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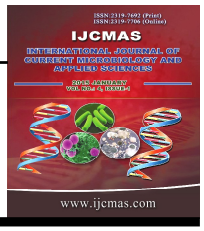

Original Research Article

Bacteriological Profile in Patients with Diabetic Foot Ulcers with special reference to their antibiotic sensitivity pattern

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A B S T R A C T

Diabetes mellitus is one of the major endocrine disorder of global concern. Foot lesions in these patients are a major medical, social and economic problems and are the leading causes for hospitalization. Severe infections lead to amputation of the affected part. Providing effective anti microbial therapy plays a major role in treating these infections. The present study was performed to isolate and the bacterial pathogens in patients with diabetic foot ulcers and to know their drug susceptibility pattern. A total of 150 pus samples were collected from all the patients with diabetic foot lesions during July to December 2014. Samples were processed as per standard guidelines. Out of 150 samples 100 samples yielded growth making a total of 120 isolates. Out of the 120 isolates 75 of the isolates were Gram negative and 45 were Gram positive. In our study among Gram negative pathogens Pseudomonas aeruginosa was the most common (25)(33.3%), followed by Escherichia coli (15)(20%), Klebsiella species (12)(16%), Citrobacter species (10)(13.5%), Acinetobacter species (8)(10.6%), Proteus species (5)(6.6%). Among 45 Gram positive isolates Staphylococcus aureus was the most common isolate (30)(66.6%), followed by CONS (10)(22.2%), Enterococcus species(5)(11.1%). Out of 75 Gram negative isolates 50 were ESBL producers (66.6%), and majority of ESBL producers were Klebsiella species (50%), followed by Citrobacter species(30%), Escherichia coli(20%). Majority of Gram negative pathogens were resistant to Gentamicin, Cefperozone+ sulbactam, Ceftazidime, Cefixime, Aztreonam, Piperacillin+Tazobactam, Ceftriaxone. All the isolates of Pseudomonas aeruginosa were sensitive to Polymyxin-B, Colistin and resistant to Ciprofloxacin, Ceftazidime, Amikacin. Citrobacter species isolated in the present study were sensitive to Colistin, Piperacillin + Tazobactam, Amikacin, Amoxy clav. Of the 30 Staphylococcus aureus isolates 20 were identified as MRSA strains and were sensitive to Cefoxitin. Although in present study Pseudomonas aeruginosa was the most common isolate, Citrobacter species over shadowed the common pathogens like Acinetobacter, Proteus with multiple drug resistance.

Keywords

Diabetes mellitus, Foot ulcers, Amputation, Drug susceptibility pattern, Multi drug resistance

Introduction

Diabetes mellitus is a endocrine disorder affecting β cells of Islets of Langerhans leading to relative deficiency of Insulin. Complications of Diabetes are multiple

which includes Diabetic retinopathy, Diabetic neuropathy, Diabetic nephropathy etc… But apart from the above said complications diabetic foot lesions are of major concern which occur following a minor trauma. Each year more than 700,000 new cases were diagnosed. According to present scenario majority of the people affected with Diabetes mellitus were young adults, children, elderly people, teenagers. Till now no permanent cure is available for Diabetes mellitus (1). Foot ulceration and infection in Diabetic patients is one of the major cause for morbidity, hospitalization and amputation(2). Diabetic foot infections include Cellulitis, abscess, Necrotizing fasciitis, Pyogenic or Suppurative arthritis, Osteomyelitis, Tendinitis,(3) lesions in patients with chronic Calcaneal spurs. The life time risk to a person with diabetes for developing foot ulcer could be as high as 25%(4). Infection is most often as a consequence of foot ulceration which typically occurs after trauma to a neuropathic foot. Infection may be caused by pathogenic bacteria originating from external environment as well as by bacteria forming physiological microflora of skin. The presence of infection depends mainly on the number of micro organisms residing in the wound, where as the healing process depends on the type of bacterial strains and their pathogenicity (5).Diabetic foot ulcer is defined as Infection, Ulceration and destruction of deep tissues associated with neurological abnormalities and various degrees of Peripheral Vascular Disease in the lower limb (WHO, 1985).

Ulcerations are prone to colonization by nearly every microorganism that can come in contact with their surface. Usually ulcerations contain mixed flora, consisting of several strains of bacteria. Most often these bacteria include Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, Proteus species, Citrobacter, Acinetobacter etc…. The mechanism by which bacteria delay the healing process is not understood(6). Infected ulcerations may also be a source of generalized infections, such as inflammation of lymph vessels or sepsis, which may eventually require amputation of limb (7).

