ORIGINAL ARTICLE

BACTERIAL CULTURE ISOLATES FROM INFECTED DIABETIC FOOT TISSUE SPECIMENS AND THEIR SENSITIVITY TO ANTIMICROBIAL AGENTS

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ABSTRACT

Objective: To find out the most common organisms responsible for Diabetic Foot Infection (DFI) and their sensitivity to antimicrobial agents for the prevention of sepsis/amputation by the administration of empirical treatment.

Material & Methods: Study was carried out to analyze the bacterial isolates of all patients admitted to the Surgical, Medical and Orthopedic wards of KHYBER TEACHING HOSPITAL, Peshawar, Pakistan presented with diabetic foot infection. The study period was from April 2016 to October 2016. We started by formulating a questionnaire that was circulated among the designated groups of people, to check for organism responsible. Convenient sampling technique is used. A 6 months long prospective study (taking the midyear population into account) was carried out. 100 patients having DFI (diabetic foot infection) were selected, their culture and sensitivity (C/S) reports were performed and analyzed using SPSS 20.

Results: According to our findings a large number of people presenting with Diabetic Foot showed the following results: A total of 62 (62%) aerobes and 38 (38%) fungal or anaerobes were isolated.

Conclusion: Staph aureus and E.coli are the most common Gram positive and Gram negative organisms, respectively, in KPK. E.coli being the subset that mainly represents the bacterial population, isolated, upon culture, with high prevalence of antimicrobial drug resistance particularly to Augmentin, cephradine, ciprofloxacin, cefutoxime and cefpodoxime and sensitive to Cefoperazone/Sulbactam Vancomycin Imipenem and Piperacilline/Tazobectam.

Key Words: Culture, infection, diabetic foot, anti-microbial agents.

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INTRODUCTION

A diabetic foot is a non healing ulcer due to the presence of diabetes in a patient. A "diabetic foot syndrome" is when several diabetic foot pathologies are present. The organism responsible for a Diabetic foot is clostridium species in the west, hence, we need to know the most common organism involved in DF in our setup. Our research project sheds light on the prevalence of most common organism responsible for diabetic infection and to find out their sensitivity to antimicrobial agents to prevent amputation and sepsis by the administration of empirical treatment. A diabetic foot is a non healing ulcer due to the presence of diabetes in a patient[2].

The most important and serious foot complications in diabetes are:

1. Ulceration (an estimate shows lifetime incidence of foot ulcers among people affected by diabetes is around 15-25%) "A Diabetic foot ulcer (DFU) affects around 15% of all the people suffering from diabetes along the course of their life and is a major factor in predisposing amputations in almost 15% of all cases[3,4,5,6].

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1. Neuropathic osteoarthropathy.

These are the significant risk factors for lower extremity amputation. Administration of antimicrobial agents, to which they are sensitive to, is very important part of the management of these patients. "Of all the methods that are proposed for the prevention of DFU, the only beneficial therapy in RCTs was foot temperature-guided avoidance therapy" a meta-analysis shows[7].

Treating DFIs with broad spectrum antibiotics is practiced worldwide; however, because of infections with resistant organisms, treating with a narrow spectrum antibiotic may be more appropriate, due to low resistance rates and high bacteriological and clinical cure rates. The fact that antibiotic sensitivity changes with time[8,9], therefore knowledge of common bacteria involved and their current sensitivity pattern will help us not only in providing the best initial empirical therapy but also in preventing the emergence of resistance[10] when taken properly and to prevent long term morbidity. Records of 2013 show that around 382 million people worldwide suffer from diabetes[11]. About 90% of these are type 2[12,13].

International Diabetes Federation (IDF) in 2014 audited that diabetes resulted in 4.9 million deaths[14]. World Health Organization (WHO) in 2012 estimated that diabetes resulted in 1.5 million deaths, what makes it the 8th leading cause of death[15]. Modeling is used by IDF to estimate the deaths amounting to diabetes[16]. Low and middle income countries amounted for around 80% deaths due to diabetes[17].

