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Antibiotic Susceptibility of Bacterial Pathogens Isolated from Diabetic Patients

V. Rajalakshmi and V. Amsaveni

Department of Microbiology, Dr.G.R. Damodaran College of Science, Coimbatore-641 048, Tamilnadu, India

Abstract: Diabetic foot wounds are a major complication of diabetes resulting in a substantial morbidity and mortality. The present study evaluated the necessity of screening the bacterial pathogens in time and to determine their antibiotic susceptibility, so as to help to identify an empirical therapy. The study aimed to screen the bacterial pathogens present in diabetic pus and to determine their antibiotic sensitivity and resistance pattern against 15 commonly used standard antibiotics; amikacin (30 µg), ampicillin (10 µg), cefotoxime (30µg), ceftazidime (30µg), cefazolin (30µg), ceftriaxone (30 µg), ciprofloxacin (10 µg), gentamycin (10 µg), imipenem (10 µg), ofloxacin (5 µg), penicillin - G (2 µg), piperacilin (100 µg), sulphamethazole (10 µg), trimethoprim (10 µg) and vancomycin (30 µg). Common pathogens isolated from the diabetic pus included Gram-positive cocci like (*Staphylococcus aureus* and *Streptococcus pyogenes*) and Gram-negative bacilli like (*Pseudomonas* sp. *Escherichia coli*. *Klebsiella* sp. and *Proteus* sp.).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.

Key words: *Staphylococcus aureus* · *Streptococcus pyogenes* · *Pseudomonas* sp · *E. coli* · *Klebsiella* sp · *Proteus* sp.

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[1].

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 [2]. An average of 5-6 strains of organisms is often involved in the diabetic foot infections with a mixture of aerobic and anaerobic organisms [3]. Selection of an effective antimicrobial agent for a microbial infection requires knowledge of the potential microbial pathogen, an understanding of the pathophysiology of the infectious process and an understanding of the pharmacology and pharmaco kinetics of the intended therapeutic agents [4] . Also, antibiotic resistance to the commonly used antibiotics is now emerging as a result of misuse and abuse of particular antibiotics [5] . 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 *Enterococci* sp. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella* sp. and *Proteus* sp. [6]. 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 pus samples of diabetic patients.

MATERIALS AND METHODS

Collection of Diabetic Foot Wound Swabs: A total of 50 wound swabs were collected from diabetic foot ulcer patients at a multispeciality hospital in Coimbatore, India.

Corresponding Author: V. Amsaveni, Department of Microbiology, Dr.G.R. Damodaran College of Science, Coimbatore - 641048, India. Mob: +9444179318.

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 [7].

Antibiotic Sensitivity Test for the Confirmed Bacterial Isolates

Preparation of Bacterial Strains Inoculums: The isolated bacterial strains; *Staphylococcus aureus*, *Streptococcus pyogens*, *Escherichia coli*, *Klebsiella* sp. *Pseudomonas aeruginosa and Proteus* sp. inoculums were prepared in 5 ml nutrient broth with 3 to 5 colonies of each bacterial strain. The inoculums were incubated at 37°C for 24 hr to get sample approximately close to 0.5% Mc Farland standard for susceptibility testing. [8].

Antibiotic Discs Used: Commercially available antibiotic discs such as amikacin (30 µg), ampicillin (10 µg), cefotoxime (30µg), ceftazidime (30µg), cefazolin (30µg), ceftriaxone (30 µg), ciprofloxacin (10 µg), gentamycin (10 µg), imipenem (10 µg), ofloxacin (5 µg), penicillin - G (2 µg), piperacilin (100) µg, sulphamethazole (10 µg), trimethoprim (10 µg) and vancomycin (30 µg) were used [8].

A sterile cotton swab was dipped into the cell suspension of the respective isolate whose turbidity was checked with 0.5% McFarland's standard and inoculated on the entire agar surface of each plate first in a horizontal direction and then in a vertical direction to ensure even distribution of the organisms. Antibiotic discs are placed after 5 min. to allowed the agar surface to dry. The inoculated plates were incubated at 37°C for 24-48 hr in an inverted position and the zone of inhibition was recorded [9]. The zone of inhibition was expressed in terms of the Mean ± Standard Deviation by using four replicas and the results were tabulated.

