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Research Article

Antibiotic Susceptibility of Bacterial Strains Isolated from Diabetic Patients

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ABSTRACT

Diabetic foot wounds are a major complication of diabetes resulting in a substantial morbidity and mortality. The present study was carried out to determine the prevalence of different pathogens in Diabetic foot wounds, and their antimicrobial susceptibility patterns. Pus swab from each patient was collected aseptically, and inoculated on culture media. Isolates were characterized, and identified, and Antibiotic susceptibility patterns were determined using the Kirby-Bauer diffusion method. The study aimed to screen the bacterial pathogens present in diabetic foot wounds pus and to determine their antibiotic sensitivity and resistance pattern against 11 commonly used standard antibiotics Augmentin (100%), Amoxicillin (100%), Gentamycin (99%), Ceftriazone (95%), Cotriomoxazole (94%), Chloramphenicol (94.4%), Erythromycin (89%) ,Tetracycline (77.8%), ciprofloxacin (100%), Ofloxacin (94.4%) and Streptomycin (50%). Common pathogens isolated from the diabetic foot wounds pus included Gram positive cocci like *Staphylococcus aureus* and Gram-negative bacilli like *Pseudomonas aeruginosa*. It can be concluded that Gram negative bacteria were present in greater number than Gram positive bacteria in the pus sample. In this study bacterial pathogens showed resistance to most of the antibiotics. It is recommended that antibiotic sensitivity testing be carried out on all isolates of surgical wounds before chemotherapy to avoid selection of drug resistant strains.

Keywords: Diabetic foot wound, Kirby-Bauer diffusion, Antibiotic susceptibility, *Staphylococcus aureus*.

INTRODUCTION

Diabetes impairs the body's ability to regulate blood glucose levels leading to high blood sugar (hyperglycemia). The word diabetes comes from the ancient Greek word meaning "to flow through". The Latin word mellitus meaning "Sweetened or honey like" was added later giving the phrase Diabetes mellitus, which describes the classic symptoms of diabeties. Diabetes mellitus is broadly classified into two type's type 1 and type 2. Of the total diabetic population, 15.20% will experience a foot ulcer in their lifetime. All diabetic foot ulcers are superficially colonized by a plethora of microbes . An average of 5-6 strains of organisms is often involved in the diabetic foot infections with a mixture of aerobic and anaerobic organisms . Selection of an effective antimicrobial agent for a microbial infection requires knowledge of the potential microbial pathogen, an understanding of the patho physiology of the infectious process and an understanding of the pharmacology and pharmaco kinetics of the intended therapeutic agents . Also, antibiotic resistance to the commonly used antibiotics is now emerging as a result of misuse and abuse of particular antibiotics .

Hence the treatment of infection in diabetic patients becomes difficult. Studies are required to assess the right kind of antibiotics and the appropriate concentrations to be used in diabetic infections, taking into consideration the etiology of the infection and the duration of the antibiotic treatment. The diabetic wounds are mostly infected by pus forming microorganisms like *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The magic bullets, the miraculous drugs, antibiotics can be used to heal the diabetic wounds and thus the complications, which are a threat to all diabetic patients and thus can be minimized to a great extent. The aim of this paper

was to substantiate the antibacterial sensitivity of different antibiotics against bacterial pathogens isolated from foot wounds pus samples of diabetic patients.

MATERIALS AND METHODS

Sterilization of Materials

Glass wares which include conical flasks, beakers, test tubes, pipettes, McCartney bottles were washed with detergent after which they were rinsed and sterilized in the oven at 160°C for 1 hour. Inoculating loops and forceps were heated to redness in a Bunsen burner. The spatula, scalpel, mortar and pestle were disinfected with 70% alcohol.

Source of Sample

A total of 20 Diabetic foot wounds swabs were collected from diabetic foot ulcer patients at a Multispecialty hospital in Bangalore, India.

Characterization of Bacterial Isolates

Wound samples were collected using sterile cotton swabs (fresh pus). The pus specimen was inoculated on blood and MacConkey agar plates. The streaked plates were incubated at 37°C for 24 hr. Identification of isolates were done based on colony morphology, Gram staining, motility, catalase test, oxidase test, coagulase test and biochemical tests.

Antibiotics Susceptibility Testing

Susceptibility of isolates to different antibiotics were tested by following Kirby Bauer disc diffusion method using Muller Hinton Agar against selected antibiotics, namely Ampicillin (A) 25mcg, Chloramphenicol (C) 50mcg, Kanamycin (K) 30mcg, Streptomycin (S) 30mcg and Tetracycline (T) 100mcg (Hi- Media, Mumbai). Amoxicillin (30 µg), amoxicillin-clavulanic acid (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), ceftriaxone (30µg), cefuroxime (30 µg), ceftazidime (30 µg), gentamicin(10µg),), ofloxacin (30 µg), levofloxacin (30 µg), ciprofloxacin (30 µg). Inhibition zone size was interpreted using standard recommendation of National Committee for Clinical Laboratory Standards now known as Clinical Laboratory Standard Institute (CLSI).