Within this backdrop we propose to study the most common organisms responsible for Diabetic Foot Infection and their sensitivity to antimicrobial agents for the prevention of sepsis/amputation by the administration of empirical treatment. This research work would hopefully give us deeper insights into further understanding, prevention and treatment of this disorder.

MATERIAL & METHODS

This study was carried out at the departments of Surgical, Medical and Orthopedic wards of Khyber Teaching Hospital, Peshawar, Pakistan. Convenient sampling technique was used and the sample size was calculated to be 100 using WHO sample size calculator. Patients who were resident of KP and were admitted to Khyber Teaching Hospital for diabetic foot treatment were included in the study and those who refused to participate in the study, patients with documented anatomical abnormalities of lower limbs (based on history and past medical record) and those without a confirm diagnosis of diabetes were excluded from the study.

Diabetes was defined as symptoms of diabetes plus random blood sugar ≥ 11.1 mmol/ L or fasting blood sugar ≥ 7 mmol/L and/or HbA1c ≥ 6.5%[18,19]. A semi structured questionnaire was used for this purpose having open-ended as well as close-ended questions. In most cases data was collected by person to person interviews with respondents. Study was conducted after approval from ethical & research committee.

RESULTS

This section revolves around meaningful facts and figures derived computational statistics of our research work. Our sample size was 100 people belonging to

Table 1: different types of organism isolated in DFU

Organisms	Frequency	Percent	Valid Percent	Cumulative Percent
No orgaism	36	36.0	36.0	36.0
E.coli	19	19.0	19.0	55.0
Staph aureus	9	9.0	9.0	64.0
MRSA	6	6.0	6.0	70.0
Proteus mirabilis	4	4.0	4.0	74.0
Citrobacter freundii	1	1.0	1.0	75.0
Klebsiella	1	1.0	1.0	76.0
Pseudomonas	7	7.0	7.0	83.0
Acinobacter	2	2.0	2.0	85.0
Coliform species	4	4.0	4.0	89.0
Proteus vulgaris	2	2.0	2.0	91.0
MRSA + E.coli	5	5.0	5.0	96.0
MRSA + pseudomonas	1	1.0	1.0	97.0
Staph aureus + coliform species	3	3.0	3.0	100.0
Total	100	100.0	100.0	

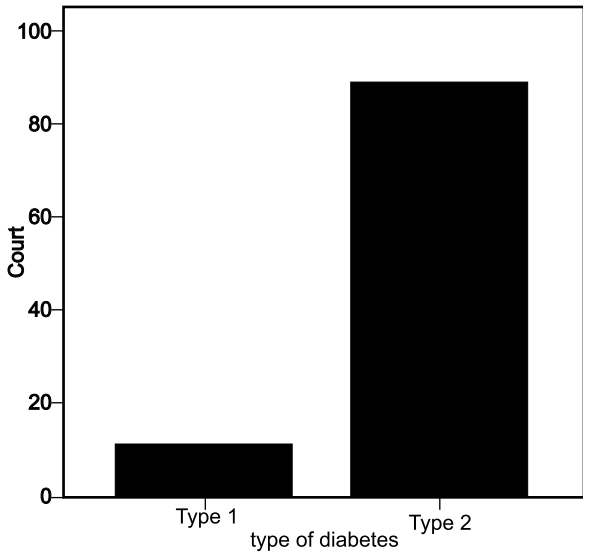

Figure 1: Type of diabetes

different walks of life with different occupations 58 were males and 42 were females. Sixty-four were married, 14 were single, 12 were divorced and 10 were widowed. If we talk about their educational background then 37 were uneducated, 26 studied up to primary, 17 were matriculate and 20 had done higher education. Demographically, out of the 100 there were 24 from Peshawar region, 5 from DI Khan, 6 each from Chitral and Charsadda, 7 from Nowshera, 8 from Kohat, 9 from Bannu, 10 each from Sawabi and Sawat, 15 from FATA.

