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ORIGINAL RESEARCH OPEN ACCESS

A study of microbiology in diabetic foot infections

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Abstract

Background: Diabetic foot ulcers and infections are a common complication of diabetes. Holistic patient care requires dissemination of knowledge about prevention to avoid amputation and associated morbidity, early diagnosis and apt treatment. Healthcare costs of diabetic ulcers are high as complete treatment requires long term monitoring and high cost of rehabilitation in amputees.

Methods: The study design is retrospective observational study. All data was retrospectively procured from the Medical Record Department. From January 2019 to December 2019, two hundred and twenty patients with diabetic foot infections who had been treated with oral or parenteral antibiotics in the previous 5 to 15 days were chosen.

Results: The gram negative bacteria (88%) were most commonly isolated. Gram positive organisms contributed to 11% and candida spp. to 1% of swab culture. All patients with *Staphylococci* in wound swab were 100% sensitivity to vancomycin & linezolid. Teicoplanin, vancomycin, and clindamycin had 100% sensitivity against MRSA in wound swab. Patients with *E. coli* in wound swab had maximum sensitivity to colistin. Ceftriaxone which has been used conventionally was found less sensitive for *E. coli* and *Klebsiella*.

Conclusion: Piperacillin tazobactam combination for gram negative coverage in accordance to sensitivity pattern of community acquired infection is recommended because conventionally used ceftriaxone is less sensitive. For gram positive diabetic foot ulcers/ infections which were seen to be hospital acquired, linezolid is recommended for full course.

Keywords: diabetes; antibiotics; microbiology; bacteria; foot

Introduction

Diabetic foot infections (DFU/Is) are a frequent complications of type-1 and type-2 diabetes. The loss of lower extremities is a serious consequence of untreated infected wounds [1]. The most likely explanation for this is the examining physician's lack of understanding that 1. Preventive and surveillance methods, 2. Inappropriate wound microflora evaluation, 3. Incorrect antimicrobial therapy.

To avoid morbidity and amputation, prevention, quick diagnosis, and treatment are required. DFU/Is are frequently associated with a longer hospital stay, a higher chance of lower extremity amputation, and substantial financial costs, and they can result in long-term morbidity and mortality. Patients with diabetes may appear with foot ulcers, and vulnerable patients with a foot ulcer of unknown origin should be evaluated for diabetes [2]. Approximately 10%–15% of diabetic people develop foot ulcers [1]. Antibiotic regimens are usually chosen on the basis of experience. A set of common guidelines can assist you to avoid choosing an antibiotic treatment regimen that is either too wide or too narrow. A wide range of species, including aerobic and anaerobic bacteria, can colonise DFU/

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Is. The development of DFU/I is attributed to several characteristics of wound microbiology.

These are some of them: 1. Diversity of microbial profiles, 2. Existence of infective organisms, 3. Source of infection, 4. Antibiotics which are used to treat the infections, 5. Sample collection method, 6. Microbial load, 7. Synergistic association amongst microbial species, 8. Laboratory techniques.

A few guidelines can assist you avoid choosing an overly broad or inappropriately narrow regimen. For high-risk individuals, antibiotic therapy for aerobic Gram-positive cocci, particularly *Staphylococcus aureus* (including MRSA), should be explored. If the illness is prolonged, therapy should also target aerobic Gram-negative microorganisms. Anti-anaerobe drugs should be utilised on an ischaemic limb with necrotic or gangrenous infections.

Using a Gram-stained smear of a wound specimen to guide empirical antibiotic treatment could be beneficial. The study objectives were to identify the microbiological spectrum in DFU/Is and to identify sensitivity and resistance pattern of antibiotic prophylaxis.

Materials and methods

It is a retrospective observational study. All data was retrospectively procured from the MRD (Medical Record Department) from patient files and from Microbiology lab using patient outpatient department (OPD)/ inpatient department (IPD) number. The data of local examination and arterial doppler was collected from MRD (Medical Records Department) and patient files. Two hundred and twenty patients presenting with DFIs (Diabetic Foot Infections) who had been treated with oral or parenteral antibiotics in the past 5 days to 15 days, were selected from January 2019 to December 2019. The size and location of the ulcer, as well as symptoms of infection such as erythema, induration, local discomfort, warmth, crepitation, and/or purulent discharge, were assessed clinically. The existence and severity of foot infection in DFU were classified using the Infectious Disease Society of America (IDSA) classification system and the infection portion of the PEDIS classification system of the International Working Group on the Diabetic Foot (IWGDF).

Table 1: IWGDF Classification.

