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Bacterial Etiology and Antibiotic Susceptibility Pattern of Diabetic Foot Infections

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Abstract

Diabetes mellitus is one of the most prevalent endocrinopathies associated with several complications. These patients are at greater risk for bacterial infection and also have an increased sensitivity for all kinds of infections. In diabetic patients, soft tissue and bone infection of the lower limbs is the so common cause for hospital admission and sometimes leadsto lower-extremity amputation. This study was designed for isolation and characterization of pathogenic strains from the wounds of diabetic foot ulcer and their antibiogram analysis against commonly used antibiotics, a total of fifty samples were collected from the wounds of diabetic patients admitted in Rehman Medical Institute, Peshawar Hayatabad by using appropriate method. The collected samples were inoculated on different types of culture media through pure culturing technique and Standard procedures of gram staining and biochemical tests were carried out for the identification and characterization of bacterial species. Six various types of antibiotics were tested which include Augmentin, Vancomycin, Erythromycin, Cefixime, fosfomycin and Ciprofloxacin against the isolated bacterial species. Eight bacterial species were isolated, among which four were Gram-negative *Klebsiella oxytoca, Proteus mirabilis, Yersinia enterocolitica* and *Providencia* and four were Gram-positive *Staphylococcus epidermidis, Staphylococcus aureus, Micrococcus kristeniae, Micrococcus mucilaginosus*. Amoung these gram positive species *M. kristeniae* (32.60%) was the most frequently prevalent species in diabetic foot infection. The only antibiotic that was effective against all isolates was Fosfomycin while all other antibiotics used in the present study were only partly or not effective against the isolated species. Only 38%, 25% and 13% of the species were sensitive to Ciprofloxacin, Erythromycin and Cefixime respectively.

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Introduction

Diabetes mellitus (MD) is one of the foremost endocrinopathies related to several complications. It is classified into 2 types, type I and types II DM. Type I diabetes mellitus is an autoimmune disorder, in which the host cell attacks on the pancreas by forming auto antibodies against it, resulting in the deficiency of insulin in body (WHO, 1999). Type II diabetes is the disease of insulin resistance in which the host tissue becomes unresponsive to physiological levels of insulin produced (Makheswar *et al.*, 2010). Currently6.94% of the population of Pakistan has diabetes and by the year 2045, it may rise to8.45% as per IDF (Cho *et al.*, 2018). By the year 2030, around 13.8 million people in Pakistan will have this disease making it the 4thlargest diabetic population in the world (Rhys *et al.*, 2019). Clinically, diabetics most commonly present with delayed wound healing increased susceptibility to all kinds of infections, and limb amputations (mostly in the distal part of limbs) as a consequence of the disease.

Diabetic foot infections clinically present with ulcers (DFU), which may progress to gangrenous necrosis and even in some cases the patient can become septic. The incidence of DFIs in diabetics is estimated to be in a range of four percent (in all patients of diabetes) to seven percent (in patients treated in a diabetic foot center yearly (Pecoraro*et al., 1990)*. Approximately, fifteen percent of the patients with this disease will develop foot ulcers (DFU) in their life (Tantisiriwat *et al., 1998)*. The presence of neuropathy (central and peripheral), ischemia and immune function disorder (acquired immunodeficiency) in diabetic patients leads to vigorous infection in the lower limbs (Pataky *et al.*, 2005). Diabetes-associated complications contribute to the improved sensitivity to many infections for example peripheral vascular disease (with accompanying ischemia), neuropathy, and a disorder of immune system (Laing *et al.,* 1998). This vigorous infection spreads by softening of the tissue and bones leading to amputation of the lower limbs. Diabetic foot infections are usually polymicrobial due to the presence of different types of microbial strains. Microorganisms that are involved in the infection of non-communicable diseases like diabetes are Gram +ve and Gram -ve bacteria. Gram–ve aerobes include *S. aureus*, Coagulase-negative *Staphylococci Streptococci* species, *Enterococcus* species, *Corynebacterium* spp, while Gram-negative aerobes include Enterobacteriaceae spp. i.e. *Klebsiella, E. coli,* Enterobacter sp. *Pseudomonas* & *Proteus* spp (Chaudhry *et al.*, 2004).

It is important to get the newest trend within the bacteriology of diabetic foot ulcers to improve their treatment and management and to save lots of patients from losing their limbs. Proper management of those diabetic infections requires proper antibiotic selection supported after culture and antimicrobial susceptibility testing results, however, the initial management comprises of empirical antimicrobial therapy (Citron *et al.*, 2007). Research is restricted regarding this aspect of the diabetic complication, whereas its impact on the patient's quality of life is huge; hence absolutely the need for this study to be conducted. Therefore, the present study was designed to analyzed antibiotic susceptibility patterns and infectious microbes isolated from wounds of diabetic patients. This study will help injudicious use of antibiotics prescribed by physicians.