RESULTS

Table 1 Show the Number of Isolates and Sex

Table 2 shows the zones of inhibition by impregnated gram positive antibiotics discs and then measured with calibrated ruler in millimeters (mm). It was found that ciprofloxacin show the highest zone of inhibition in positive antibiotic discs while cotriazone show the least zones of inhibition. Zones of inhibitions exerted by negative antibiotic discs were shown in table 3. They were measured with calibrated ruler in millimeter (mm). It showed that ciprofloxacin and ofloxacin were most active on the isolated organism with highest zones of inhibition, while the organisms showed resistance to augmentin and gentamycin with the least zones of inhibition.

Table 4 shows the antibiotic susceptibility pattern from positive antibiotic discs. It was found that Gram – Positive and Gram – Negative organism isolate such as staphylococci species and *pseudomonas species* were 100% sensitive to the to the ciprofloxacin and ofloxacin while it was least sensitive to cotrimoxazole.

Table 5 shows the antibiotic susceptibility pattern to Negative antibiotic discs. It was found that gram – positive and gram – negative organism isolated such as *staphylococci species* and *pseudomonas species* were sensitive to ciprofloxacin, ofloxacin and pefloxacin, while it was least
sensitive to augmentin and gentamycin.

DISCUSSION

This study reveals the pattern of the antibiotics susceptibility of bacteria isolated from the Diabetic foot wounds. The use of antimicrobial drugs is often essential and indeed sometimes mandatory in order to achieve desired therapeutic objectives or to treat co-existing diseases . The Microbes causing the diseases may either be sensitive or resistant to the Drugs. Bacteria were isolated in 80% of Diabetic foot wounds, in this study and these organisms were parable to the study done by which reported *Pseudomonas aeruginosa as* the commonest organisms followed by *Staphylococcus aureus.* However, isolated *Staphylococcus aureus* as the commonest isolate followed by *Pseudomonas aeruginosa* and *Streptococcus pyrogenes.* In a study by on 124 patients clinically diagnosed as infections Diabetic foot wounds found *Staphylococcus aureus* and *Pseudomonas aeruginosa* with equal frequency. Other organisms isolated in their study were *Proteus* sp., *Klebsiella* sp and *Escherichia coli*. Furthermore, this study reveals the females were affected slightly more frequent than males. also found that females were more frequently affected than males found that pain, itching and discharge of pus were the most common present complaints in Diabetic foot wounds.

The antibiotic susceptibility test in this study shows that positive antibiotic disc used on gram-positive bacteria isolated were sensitive to ciproflaxcin (100%) and Chloramphenicol (94%). Similarly , the study reveal that negative antibiotic disc used don gram negative bacteria isolated are sensitive to Ciprofloxacin (95%), Pefloxacin (94.4%) and Ofloxacin (89%). Also gram-negative bacteria isolated are resistant to Agumentin (100%) and Gentamycin (99%). also found that majority of organisms isolated from infections Diabetic foot

wounds were resistant to trimoxazole, amoxicillin and erythromycin while 100% were sensitive to impenem and 92% were sensitive to ciprofloxacin and ofloxacin. In conclusion, it is obvious that ciprofloxacin is the most sensitive to both gram-Positive and gram negative bacteria isolated followed by ofloxacin and pefloxacin. However ciprofloxacin can be used empirically for the treatment of acute infections Diabetic foot wounds and the susceptibility will cover both isolated gram-positive and Gram negative bacteria respectively.

CONCLUSION

In conclusion Proper management of diabetic foot infection with the appropriate antibiotic must be implanted keeping in mind the incidence of drug resistance in this population.

Table 1: Show the Number of Isolates and Sex

Samples	No. of Isolates	Sex
1	ACP1	F
2	ACP2	F
3	ACP3	F
4	ACP4	F
5	ACP5	F
6	ACP6	F
7	ACP7	M
8	ACP8	M
9	ACP9	M
10	ACP10	M
11	ACP11	M
12	ACP12	M
13	ACP13	F
14	ACP14	F
15	ACP15	F
16	ACP16	F
17	ACP77	F
18	ACP18	F
19	ACP19	F
20	ACP20	F
Total	**20**	

