of Grade 3 and Grade 4 infections were caused by Klebsiella and Pseudomonas. Most of Grade 1 and Grade 2 had E. coli and Staph aureus as predominant pathogen. All of grade 5 ulcers yielded growth of either Klebsiella or pseudomonas or MDR Acinetobacter. Proper assessment of ulcer and targeted antibiotics against these organisms can aid in early healing and avoidance of diabetic foot related complications.

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