Materials and methods

The study was performed at the Department of Microbiology and Biotechnology, Abasyn University Peshawar. The samples were collected from Rehman Medical Institute, Peshawar using sterile cotton swabs in a screw-capped bottle bearing the patient's name, age, and sex and transported to Microbiology Research Laboratory under sterile conditions for culturing preservation and further processing like sub-culturing, identification, and characterization of the organism. After the initial examination, a Stained slide was observed under a microscope and determined gram +ve and gram-ve bacteria on the base of Pink and blue color and characterized through inoculation on three different types of culture media MacConkey media, Nutrient media, and Mannitol Salt media. After streaking the plates were incubated at 37°C for 1 day. The bacterial growth was later Gram

stained. The characterization of bacterial isolates was based on standard microbiological methods.

Nutrient media was utilized for the cultivation of the microbes supporting the growth of a wide range of non-fastidious organisms and many types of bacteria and MacConkey medium is a differential culture medium. It was used to selectively isolate Gram-ve and enteric Bacilli and to differentiate them based on lactose fermentation while Mannitol Salt media (MSA) contains a high concentration (7.5 to 10%) of salt (Sodium chloride), making it selective for Gram +ve bacteria (*Staphylococcus* and *Micrococaceae*) *S. aureus* produced yellow colonies, where other Coagulase-ve *Staphylococci* produced small pink or red colonies. For the Identification and confirmation of the cultured organisms,relevant biochemical tests i.e Catalase, Oxidase, Coagulase, Triple sugar iron, Urease, Citrate, Indole tests and procedures were performed.

In last antibiotic sensitivity test was carried out through the Disc diffusion method for determination of the antibiotic susceptibility profile of bacterial isolate. Isolated bacteria were inoculated on Muller Hinton agar media with the use of a sterile swab to create a uniform bacterial lawn and antibiotic discs were placed over the MHA plates with the use of sterile disc dispenser at equal distance. The plates were shifted to an incubator for 24 hours at 37°Cand Zone of inhibition around the antibiotic discs showed the resistance of bacteria to a particular antibiotic. The zone was measured in mm by following the guidelines of CLSI. Following antibiotics were used to find the susceptibility profile of the isolates Augmentin, Vancomycin, Cefixime, Erythromycin, Ciprofloxacin and Fosfomycin.

Results

Bacteriological analysis of wound samples

Isolated bacterial strains were characterized according to Bergey's Manual of Determinative Bacteriology (9th Edition). Based on microscopy, cultural characteristics on different media and biochemical tests, 08 different isolates were identified in wounds sample of diabetic patients**.**

