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Microbiology

To Study the Antibiotic Sensitivity to Isolated Organism among Diabetic Foot at RKDF Medical College, Bhopal

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Abstract: The study was carried out in the Department of Microbiology. In 100 diabetic foot patients studied, 73% were male and 27% were female. The age ranged from 31-80 yrs and majority of patients were in the age group o 51- 60 yrs. All strains of *S. aureus* were sensitive to Vancomycin, 63.6% were sensitive to gentamicin and 60.60% were sensitive to cotrimoxazole. Erythromycin and penicillin showed least sensitivity 36.36% and 36.3% respectively. *Coagulase Negative Staphylococci* showed relatively high sensitivity to all antibiotics, gentamicin 56.5% ciprofloxacin 82.6% penicillin 56.5% erythromycin 52% and cotrimoxazole 52%. Among Pseudomonas spp. 90% strains showed sensitivity to Imipenem, 90% were sensitive to Amikacin, 70% were sensitive to Gentamicin. Pipercillin - Tazobactam combination showed sensitivity of 62.5 % Ceftazidime and Piperacillin had least acivity 45% and 30 % respectively.
Keywords: Antibiotic, Antimicrobial susceptibility & Isolated Organism.

INTRODUCTION

Diabetes mellitus is a syndrome consisting of metabolic, vascular and neuropathic components that are interrelated. It is defined as a group of metabolic diseases that are characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both [1]. The major age group at present affected by diabetes is stuck between 40-59 years. By 2030 this record is expected to move to the 60-79 age groups with some 196 million cases [2].

Foot pathology remains the leading diabetic complication requiring hospitalization. As the incidence of diabetes in general population is expected to raise, the prevalence of diabetic foot complications will follow [3]. It is estimated that 15% of diabetic patients will develop a foot ulcer during their lifetime. The prevalence of diabetic foot ulceration has been reported to range from 5 to 25% in diabetic patients. Foot ulcers are lesions that involve a skin break with loss of epithelium; they can extent into dermis and deeper layers, sometimes involving bone and muscles [4,5].

Diabetic foot is characterized by means of numerous pathological complications such as neuropathy, peripheral vascular disease, foot ulceration & infection with or without osteomyelitis, primary to development of gangrene & still necessitating limb amputation. Infection is a frequent (40%-80%) and complication of these ulcers and represents a major cause of morbidity and mortality [5]. All foot ulcers are colonized with potentially pathogenic organisms. The impaired micro-vascular circulation in patients with diabetic foot limits the access of phagocytes favoring development of infection. *Pseudomonas spp., Staph aureus, Esch coli, Proteus spp*. and *Enterococcus spp*. are the most frequent aerobic pathogens contributing to progressive and widespread tissue destruction. Diabetic foot infections are often polymicrobial [6].

Diabetic ulcers have 15 to 46 times higher risk of limb amputation than foot ulcers due to other causes every year more than a million diabetic patients requires limb amputation [6]. The increasing association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers further compounds the challenge faced by the physician or the surgeon in treating diabetic ulcers, even may lead to amputation of the effected part. Infection with MDR pathogens is also responsible for the increased duration of hospitalization, cost of management, morbidity and mortality of the diabetic patients [6]. The resulting cost to the society can be measured in direct cost attributed to treatment as well as

indirect cost in lost productivity. However the costs are measured, diabetic foot problems represent a major public health challenge of growing proportions [3,7]

Appropriate selection of antibiotics based on the antiprogram of the isolates form the lesions are most critical for the proper management of these infections. Nevertheless, the initial empirical therapy is often decided based on the knowledge of the susceptibility profile of the prevalent microbial flora recovered from the previous cases [6].

MATERIALS AND METHODS

The study was carried out in the Department of Microbiology at RKDF, Medical College, and Bhopal.