F-Female
M-Male

Table 2: Zones of Inhibition by Positive Antibiotic Sensitivity Disc

No. of plates	Antibiotic Discs									
	AMX	**OFL**	**STR**	**CHL**	**CEF**	**GEN**	**PEF**	**COT**	**CPX**	**ERY**
1	12	24	26	0	0	0	22	0	30	16
2	0	18	18	0	0	0	10	0	22	0
3	0	20	0	0	0	0	14	0	36	0
4	0	28	28	0	0	0	20	0	30	0
5	22	22	24	0	0	0	18	0	32	0
6	0	24	0	10	0	0	16	0	26	0
7	0	0	0	0	0	0	0	0	20	0
8	0	30	0	0	0	0	24	0	26	0
9	0	24	0	0	0	30	20	0	32	0
10	0	22	0	0	0	0	20	0	30	0
11	22	30	0	0	0	0	0	0	26	0
12	0	24	0	0	0	0	32	26	30	0
13	0	24	0	0	0	0	20	20	28	0
14	0	26	0	0	0	0	28	18	32	26
15	0	32	0	0	0	0	28	0	26	0
16	0	28	0	0	0	18	30	0	34	0
17	0	30	20	20	0	0	34	20	32	24
18	0	28	0	0	0	0	24	20	30	0
Total	**56**	**434**	**30**	**30**	**0**	**62**	**360**	**104**	**522**	**66**

AMX-Amoxyxillin 25 mcg;**OFL**-Ofloxacin 5 mcg;**STR**-Streptomycin 10 mcg; **CHL**-Chloramphenicol 30 mcg; **CEF**-Ceftrizone 30 mcg;**GEN**-Gentamycin 10 mcg; **PEF**-Pefloxacin 5 mcg; **COT**-Cotrimoxazole 25 mcg; **CPX**-Ciprofloxacin 10 mcg; **ERY**-Eryrhromycin 5 mcg.

Table 3: Zones of Inhibition by Negative Antibiotic Sensitivity Disc

No. of plates	Antibiotic Discs									
	AUG	CRO	NIT	GEN	COT	OFL	AMX	CPX	TET	PFX
1	0	0	0	0	20	22	0	24	0	24
2	0	0	0	0	0	28	0	28	0	28
3	0	0	0	0	0	20	0	30	0	18
4	0	0	0	0	0	24	0	34	0	22
5	0	18	0	0	0	20	0	34	28	28
6	0	0	0	0	0	16	0	34	0	26
7	0	0	22	0	0	28	0	22	0	28
8	0	0	0	0	0	20	0	30	0	20
9	0	0	0	0	0	20	0	32	0	22
10	0	0	0	0	0	26	0	22	0	22
11	0	0	0	0	0	18	9	24	0	22
12	0	0	0	0	0	24	0	26	0	12
13	0	0	0	0	0	26	0	36	22	28
14	0	0	0	0	0	12	12	32	0	22
15	0	0	0	0	0	28	0	32	0	30
16	0	0	0	0	0	14	10	28	0	18
17	0	0	0	0	0	30	10	18	24	32
18	0	0	0	0	0	28	0	24	24	18
Total	**0**	**18**	**22**	**0**	**20**	**402**	**22**	**510**	**106**	**402**

AUG –Augmentin 30 mcg; **CRO** –Cenftrizone 30mcg; **NIT** –Nitrofuranton 200 mcg; **GEN** –Gentamycin 10 mcg; **COT** –Cotrimoxazole 25 mcg; **OFL** –Ofloxacin 5 mcg; **AMX** –Amoxycillin 25 mcg; **CPX** –Ciproflooxacin 10 mcg; **TET** –Tetracyclin 30 mcg; **PFX** -Pefloxacin 5 mcg.

Table 4: Antibiotic Susceptibility Pattern from Positive Antibiotic Discs in Percent

Antibiotic Discs	No. of Isolates	No. of Sensitive in (%)	No. of Intermediate In(%)	No. of Resistance in (%)
Amoxyxillin	18	16.7	0.0	5.6
Ofloxacin	18	94.4	0.0	5.6
Streptomycin	18	50.0	0.0	0.0
Chloramphenicol	18	5.6	0.0	94.4
Ceftrizone	18	0.0	5.6	94.4
Gentamycin	18	16.7	0.0	77.4
Pefloxacin	18	17.2	0.0	16.7
Cotrimoxazole	18	27.8	0.0	72.2
Ciprofloxacin	18	100.0	5.6	0.0
Eryrhromycin	18	5.6	0.0	89.0

Table 5: Antibiotic Susceptibility Pattern from Negative Antibiotic Discs in Percent

Antibiotic Discs	No. of Isolates	No. of Sensitive in (%)	No. of Intermediate In(%)	No. of Resistance in (%)
Augmentin	18	0.0	0.0	100.0
Cenftrizone	18	5.6	0.0	94.4
Nitrofuranton	18	2.2	0.0	77.8
Gentamycin	18	0.0	0.0	100.0
Cotrimoxazole	18	5.6	5.6	94.4
Ofloxacin	18	88.8	0.0	5.6
Amoxycillin	18	0.0	0.0	100
Ciproflooxacin	18	94.4	5.6	0.0
Tetracyclin	18	22.2	0.0	77.8
Pefloxacin	18	94.4	0.0	5.6

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