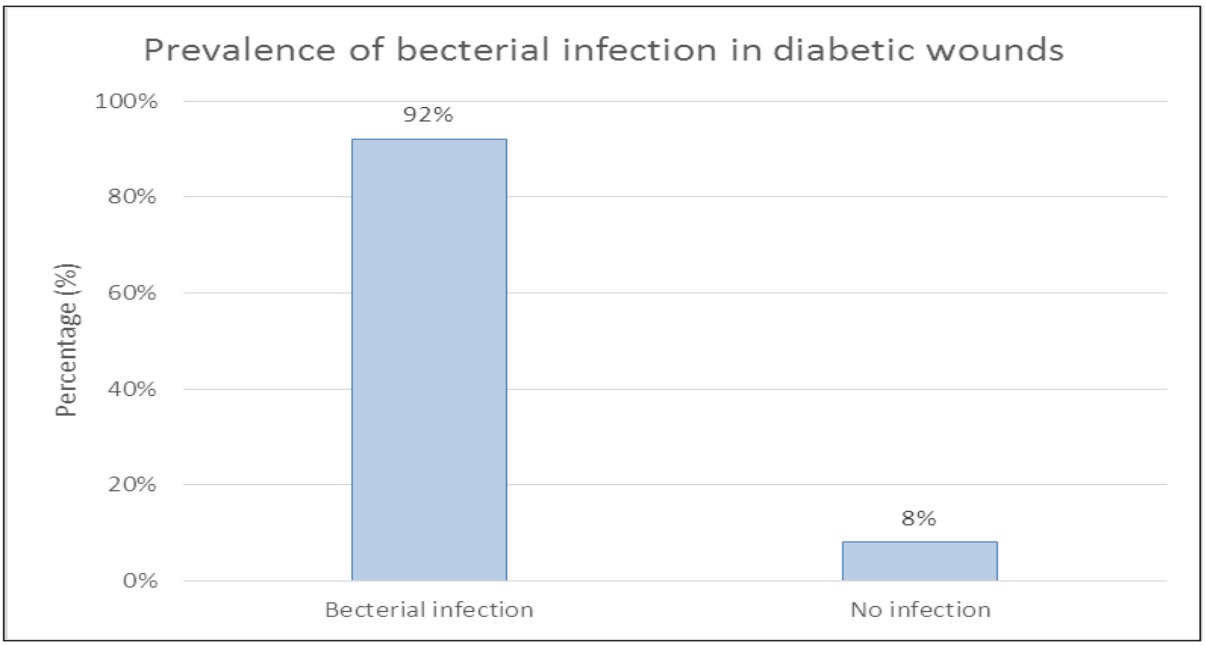

Fig. 1. Prevalence (%) of infection in diabetic wound samples.

Out of 50 collected samples, 46 (92%) were positive for microbial growth and four samples showed no growth (Fig.1). Both types of Gram +ve and Gram -ve species were isolated from diabetic foot ulcers. In the majority of the samples positive for growth, the isolated bacterial species were Gram-positive 32/46 (70%) while Gram-negative were 14/46 (30%) (Fig. 2). The identified Gram-positive species were *S.*

epidermidis, *S.aureus*, *M.kristeniae and M.mucilaginosus* and gram negative species were *K. oxytoca*, *P. mirabilis*, *Y. enterocolitica* and *providencia*. Among gram positive species the most prevalent specie was *M.kristeniae* 32.60% while *P.mirabilis* was the most prevelant species among gram negative which is 17.3 % as shown in (Fig. 3).

Antibiotic sensitivity

Different antibiotics were used against identified species. The only antibiotic that was effective against all isolates was Fosfomycin. All other antibiotics used in the present study were only partly or not effective against the isolated species. Only 38%, 25% and 13% of the species were sensitive to Ciprofloxacin, Erythromycin and Cefixime respectively. While all species were resistant towards Vancomycin and Augmentin as shown in (Fig. 4).

Discussion

In this study, 50 samples were obtained from the ulcer of diabetic patients admitted in RMI Peshawar Hayatabad at Diabetology ward at KPK (Khyber Pakhtunkhwa) Province, by using sterile cotton swabs. Eight different types of bacterial isolates from DFI, half of which were Gram + ve and a half Gram (– ve). The difference in speciedistribution in each sample in this study is striking. As noted, the frequency of the bacteria is likely to be influenced by many predisposing underlying factors.