NUMBER OF CASES STUDIED: 100

INCLUSION CRITERIA

Diabetic foot infection with open lesions

EXCLUSINON CRITERIA

Diabetic foot infection with only cellulitis, no open lesion Limbs with amputation

SPECIMEN COLLECTION [8-14]

Specimens were collected, after thorough cleaning of the lesion with sterile normal saline, preferably before administration of antibiotics.

The specimens were as follows.

- Wound curettage by using a sterile scalpel.
- Aspiration from abscesses by using needle and syringe.
- Pus by using sterile swab.

Two specimens were collected from each patient. The two specimens were used for Gram stain and aerobic culture. The specimens were immediately transported to the microbiology laboratory.

SPECIMEN PROCESSING [8-14]

Grams staining: One of the specimens was smeared over a clean, dry microscopic slide and was stained by Gram staining technique. The film was examined for the presence bacteria and polymorphs.

Aerobic culture was carried out by directly inoculating the specimen onto blood agar and Mac Conkey agar which was incubated over night at 37^0C. All types of colony grown on these plates were read and colony description was recorded. Identification of the isolates was done by using standard conventional biochemical methods.

OBSERVATION & RESULTS

Antibiotic sensitivity test [15]

The antibiotic sensitivity testing was done by Kirby Bauer disk diffusion method with commercially available Hi Media disks according to clinical laboratory of standard institute (CLSI) guidelines.

The antibiotics to be tested against the isolates were determined according to the standard guidelines and also considering the local susceptibility pattern of the organism. The set of antibiotics tested for susceptibility against different organisms were as follows.

Table-01: Antimicrobial agents tested for different isolates in present study

Isolates	Antimicrobial agent tested
Staph. Aureus	Penicillin, cefazoline, erythromycin, ciprofloxacin, and co-trimoxazole, gentamicin and vancomycin
Coagulase Negative Staphylococci Staphylococci (CoNS)	Penicillin, cefazoline, erythromycin, ciprofloxacin, co-trimoxazole, gentamicin and vancomycin
Enterococci spp.	Penicillin, gentamycin, amikacin, vancomycin.
Pseudomonas spp.	Piperacillin, Piperacillin- tazobactam, amikacin, gentamicin, ceftazidime, ciprofloxacin and imipenem.
Other Gram negative bacilli	Piperacillin, amoxicillin-clavulanicacid, gentamicin, amikacin, ciprofloxacin, ceftazidime, cefuroxime, co-trimoxazole, imipenem.

Table-02: The antibiotics and disc strength

Antibiotic	Disc strength
Penicillin(P)	10U
Amoxicillin-clavulanic acid(AC)	20/10μg
Piperacillin Tazobactem (PT)	100/10μg
Erythromycin (EM)	15μg
Cefazoline (CFZ)	30 μg
Ceftazidime (CZ/CAZ)	30 μg
Cefuroxime (XM)	30 μg
Ciprofloxacin (CL)	5 μg
Co-trimaxazole (SXT/CT)	1.25/23.75 μg
Gentamicin (GM)	10 μg/ 120 μg
Amikacin (AK)	30 μg
Vancomycin (VA)	30 μg
Imipenem (I)	10 μg

Procedure

Two to three well isolated colonies were emulsified in sterile test tube and incubated at 37 ^{0}C for 2-4 hours. The inoculum was matched with Mc Farland 0.5 standard for turbidity and a lawn culture was made in a Mueller Hinton agar plate using a sterile cotton swab after dipping into the inoculum and removing the excess amount by squeezing on to the walls of the test tube. Six antibiotic discs were placed in a 90 mm plate. The plates were incubated at 30 ^{0}C for 18-24 hours.

Interpretation

Measurement of zone diameters

After overnight incubation, zone diameters were measured using caliperor scale. The zone of the complete growth inhibition around each of the discs was measured to within the nearest millimeters. The diameter of the disc was included in the measurement. An interpretative correlation (Sensitive, intermediate or resistant) was done by using reference chart.