Occupationally, out of 100 there were 8 who were students, 8 others were self employed, 10 were unemployed, 38 were employed, 36 were house workers. Out of the 100 patients, 87 presented with Type 2 diabetes and 13 presented with Type 1 diabetes (Figure 1). Thirty-five had no amputation, 35 with amputation below ankle, 16 with below knee amputation and 14 with above knee amputation. The different organisms found are shown in Table 1. The most frequently found Gram positive bacteria are Staph. Aureus (9%), MRSA (6%), the most common Gram negative bacteria are E.coli (19%), Pseudomonas (7%), and Proteus (4%).

Most effective drugs against Gram positive are Cephalosporin's {Generation II and III (100%)}, Vancomycin (100%), Imipenem (100%), Piperacilline/Tazobectam (100%). Most effective drugs against Gram negative are Cefoperazone/Sulbactam (94.4%), Vancomycin (92.3%), Imipenem (89.4%), and Piperacilline/Tazobectam (89.4%), Most effective drugs against MRSA are Vancomycin (100%), Chloramphenicol (100%), Amikacin (100%), and Minocyclin (100%), Most effective drug against polymicrobial infection is Vancomycin (100%), Chloramphenicol (80%), and amikacin (50%).

DISCUSSION

The global burden of non communicable disease is serious public health issue resulting in increased morbidity & mortality, amongst which diabetes is one big problem rather it's a global epidemic resulting in 4.9 million deaths each year[14]. Every year the incidence of diabetes is increasing and it is expected that this ratio will double in the coming years and diabetic foot is one of the most serious complication of diabetes which results in high social and economic costs[11]. All the patients once diagnosed diabetic are at risk of developing diabetic foot ulcer irrespective of duration of disease, repeated interventions result in progressive disability. A diabetic foot is a non healing ulcer below the ankle due to the presence of diabetes in a patient.

A United States multicenter Clinical Trial was carried out to find out the organism cultured from diabetic foot infections and to compare the effects of Ertapenem and piperacillin-tazobactam for their treatment, especially in cases of moderate to severe diabetic foot infections. A total of 433 patients were brought under observation for this trial. The specimens were mostly collected by biopsy or curettage after the debridement of the wound. The aerobic and anaerobic culture were performed on those samples. After the results were compiled it was evident that almost 84% of the samples were polymicrobial (a huge no, contrary to our study). The predominant organism isolated were oxacillin-susceptible Staphylococcus aureus and Corynebacterium species. The study further sheds light on the antimicrobial therapy. Ertapenem and piperacillin-tazobactam were each active against >98% of the enteric gram-negative rods. Most of the DFIs are polymicrobial and hence need due attention. These antibiotic susceptibility results can help inform therapeutic choices. Initially when a patient presents with DFI, the patient is treated empirically meanwhile a therapy directed at known causative agent is liable to better outcomes, better prognosis and is in better interest of the patient. An interesting fact here is the role of anaerobes which is pretty unclear, because this is believed that in most such studies the specimen collection was not up to the mark. The studies that used appropriate methods are consistent with the findings in favour of a minimal role while others suggest B. Fragilis to be the predominant anaerobe isolated. The bacteriology of DFIs has been the talk of the century and is under taken by different researchers for over three decades now[15,16] but it is very interesting to see that the results have varied, misleading and contradictory to the popular belief. We mostly see that a discouraging pattern is observed for commonly used antibiotics on antibiogram in cases of DFIs. Knowing the organism and its sensitivity to the

microbes is very vital in instituting a treatment plan for the patient. These ulcers and infections may require use of combined antimicrobial therapy for initial management, repeated dressing and wound debridement may be required[17].

