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Culturing and sensitivity of diabetic foot ulcer of the patients visiting to Ayub medical complex Abbottabad, Pakistan

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Abstract
Diabetic foot ulcer is one of the most serious complications occurring in diabetic patients causing amputation. This study was done to identify the different species of microorganisms in infection of patients visiting to Ayub Medical Complex. Gram positive and Gram negative bacteria were isolated such as Streptococcus, Staphylococcus, Escherichia coli and Klebsiella pneumonia by swab culturing method on different media such as blood agar and MacConkey agar media and sensitivity test were performed by disc diffusion method on nutrient agar for both Gram positive and Gram negative bacteria. In this study Gram positive is mostly present in diabetic foot ulcer patients. Staphylococcus was mostly present in patients i.e. 63 out of 100 patients have Staphylococcus in their infection. By performing sensitivity test for these bacteria Staphylococcus is mostly resistive to Ampicillin, Cefazolin, Oflaxacin, Streptomycin while mostly sensitive to Amikacin (53.96 %), Gentamycin (25.39 %), Cifroflaxin (34.92 %) and Vancomycin (15.87 %). Streptococcus was sensitive to Gentamycin (27.27 %), Linezolid (36.3 %), Pipracillin tozobactum (36.3 %), Vancomycin (27.2 %) and resistive to Cefazolin, Ampicillin and Doxycycline. Gram negative bacteria Klebsiella pneumonia was sensitive to Amikacin, Vancomycin, Lincomycin and mostly resistive to Doxycycline, Meropenem and Cefazoline. Escherichia coli was mostly sensitive to Erythromycin (25 %), Oflaxacin (50 %), Azactam (50 %), Amikacin (50 %) and resistive to Doxycycline, linomycin and Gentamycin. This study conclude that Amikacin, Vancomycin and Erythromycin are mostly sensitive against both Gram negative and Gram positive bacteria.

Keywords: Medical Micro Biology, Culture and Sensitivity.

Introduction

Diabetes Mellitus is a group of metabolic disease characterized by hyperglycemia due to abnormal insulin secretion or insulin action or both (1). The term "Diabetes" is Greek word and means „toran through a Siphon" was first used by Aretaeus of cappadocia (81-138 A.D.). William Cullen (1710-1790) added a Latin word "mellitus" means "honey" to diabetes, due to the sweet test of urine of the diabetic patients (Bruno-w *et al*., 1977) [3]. Hyperglycemia in diabetic patients produces serious chronic complications and failure of various organs like eyes, kidneys, nerves, heart and blood vessels. Peripheral neuropathy is one of the most serious disorder of these which leads to planter foot ulceration (Weiman 2005) [18]. Diabetic patients have a life time risk as high as 25% for developing foot ulceration (Singh *et al*., 2005) [16]. Diabetic ulcers have 15–46 times higher risk of limb amputation when compared with foot ulcers due to other causes (Alavi *et al*., 2007) [1]. Every year, more than a million of diabetic patients require limb amputation (Khanolkar *et al*., 2008) [9]. The clinical manifestations include sepsis, blistering, malodorous smell, and pus production (Brem *et al*., 2000) [2]. The reported rate of foot ulcer prevalence was as high as 11.6 % by Centres for Disease Control and Prevention 2003 in United States. The 10.6% prevalence rate of Diabetic Foot Ulcer was reported in United States of America in a population based study, with 2.2% an annual rate on incidence (Moss *et al*., 1992) [10]. In the same year that is 2003, 4% prevalence rate of Diabetic Foot Ulcer was reported by Walter (Walters *et al*., 1992) [17] in United Kingdom. The average risk of foot ulcer development in peoples with diabetes is estimated to be 15% (Palumbo and Melton 1995) [11]. Common age of diabetic foot disease is 40 to 60 years and 98% occurs with Diabetic Mellitus type-2. Approximately 10-25% of all diabetic mellitus types develop foot infections during the period of their illness from simple to major abscesses and osteomyelitis (Rooh-Ul-Muqim *et al*., 2003) [14]. 40-80% of ulcers eventually become infected. The reasons for increased incidence of this disorder in Diabetic Mellitus involve interaction of several pathogenic factors that is neuropathy, abnormal foot biomechanics, peripheral arterial disease and poor wound healing (Kasper *et al*., 2005; Yusof *et al*., 2008) [12, 20]. Global prevalence of diabetes in 2003 was estimated to be 194 million (14). By 2030, this figure is

predicted to rise to 366 million due to longer life expectancy and changing dietary habits (Wild *et al*., 2004) [19].