Clinical manifestations	*Infection severity*
Wound lacking purulence or any manifestations of inflammation	Uninfected
Presence of 2 or more manifestations of inflammation (purulence, or erythema, tenderness, warmth or induration), but any cellulitis/erythema extends less or equal to 2cm around the ulcer, and infection is limited to the skin or superficial subcutaneous tissues; no other local complications or systemic illness	Mild
Infection (as above) in a patient who is systemically well and metabolically stable but which has 1 or more of the following characteristics: cellulitis extending >2cm, lymphangitic streaking, spread beneath the superficial fascia, deep-tissue abscess, gangrene and involvement of muscle, tendon, joint or bone	Moderate
Infection in a patient with systemic toxicity or metabolic instability (e.g. fever, chills, tachycardia, hypotension, confusion, vomiting, leukocytosis, acidosis, severe hyperglycemia, or azotemia)	Severe

Inclusion criteria: 1. Male and female patients above 18 years of age. 2. Patient should be a k/c/o Diabetes Mellitus with DFU/Is undergoing management for diabetic foot infections.

Exclusion criteria: 1. Patients of Type 1 DM, 2. Pregnant women with DM, 3. DM patients involved in RTA (Road Traffic Accident)/ significant (non-trivial) trauma leading to lower limb infections.

Ethics approval

The study had been initiated after approval from the Institutional Ethics Committee (IEC) of Seth G S Medical College and KEM hospital, Mumbai, India EC/49/2020 dated on 9th October 2020.

Tissue sampling and culture technique

All data shall be procured from the MRD (Medical Record Department) from patient files and from microbiology lab using patient OPD/IPD number. Sending of tissue samples for microbiological examination of Diabetic Foot Infections is a routine practice in all tertiary care centres. Tissue sample is sent by the treating physician to the microbiology lab after collecting it with aseptic precautions in a sterile container.

Bacteriology

For operated patients a sample of tissue was sent for culture/sensitivity and for patients with superficial ulcers a tissue swab was sent for culture/sensitivity.

The swab/tissue specimens were first subjected to Gram's staining and microscopy. The results were recorded. In isolation of aerobic bacteria, the specimen was inoculated as per conventional method by streaking and incubation at 37 degree celsius and observed at 24 & 48 hours respectively for growth. For isolation of anaerobic bacteria the specimen was simultaneously also inoculated into Robertson's cooked meat medium (RCM) and Neomycin blood agar (NBA) which were incubated anaerobically.

NBA plate will be read at 24 and 48 hrs. The inoculated RCM after 24 hours of anaerobic incubation was sub-cultured onto neomycin blood agar plate and examined at 24 and 48 hrs. Identification and antimicrobial susceptibility testing- All bacteria were identified up to species level phenotypically as per standard protocol.

Antimicrobial susceptibility testing of aerobic isolates was performed in accordance with Clinical and Laboratory Standards Institute (CLSI) standards using Kirby Bauer Disk Diffusion method [3].

Statistical analysis

This is an observational study. The data will be entered and analysed by Microsoft EXCEL in MS Office 2010 version with built in tests for descriptive statistics. Spearman rank correlation for type of infection and outcome using SPSS software version 16. Type of DFU/Is shall be correlated with C&S (Culture and Sensitivity) data of organisms found in primary case of DFU/Is as well as cases previously received antibiotics for the same. The C/S data shall be used to identify the spectrum, sensitivity and resistance of Microbiology in DFU/Is.

Results

Our study was conducted from 1st January 2019 to 31st December 2019 on 220 patients who developed DFU/Is.

Approximately 35 patients were excluded from the study as they were lost to follow-up/dropped out.

All our patients were treated at other centres for DFI/U. Most of them had multiple cycles of treatment at different centres with varying antibiotics each time. The antibiotic used in 92.1% of our patients was amoxycillin + clavulanic acid. Ciprofloxacin was used in 4.5% patients. Metronidazole was used in 60.2% patients. Linezolid was used for 40.25 % patients. Vancomycin was used in 34.5% patients. Ceftriaxone was used in 74.5% patients. Meropenem was used in 3% patients.

Table 2: Table representing patient data.

Parameters	*Value*
Age (Average)	52 ± 10 years
Duration of diabetes	15 ± 5 years
HbA1C (Average)	8.2
Amputated limb/toes previously	7%
Previous History of admission for DFI	10%
IWDGF Grade 1	12%
IWDGF Grade 2	70.2%
IWDGF Grade 3	17.8%
Polymicrobial infection	85%
Monomicrobial infection	13.15%
Fungal infection	1.85%

Most incidences of DFI were in patients above 50 years of age. The most common organism isolated overall was *Escherichia coli* (59.1%) followed closely by *Klebsiella pneumoniae* (27.3%) and *Pseudomonas aeruginosa* (7%) subsequently.