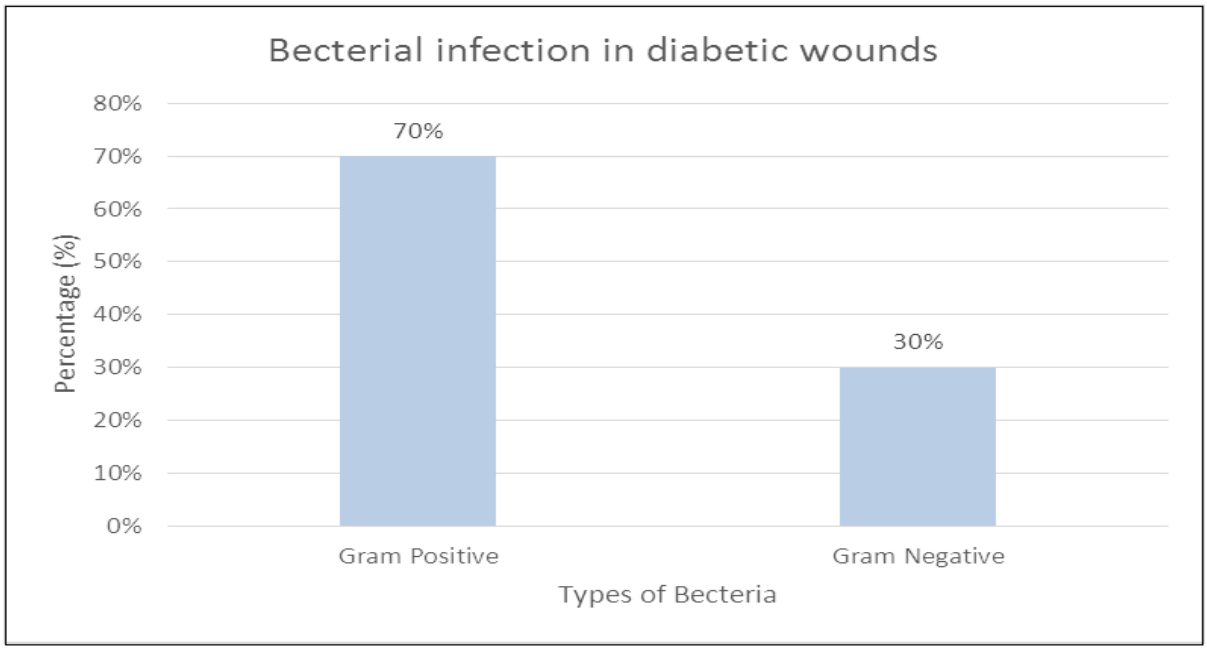

Fig. 2. Frequency distribution of the types of bacterial infection in diabetic wound samples.

In studies documented by (Ramsamy *et al*., 2013) in diabetic patients, *Escherichia coli*was isolated in maximum whereas other studies report *Klebsiella pneumonia,Pseudomonas aeroginosa*as the common species in DFI (Rampal *et al*., 2019). On the contrary, our study reports *M. Kristinae* is the most prevalent species.

It has been reported as an opportunistic pathogen in diabetes and we observed a high (%) incidence of the specie in diabetic foot ulcers. However the second most common species in DFI was Staph aureus similar to previous work (Ramsamy *et al*., 2003; Rampal *et al*.,2019) Given this observation, differences in society, living style, and geography, age and sex are responsible for the difference in species in particular samples all over the world (Miyan *et al*.,2017). In this research we used different classes of antibiotics for antimicrobial analysis which included; Fosfomycin, Ciprofloxacin, Erythromycin, Vancomycin, Cefixime and Augmentin. (Miyan *et al*., 2017) has reported the presence of antimicrobial resistance in species isolated from the diabetic foot. In line with this, antimicrobial resistance was also

observed in the species isolated. The only antibiotic that was effective against all isolates was Fosfomycin. Previously, it has been reported that the efficacy of Fosfomycin in the treatment of deep-seated diabetic foot infections is very effective (Schintle *et al.*, 2009). In this study, some of the antibiotics were ineffective while some were partly less effective against the isolated species. Only 38%, 25% and 13% of the species were sensitive to Ciprofloxacin, Erythromycin and Cefixime respectively.

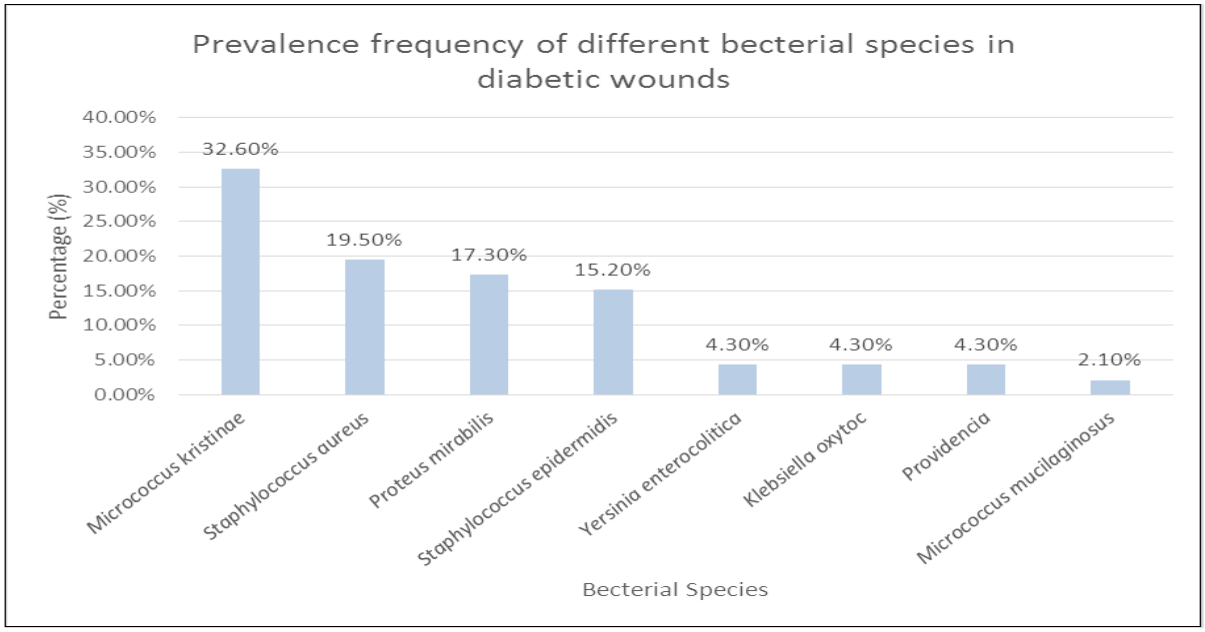

Fig. 3. Frequency distribution of different bacterial species in diabetic wounds.