Infection may be caused due to pathogenic bacteria originating from the external environment as well as by bacteria forming physiological microflora of the skin for example *Staphylococcus epidermidis, Staphylococcus aureus, and Propionibacterium* acnes. Pathogenic microflora may be transferred unconsciously by medical personnel and through materials and substances used for treatment. The presence of infection depends mainly on the number of microorganisms present in the wound, whereas the healing process depends on the type of bacterial strains present in infection and their pathogenicity (Citron *et al*., 2007) [4].

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been commonly isolated from 30% of the diabetic wounds (Shankar *et al*., 2005) [15] The presence of MRSA and ESBL strains further worsen the prognosis and increase the risk of amputation (Juan *et al*., 2012) [8].

Within the same context, we designed the present study to evaluate culturing of different samples taken from diabetic patients on different Medias such as Blood Agar and Mackonkyes and identification of microorganisms appear on these cultures, the prevalence of microorganism in infected diabetic foot and its sensitivity pattern to the routinely used antibiotics in Abbottabad and Mansehra.

Methodology

The diabetic patients were visited to the Ayub Medical Complex for the treatment of diabetic foot ulcer. The history of the participants such as age, sex, diagnosis, and complications of diabetes were collected. Specimens of pus were collected from patients during initial admission to the Ayub Medical Complex (provided that no antibiotics were taken within the past 2 days). Samples were collected by swab from infected foot wound of diabetic patients, pus swabs were taken at the deepest part of the wound. The specimens were collected by using sterile swabs and transported at the microbiology laboratory immediately for preventing contamination. All pus swabs were firstly Gram stained for direct examination and microscopy. Then they were cultured on blood agar plates, on MacConkey medium. The media were incubated at 37 °C overnight in incubator.

The colonies were identified firstly by their physical appearance. Colonies appeared after incubation shows both gram positive and gram negative bacteria. For further identification of gram positive and gram negative more chemical tests are performed such as API, cogulase and oxidase tests etc.

The Gram-negative colonies were further identified using the API system. *Staphylococcal* isolates were additionally tested for coagulase test to confirm the presence of *Staphylococcus aureus*. MRSA was confirmed by the sensitivity test on nutrient agar after applying drugs to culture on nutrient agar. All organisms isolated were subjected to antibiotic sensitivity testing by the Kirby–Bauer disc diffusion method using commercially purchased antibiotic discs and interpreted according to Clinical and Laboratory Standard recommendations after applying on nutrient agar. All patients received proper antibiotics according to the culture and sensitivity results as well as metronidazole for associated anaerobic organisms (Hefni *et al*., 2013) [6].

Imipenem, Ciprofloxacin, Levofloxacin, Cefotaxime, Ceftazidime, Ceftriaxone, Amikacin, Gentamycin, Amoxcillin, Tetracycline, Pipracillin, Sulph-trimethoprim, Cefuroxime were tested for gram-negative bacteria. Penicillin, Amoxicillin-clavulanic acid, Erythromycin, Trimethoprim-sulphamethazole, Tetracycline, Ciprofloxacin, Levofloxacin, Gentamicin, Ceftriaxone, Oxacillin, Vancomycin, Cefotaxime, and Ceftazidime were tested for *Staphylococcus species and Enterococcus species* (Esmat and Saif Al Islam 2012) [5]

Results

The present study includes 100 diabetic patients out of which 59 were males and 41 females. The pus samples were taken from these patients and cultured on media to identify microorganisms present in their samples. The age ranged from 32 to 61 years mean of which is 46.5. All of patients have foot infections and their diabetic degree was searched by different specialist at Ayub medical complex Abbottabad.

After culturing their samples on media and then studied overall of which most of patient have *staphylococcus* that is 63 patients have *staphylococcus* while 11 have *streptococcus*. Gram negative also identified in this study but very less as compared to gram positive that is only 1 patient have *Klebsella* and 4 patients have *Escherichia coli*. Some cultures also show no growth of any organisms that is 21 patients' samples have no growth. The data in table 1 shows the frequency of microorganisms in male and female separately.