Table-03: Invitro antimicrobial susceptibility patterns of Gram positive bacteria

Antimicrobial agent	*Staphylococcus aureus;* η = 33		CoNS; η =23		*Enterococcus spp.;* (η=17)	
	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)	Sensitive No. (%)	Resistant No. (%)
Penicillin	12(36.4)	21(63.6)	13(56.5)	10(43.5)	4(23.5)	13(76.5)
Ampicillin	-	-	-	-	6(35.3)	11(64.8)
Erythromycin	12(36.4)	21(63.6)	12(52.2)	11(47.8)	-	-
Cefazoline	11(33.3)	22(66.7)	13(56.5)	10(43.5)	-	-
Cotrimoxazole	20(60.7)	13(39.3)	12(52.2)	11(47.8)	-	-
Ciprofloxacin	15(48.5)	17(51.5)	19(82.6)	4(17.4)	-	-
Gentamicin	21(63.6)	12(36.4)	13(56.5)	10(43.5)	12 (70.6)	5 (29.4)
Amikacin	-	-	-	-	12 (70.6)	5 (29.4)
Vancomycin	33(100)	0	23 (100)	0	17 (100)	0

Table-04: Invitro antimicrobial sensitivity pattern of Gram negative bacteria

AMA	P.aeruginosaη = 40	E.coli; (η = 18)	Klebsiella spp.(η = 16)	Citrobacter spp. (η = 13)	Proteus spp. (η = 10)
	Sensitive No.(%)	Sensitive No.(%)	Sensitive No.(%)	Sensitive No.(%)	Sensitive No.(%)
PC	12(30)	4(22.2)	6(37.5)	6(46.1)	3(30)
AC	-	8(44.4)	9(56.2)	9(69.2)	6(60)
PT	25(62.5)	-	-	-	-
GM	28(70)	12(66.7)	11(68.7)	9(69.2)	6(60)
AK	36(90)	13(72.2)	11(68.7)	10(76.9)	7(70)
CL	22(55)	6(33.3)	7(43.7)	8(61.5)	4(40)
XM	-	6(33.3)	8(50)	7(53.8)	5(50)
CZ/CAZ	18(45)	11(61.1)	11(68.7)	10(76.9)	7(70)
SXT/CT	-	10(55.5)	10(62.5)	7(53.8)	6(60)
I	36(90)	18(100)	16(100)	13(100)	10(100)

AMA- Antimicrobial agent, PC- Piperacillin, AC-Amoxicillin - Clavulanicacid, PT- Piperacillin tazobactam, GM-Gentamicin, AK- Amikacin, CL- Ciprofloxacin, XM-Cefuroxime, CZ/CAZ- Ceftazidime, SXT/CT- Co-trimoxazole, I-Imipenem.

DISCUSSION

Diabetic foot infections are of the most feared complications of diabetes. This study was undertaken to determine the vitro antibiotic susceptibility pattern. A total of 100 patients with diabetic foot ulcers were studied. This study showed that chronic, complex and previously treated wound infections were generally polymicrobial with mixed Gram positive and Gram negative organisms and these organisms are resistant to multiple drugs.

CONCLUSION

- As the incidence of diabetes will increase expected (196 million cases in a age group of 40-59 to 60-79) to be rise in general population incidence of diabetic foot may also increase up to 15%.
- There is a rising prevalence of multidrug resistant bacteria isolated form diabetic foot infections.
- Gram positive bacteria are found to be most sensitive to vancomycin followed by gentamicin. Gram negative bacteria are found to be most sensitive to imipenem amikacin and gentamicin.
- A combination regimen consisting of amikacin or imipenem and vancomycin seems to be an effective combination for empirical treatment of diabetic foot.
- This study recommends antibiotic should be empirical treatment of choice for Gram-positive isolates and amikacin, cefoperazone/sulbactam, and meropenem should be considered for most of the Gram-negatives aerobes.

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