DFIs are a major issue worldwide. The role of antibiotic in treating such infections is very important but on the other hand consideration must be given to the use of selective antibiotics that foster the emergence of resistance against such drugs and lead to difficult situations in the clinical setting during treatment of such patients or in cases of recurrent infections. There are researchers which advocate the use of only clinically infected wounds and suggest the use of the narrowest spectrum treatment options. The organism responsible for diabetic foot infection in most part of the western world is clostridium species and other related organisms[18]. The Study took into account samples from 61 patients with DFI. They used the polymerase chain reaction (PCR) technique for the detection of microbes. Patients that were PCR positive were randomised into two groups: Metronidazole and non-Metronidazole. Antibiotics for the control of infection were given in both groups according to the need of each patient. Treatment outcome was assessment of the wound. The study emphasized the significance of the PCR technique over culture methods in detection of microbes. It was found out that among the organisms isolated the maximum prevalent was Clostridium (75%) followed by Bacteroides. Clostridium was also found to be the most prevalent among all Wagner Ulcer Classification grades. This was found to be significantly associated with age and the total leukocyte count. There was no healing difference found between the different groups undertaken in the study. Metronidazole was found to be sensitive for a variety of anaerobes and aerobic bacteria. The study suggests that since there has been a debate over the use of culture and molecular microbial studies for organism detection, it is very important to take into account the role of empiric treatment. The time taken for cultures to render a specimen negative or positive is very crucial and plays a vital role in the clinical outcome of the case. Where cultures might take from 4 days to a week, a molecular microbial study gives you results in 24 hrs. Hence it is very important in a clinical paradigm to evaluate, diagnose, treat and reevaluate for better patient outcomes. The main focus of the physician should be timely curative intervention and rehabilitation for the good of his patient[19].

Our study was aimed to assess the most common organisms involved in diabetic foot infection and their sensitivity to antimicrobial agents for prevention of sepsis/amputation by administering empirical treatment. Bacteriological specimens were obtained and processed using standard hospital procedure for microbiological culture and sensitivity testing. This study was also aimed to improve hospital based framework for the purpose of diagnosis and treatment of patients with diabetic foot. Cultures from the ulcers were grown in aerobic incubation at 37 degree Celsius. The most common organisms found were aerobic bacteria E.coli (19%), Staph Aureus (9%), Pseudomonas (7%) and Proteus (4%) for which the effective treatment was Cephalosporins 2nd and 3rd generation, Vancomycin, Imipenem, Piperacillin giving a 100% coverage, these findings are consistent with the studies carried out worldwide[20].

The study result showed that the mostly the patients were affected by gram positive organisms rather than gram negative in Khyber Pakhtunkhwa area of Pakistan. Anaerobes are still the most common cause for this infection, although the prevalence is less. Our study helps shed light to choose empirical treatment for patients with diabetic foot infection and also in the management of patient who comes with sepsis that is caused from diabetic foot. Further studies need to be under taken to have better patient outcomes. Now that the researchers and physicians are realizing the diversity they are facing or to face in the near future in treating infections from new bacterial strains and diversity observed in bacterial populations, it might be an important factor in the chronicity of wounds, as is the case with diabetic ulcers.

CONCLUSION

Staph. Aureus and E.coli are the most common Gram positive and Gram negative organisms, respectively, in KP. Anaerobes are still the most common cause for this infection. These ulcers and infections may require use of combined antimicrobial therapy for initial management and repeated dressing and wound debridement.

RECOMMENDATIONS

Diabetic foot infection patients should be educated to keep their feet clean and healthy. The attendants of the patients should be advised to take proper care of their patient. The patient should be compelled to check their limbs specially lower extremities for any ulcers, wounds or cuts. Good compliance to therapy will yield positive results and would eradicate the complications before it causes further damage to health.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Ayub R: Main Idea
Raza SS: Data collection
Shafiullah: Statistics
Ahsan J: Bibliography
Hussain AK: Data collection
Nadeem MD: Follow-up.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.