Table 1: Frequency of microorganisms in male and female separately

Microorganisms	Male	Female	Total
Staphylococcus	36	27	63
Strephtococcus	6	5	11
Escherichia coli	3	1	4
Klebsella	1	0	1
No growth	13	8	21
Total	59	41	100

The sensitivity tests were performed on nutrient agar and their results are given in tables. Table 2 and 3 shows the resistive and sensitive drugs separately for gram negative and gram positive bacteria both which are isolated from patients' samples. While table 4 and 5 shows the frequency of different sensitive drugs used for gram negative and gram positive bacteria. The most sensitive drug is Amikacin for both *staphylococcus* and *streptococcus* which are about sensitive in 34 patients out of 63 i.e. 54 % patients having *staphylococcus* and about 5 patients out of 11 i.e. 45 % patients having *streptococcus* are sensitive in our study. Other drugs to which patients having *staphylococcus* shows sensitivity are ciprofloxacin, gentamycin and pipracillin tozobactum. While cefazoline, linezolid and pipracillin tozobactum are more sensitive to *streptococcus species*. 22 patients are susceptible to ciprofloxacin while just patients show resistivity to ciprofloxacin. 16 patients are susceptible in our study to gentamycin. The more resistive drug that is to which most of patients shows resistivity is cefazolin and oflaxacin. About 30 patients having *staphylococcus* are resistive to cefazolin and 17 patients are resistive to oflaxacin. Cefazolin is also resistive in patients having *streptococcus* about 5 patients out of 11 patients are resistive to cefazolin.

Table 2: Sensitive and resistive antibiotics to staphylococcus and streptococcus

Bacteria	Sensitive Antibiotics	Resistive Antibiotics
Staphylococcus	Amikacin Ciproflaxin GentamycinMeropenem MEM Pipracillin Pipracillin SulbactumPipracillin Tozobactum VancomycinErthromycin	Ampicillin Cefazolin Oflaxacin Streptomycin Sulphmethazole trimethoprim
Streptococcus	Amikacin Gentamycin Erthromycin LinezolidPipracillin Pipracillin Tozobactum VancomycinMeropenem MEM Oflaxacin Streptomycin	Cefazolin Doxycycline Ampicillin

While in case of gram negative bacteria our study shows that amikacin erthromycin vancomycin oflaxacin are sensitive. Patients are susceptible to amikacin having *Klebsiella pneumonia* but those patients having *Escherichia coli* shows resistivity to amikacin. Linomycin is also sensitive in *Klebsiella pneumonia* patients while patients with *Escherichia coli* shows resistivity to linomycin. Gram negative bacteria show resistant to doxycycline, meropenem sulphamethazole trimethoprim and gentamycin.

Table 3: Sensitive and resistive antibiotics to Klebsiella pneumonia and Escherichia coli

Bacteria	Sensitive Antibiotics	Resistive Antibiotics
Escherichia coli	Erthromycin Oflaxacin Sparfloxacin Azactum cefuroxime	Doxycycline Linomycin Gentamycin
Klebsiella pneumonia	Amikacin Cifrofloxin Linomycin Vancomycin	Cefazolin Doxycycline Meropenem Pipmedic acid Sparfloxacin Sulphamethazole trimethoprim

Table 4: Frequency of antibiotics sensitivity to Gram Positive Bacteria

ANTIBIOTICS	STAPHYLOCOCCUS	STREPHTOCOCCUS
Amikacin	34 (53.96 %)	5 (45.45 %)
Ciprofloxacin	22 (34.92 %)	-
Gentamycin	16 (25.39 %)	3 (27.27 %)
Meropenem	13 (20.63 %)	2 (18.1 %)
Pipracillin	11 (17.46 %)	2 (18.1 %)
Pipracillin sulbactum	9 (14.28 %)	-
Pipracillin tozobactum	16 (25.39 %)	4 (36.3 %)
Vancomycin	10 (15.87 %)	3 (27.2 %)
Erthromycin	4 (6.34 %)	2 (18.18 %)
Ampicillin	3 (4.76 %)	0
Cefazoline	16 (25.39 %)	4 (36.3 %)
Oflaxacin	10 (15.87 %)	3 (27.2 %)
Streptomycin	6 (9.52 %)	1 (9.09 %)
Linezolid	-	4 (36.3 %)
Doxycycline	-	2 (18.1 %)
Sulphamethazole trimethoprim	6 (9.52 %)	0