RESULTS AND DISCUSSION

Bacterial pathogens could be isolated from 40 out of the 50 examined samples (Table 1). Diabetic patients with foot ulcers are subjected to several factors that may be associated with multidrug resistant microorganisms carriage, such as inappropriate antibiotic treatment, chronic course of the wound and frequent hospital admission [10]. *Staphylococcus aureus* showed the high degree of sensitivity to ciprofloxacin, gentamycin, ofloxacin and piperacilin. *Streptococcus pyogenes* also showed the high degree of sensitivity to ciprofloxacin, ofloxacin, piperacilin and cefotoxime (Table 2).

Sharma *et al*. [11] reported that the bacterial isolates; *Staphylococcus aureus*, *Pseudomonas* sp. and *Proteus* sp. are present in diabetic pus. In his study, imipenem showed the highest antibacterial activity to Gram-negative organisms. Regarding Gram-negative organisms, *Escherichia coli* showed high degree of sensitivity to imipenem and piperacilin. *Klebsiella* sp. showed high sensitivity to imipenem, piperacilin, sulphamethazole and amikacin. *Pseudomonas aeruginosa* also showed high degree of sensitivity to ciprofloxacin, imipenem, ofloxacin and piperacilin. *Proteus* sp. showed high degree of sensitivity to amikacin, imipenem, ofloxacin and piperacilin (Table 2).

Table 1: Prevalence of microbes in the collected samples

Organisms isolated	No. of positive samples	%
Staphylococcus aureus	11	27.5
Pseudomonas sp.	8	20
Escherichia coli	7	17.5
Klebsiella sp.	6	15
Proteus sp.	5	12.5
Streptococcus pyogens	3	7.5
Total No. of positive samples	40	80
Total No. of samples	50	

Table 2: Antibiotic sensitivity of the bacterial isolates

S.No	Bacteria	AMP	AK	CIP	CTX	CZ	CAZ	CTR	GEN	IMP	OF	P	PI	S	TR	VA
1.	SA	6.5±1.3	18.3±1.3	23.3±6.2	-	-	-	12.5± 6.8	22.3± 6.4	-	20± 6.6	10.3± 6.9	21.3± 6.6	18.5± 6.3	18.5± 6	14.3± 5.7
2.	SP	-	12± 5.7	18.3± 5.5	17.3± 5.3	-	11.3± 0.9	-	16.3± 3.8	-	18.3± 5.2	-	22± 2.7	14± 1.8	15.3± 0.9	-
3.	EC	6.3± 1.5	15.3± 2.4	23.5± 3.3	-	-	-	21.3± 5.1	18± 5.5	26.3± 3.7	17.5± 4.2	-	21.3± 2	-	14.8± 3	-
4.	K	9± 0.8	16.3± 3.3	13 ±4	16.3± 2.5	-	-	10.5± 1.7	15± 2.4	23.8± 4.8	14.3± 1.7	-	20.8± 0.9	18± 3.6	-	-
5.	PA	-	15± 2.8	25.5± 8.2	-	-	16.3± 0.9	-	18.3± 0.9	21.3± 9.6	21.3± 2.6	-	23.8± 3.5	-	-	-
6.	P	-	16.3 ±1.3	18± 1.4	14.8± 2.4	-	13.3± 1.5	-	17.3± 1.5	27.5± 6.4	19.5± 1.2	-	21.3 ±2.5	-	-	-

Zone of inhibition was calculated by using four replicas. (SA-*Staphylococcus aureus,* SP- *Streptococcus pyogenes*, PA- *Pseudomonas aeruginosa,* EC-*Escherichia* coli*, K-Klebsiella* sp*,, P-Proteus* sp.).(AK- amikacin, AMP- ampicillin,CTX- cefotoxime, CAZ-ceftazidime, CZ-cefazolin, CTR-ceftriaxone, CIP-ciprofloxacin,GEN- gentamycin,IMP- imipenem, OF-ofloxacin, P- penicillin - G, PI- piperacilin, S-sulphamethazole,TR- trimethoprim and VA-vancomycin).

It was reported that imipenem, meropenem, amikacin, piperacillin and tazobactam are the most effective agents against whole Gram-negative organisms including *Pseudomonas aeruginosa* and *Acinetobacter* sp. While vancomycin, teicoplanin and chloramphenicol are the most effective agents against Gram-positives. Imipenem, meropenem, vancomycin were reported to be the most effective agents against bacteria isolated in diabetic foot infection in several studies [12-14].

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

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