The most common gram-positive organism isolated from wound swab was *Enterococcus spp.* (71%), followed by, Methicillin resistant *staphylococcus aureus* (21.6%) followed by Methicillin sensitive *staphylococcus aureus* (13.5%), *Streptococcus spp.* (8.4%).

Antibiotic sensitivity pattern

77% of *K. pneumoniae* was sensitive to piperacillin tazobactam, followed by sensitivity to imipenem-cilastin (12%), meropenem (8%), colistin (3%).

In our study all patients with MRSA in diabetic foot infection wound swab were 100% sensitivity to vancomycin, teicoplanin and linezolid. Tetracycline was most resistant to *Staphylococci* in DFU/Is. However high-level gentamycin was most resistant to *Staphylococci* in DFU/Is swab. Patients with MRSA in Primary DFU/Is and previously treated DFU/Is swabs were 100% resistance to penicillin, ampicillin and ciprofloxacin. Teicoplanin, vancomycin, linezolid, gentamicin and clindamycin had 100% sensitivity against MRSA in both swabs. MSSA were 100% sensitive to methicillin, gentamicin, netilmicin, clindamycin, teicoplanin, linezolid, and vancomycin in both swabs.

Patients with *E.coli* in previously treated DFU/Is had maximum sensitivity to colistin (100%), followed by aminoglycosides (Amikacin-81%, Gentamicin -71%, Netilmicin -90%) and carbapenems (Meropenem-75%, Imipenem -85%).

Antibiotic sensitivity pattern of *E. coli* and *Klebsiella* in Primary DFU/Is were similar except for levofloxacin, imipenem, amikacin (more effective in *E. coli*) and cotrimoxazole (more sensitive to *Klebsiella* species).

Piperacillin + tazobactam combination for gram negative coverage in accordance to sensitivity pattern of community acquired infection is therefore recommended. Most gram negative species are sensitive to piperacillin/tazobactam.

Enterococcus species (71%) was the most common gram-positive organism seen. MRSA was seen in 4 out of 14 patients with DFIs. In our study all patients with *Staphylococci* in wound swab were 100% sensitivity to vancomycin, and linezolid.

Polymicrobial infections in DFU/Is are certainly due to the chronicity of the wounds.

One to three intravenous doses of a second-generation cephalosporin with or without metronidazole have been recommended in most studies. This could be due to the fact that these wounds were polluted, posing a higher risk of polymicrobial development.

Discussion

On tissue culture, we discovered that previously treated diabetic foot infections have a polymicrobial flora. The majority of these patients had previously been treated empirically with many antibiotic courses without prior tissue culture or antimicrobial sensitivity testing. Various authors from Asia [4 -14] have reported similar findings but studies conducted in western countries show predominantly gram–positive infections.

Considering that most patients are from rural communities and walk bare-feet we could consider the effect of socio-economic status on diabetic foot infections. Almost all of the infections tested were resistant to routinely administered antibiotics like ampicillin and doxycycline, according to the majority of the research. This study adds to the high incidence of antibiotic-resistant nosocomial infections in our environment, and it may imply widespread antibiotic abuse in the general population. Empirical antibiotic therapy without adequate tissue cultures and a lack of facilities for diabetic foot care, such as a dedicated surgical team, physiotherapists, and occupational therapists, could be the cause of growing resistance to various medications. According to a literature review, medication resistance to several antibiotics is gradually increasing in majority of the organisms isolated from Diabetic foot infection patients [9, 10]. In our study, we have observed that polymicrobial infections were the most common infections. The reason for this could be history of multiple cycles of treatment in the past.

Our study reconfirms the fact that gram negative bacteria are the most common organisms isolated in DFI. People in India walk barefoot with a lot of exposure to water. Hence the predominance of gram negative bacteria.

Multidrug resistant organisms (MDRO) are on the rise and make treatment of diabetic foot infections extremely difficult. Hospital antibiotic policy should reflect the steps being taken to prevent the same. Culture sensitivity reports should be aggressively traced and specific antibiotics should be started according to the report.

Limitations

This is a small study population group, so the results may not be applicable to the generalized population suffering with the same disease. Our study does not take into consideration the reinfection hence we don't know the microbacterial flora in the same therefore these results may not be comparable with patients with reinfection. Our study has only a short term follow up. Long term follow up may be advised for the more conclusive results.

Conclusion

The prevalence of multi-drug resistant organisms has noticed a change in the prescription of antibiotics from beta lactams like augmentin to third generation cephalosporins. Antibiotics are usually effective in the IWGDF Grade I and II infections. Poor patient characteristics like multiple other co-morbidities, grade III IWGDF infection often respond poorly to antibiotics, requiring multiple interventions (amputation/ revascularization) at times.

Conflicts of interest

Authors declare no conflicts of interest.

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