Table 5 Frequency of antibiotics sensitivity to Gram Negative Bacteria

ANTIBIOTICS	Escherichia coli	Klebsiella pneumonia
Amikacin	2 (50 %)	S
Cifrofloxin	1 (25 %)	S
Linomycin	0	S
Vancomycin	-	S
Cefazolin	2 (50 %)	R
Doxycycline	1 (50 %)	R
Meropenem	1 (25 %)	R
Pipemedic acid	1 (25 %)	R
Sparfloxacin	2 (50 %)	R
Sulphamethazole trimethoprim	1 (33.3 %)	R
Erthromycin	1 (25 %)	-
Oflaxacin	2 (50 %)	-
Azactam	2 (50 %)	-
Cefuroxime	1 (25 %)	-
Gentamycin	0	-

Discussion

Diabetic foot infection is major and increasing problem worldwide. Diabetic patients have a life time risk as high as 25% for developing foot ulceration. Every year, more than a million of diabetic patients require limb amputation. Identification of food wound as diabetic foot infection is more important because most of consultant treat these wound as a normal infection which can lead to life threatening infection. Therefore first of all identification is necessary of these patients by taking their samples from infection we take pus

samples from their site of infection by swab. Because swab sampling have less chances of contamination and we must take great care of samples during culturing to inhibit contaminations. After taking samples from site of infection we cultured these samples on different media such as blood agar and MacConkey in microbiology laboratory. Blood agar can support both gram positive and gram negative growth therefore we used MacConkey media as differential media to differentiate gram negative and gram positive so we identified gram negative from mackonkey and gram positive from blood agar. After inoculation of swab samples on media by streaking method we incubate the Medias in incubator for overnight. After overnight we observe the cultures and identify them by different methods such as by physical appearance and then more chemical tests were performed for identification of different strains such as gram staining, oxidase test, catalase test also performed.

After identifying different strains of bacterium as discussed above earlier that in our study gram positive is more as compared to gram negative and in gram positive *staphylococcus spp* is observed commonly that is 63 samples have staphylococcus while only 11 have *streptococcus*. After *staphylococcus* and *strephytococcus*, *Escherichia coli* present in 5 patients and only one patient have *klebsella* in sample. So in our study gram positive is more common than gram negative.

After identification of gram negative and gram positive species the sensitivity test is performed by culturing the bacteria on nutrient agar and applies the drugs discs on them according to gram positive and gram negative bacteria respectively. After incubation of overnight the next day we check the zones of drugs and see which bacteria shows susceptibility to drugs and resistivity to drugs the clear zone created by bacteria shows that drugs is sensitive to bacteria and if there will no zone then it means the bacteria is resistant to that drug. *Staphylococcus aureus* isolates in our study were found to be uniformly susceptible to vancomycin, hence, vancomycin can be considered as an important drug in the empirical regimen for treatment of diabetic foot infections especially in settings with high resistance to other antibiotics.

The most sensitive drug in our study for gram positive bacteria is amikacin for both *staphylococcus* and *streptococcus* while most resistive drug is cefazolin and oflaxacin.while most sensitive drug in gram negative bacteria is vancomycin.

Amikacin.

In our study the most susceptible drug in both gram negative and gram positive bacteria is amikacin. About 54 % of *staphylococcus* patients are susceptible to amikacin and 45 % of patients having *streptococcus* also susceptible to amikacin while gram negative bacteria *Klebsiella pneumonia* also susceptible to this drug. So our study shows that amikacin can be very helpful in treating the diabetic foot ulcer.

The gram positive specie is also highly susceptible to pipracillin and pipracillin tozobactum. Both the *staphylococcus* and *streptococcus* are susceptible to pipracillin and pipracillin tozobactum. About 25 % of patients are susceptible to pipracillin tozobactum having *staphylococcus* while 36 % patients having *streptococcus* are susceptible to this drug.

The most resistive drug in case of gram positive bacteria is ampicillin and sulphamethazole/trimethoprim in both *staphylococcus* specie and *streptococcus specie*. While in gram negative bacteria both our isolated organisms *Klebsiella pneumonia and Escherichia coli* shows resistant to doxycycline.

Ciprofloxacin (34.92 %), Gentamycin (25.39 %), Meropenem (20.63 %), Cefazoline (25.39 %) are susceptible to *staphylococcus* and Meropenem (18.1 %), Pipracillin tozobactum (36.3 %), Erthromycin (18.18 %), Streptomycin (9.09 %), Linezolid (36.3 %) are susceptible to strephtococcus. While Cefazolin (50 %), Sparfloxacin (50 %), Oflaxacin (50 %), Azactam (50 %), Cefuroxime (25 %), Sulphamethazole trimethoprim (33.3 %) are susceptible to *Escherichia coli.*

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