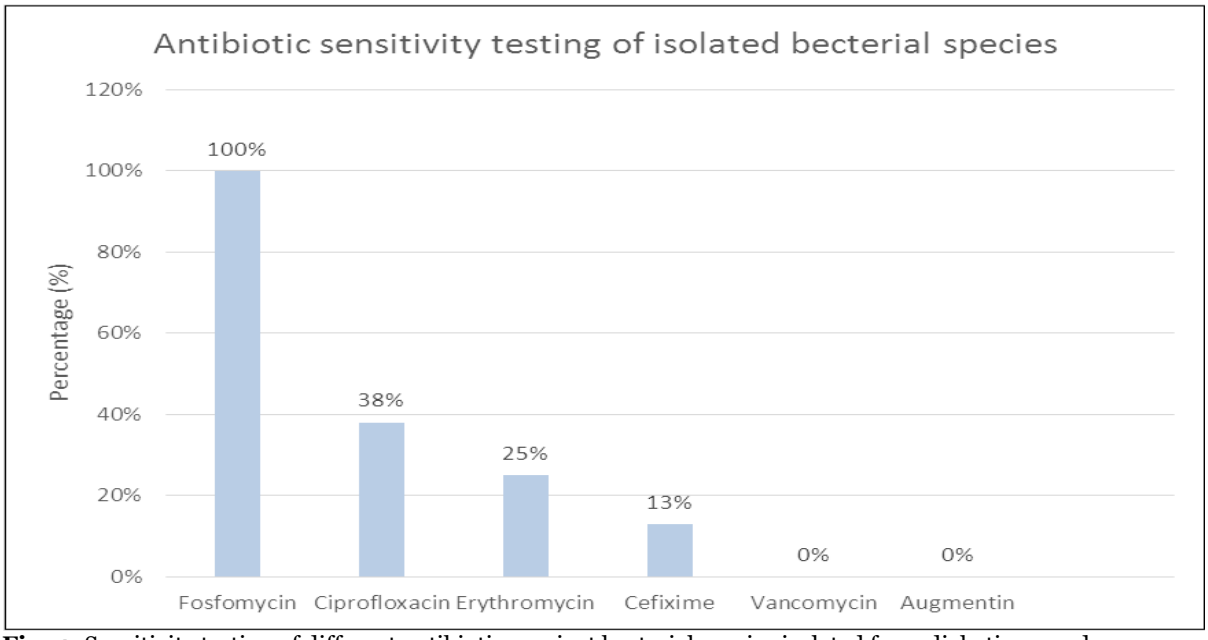

Fig. 4. Sensitivity testing of different antibiotics against bacterial species isolated from diabetic wounds.

All species were resistant towards Augmentin and Vancomycin. Many studies are carried out on microbial surveillanceand these studies have shown the effect of pathogens on a particular society including KPK Province of Pakistan. The data from these studies have shown that the difference in pathogenic speciesin similar samples is due to the geographical and climatic differences of those regions from where these samples were collected. So far from our research work, it is obvious that the common

cause of morbidity in this region of KPK is due to microbial infections, so regarding the diagnosis and treatment of diabetics,a physician needs to rule out any sort of microbial infection (Hamdan *et al., 2018)*.

It was very much apparent that the positive rate of infection was higher in those patients who were severe or chronically ill. From a lesser % of culture, it shouldn't be assumed that people in a particular region are less afflicted with infections, rather it may be due to the inaccessibility of sample collection in diabetic patients of those regions. Hence the goal for a physician is to take into account even a single number of the pathogen critical for the selection of antibiotics (Yazdankhah*et al.*, 2001).

Conclusion

The study is very significant in providing a detailed description of the microorganism found in diabetic patients having foot ulcers. Diabetic wound care is very important. Other than that, diabetic septic wounds are one of the most dangerous problems of diabetes mellitus. Due to the irrational uses of antibiotics, many bacteria developed different degrees of resistance to most known antibiotics.

The severity of the infection and growth of organisms can be correlated with social activity, living habits and geographical location of the patients. There is a need for improved hygiene in the hospital environment to discourage the multiplication of these pathogenic bacteria in wounds and encourage the early healing of infected areas.

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