Based on the presence of bacteria in the ulcers and clinical signs of inflammation, wounds are classified as : 1. Uncolonized ulcerations with out clinical signs, 2. Colonized ulcerations with out visible clinical signs,3. Infected ulcerations with clinical signs of infection

Materials and Methods

A total of 150 pus samples were collected from all the patients with diabetic foot ulcers attending Narayana General Hospital, Nellore. Complete history of the patients were recorded which includes Age, Sex, Socio-economic status, Duration of ulceration, Treatment (if any) for Diabetes mellitus and duration of Diabetes mellitus. The study was done for over a period of 6 months from July 2014 to December 2014. Sample collection : All swabs were collected before applying an antiseptic dressing to the wound and before starting treatment. Infected area should be decontaminated with 70% ethyl/ isopropyl alcohol.Wearing a sterile, disposable glove separate the wound margins with the thumb or fore finger and making a small opening in a closed abscess with a sterile scalpel, blade. The tip of the swab is extended deeply in to the depths of the lesion with other hand. Care was taken not to touch the adjacent skin margins to avoid contamination with the skin commensals. Two swabs were collected from each case. Organisms were identified by Gram's staining. Culture : Samples were inoculated on Blood agar, MacConkey agar, Nutrient agar. Isolates were further identified by bio- chemical reactions and

further processesed for Anti biotic susceptibility testing by Kirby- Bauer Disc Diffusion method. Anti biogram : Antibiotic susceptibility testing was done on Mueller-Hinton agar. The turbidity of the inoculated broth is compared with 0.5McFarland standards. Lawn culture of the broth suspension was made over the surface of the media and the antibiotic discs were placed and the plates were incubated at 37c for 24 hrs.Results & analysis : Isolates were identified based on their Gram's staining morphology, bio-chemical tests & antibiotic susceptibility testing. The results were analysed and tabulated. A total of 120 isolates were identified from the collected 150 pus samples. Of the 120 isolates 75 were Gram negative pathogens and 45 of the isolates are Gram positive. Of the 75 Gram negative pathogens isolated Pseudomonas aeruginosa(33.3%) was the major pathogen, followed by Escherichia coli (20%), Klebsiella (16%), Citrobacter(13.5%), Acinetobacter(10.6%), Proteus species(6.6%). Among 45 Gram positive isolates Staphylococcus aureus was the most common isolate (30)(66.6%), followed by CONS (10)(22.2%), Enterococcus species(5)(11.1%). Out of 75 Gram negative isolates 50 were ESBL producers (66.6%), and majority of ESBL producers were Klebsiella species (50%), followed by Citrobacter species(30%), Escherichia coli(20%). Majority of Gram negative pathogens were resistant to Gentamicin, Cefperozone+ sulbactam, Ceftazidime, Cefixime, Aztreonam, Piperacillin+ Tazobactam, Ceftriaxone. All the isolates of Pseudomonas aeruginosa were sensitive to Polymyxin-B, Colistin and resistant to Ciprofloxacin, Ceftazidime, Amikacin. Citrobacter species isolated in the present study were sensitive to Colistin, Piperacillin + Tazobactam, Amikacin, Amoxy clav. 60% of isolates of Pseudomonas aeruginosa were resistant to Gentamicin, 32% to cefixime, 28% to Ceftazidime and 24 % to Ciprofloxacin. 53.1% of isolates of Escherichia coli were resistant to Gentamicin, 46.6% to Aztreonam, 66.6% to Ceftriaxone and Cefixime. Of the 30 Staphylococcus aureus strains isolated in the study 20 were identified as MRSA strains and were sensitive to Cefoxitin. 5 of the isolates among Gram positive cocci were identified as Enterococcus species based on the positive results for Bile Esculine Agar and were sensitive to Vancomycin (60%), Sparfloxacin (40%), Linezolid (40%).

Multi drug resistance is common among the isolates belonging to Enterobacteriaceae especially ESBL producing Klebsiella, Citrobacter, Escherichia coli. The results of our study is in correlation with studies done by Sharma VKetal, Katmandu University Medical Journal, 2006, vol:4, No: 3., studies done at Egypt, 2013, Singh N, Arm strong studies on prevention of diabetic foot ulcers, 2005.

Pseudomonas aeruginosa was the most common pathogen associated with foot ulcers in patients with Diabetes mellitus. All the isolates of Pseudomonas were sensitive to Colistin, Polymyxin-B, which are the current choices of treatment.

Diabetes mellitus is the most common of the serious metabolic disorders characterized by long term complications involving eyes, kidneys, nerves and blood vessels. (9-14).Diabetic foot infections are Polymicrobial. Among the most frequently isolated bacteria from the lesions are Pseudomonas aeruginosa, Escherichia coli, Klebsiella, followed by Citrobacter, Acinetobacter etc… Among Gram positive isolates Staphylococcus aureus, Enterococcus were the predominant isolates. In the present study Gram negative aerobic bacteria were most frequently isolated,

which is correlating with study of Ravisekhar Gadepalli etal (8). Aerobic Gram negative bacilli are common in mixed infections with Pseudomonas aeruginosa, Escherichia coli, Klebsiella species being the most common to be isolated. It is often difficult to distinguish true infection caused by these organisms from surface colonization. Hence cultures must be inspected carefully. Based on the findings in sensitivity pattern it is clear that among Gram positive cocci isolated Staphylococci, Enterococci were sensitive to vancomycin. MRSA strains isolated were tested with Oxacillin and Cefoxitin and in our study it is shown that Cefoxitin is the antibiotic of choice for MRSA strains rather than Oxacillin. All the isolates of Citrobacter were sensitive to colistin, Pip+Tazobactam, Amikacin. Among ESBL producers Klebsiella species is most common, followed by Citrobacter and Escherichia coli. Citrobacter is found to be the pathogen which will be responsible for multi drug resistance in future. For infections with multiple organisms, including Gram positive and Gram negative organisms the better response will be when treated with antibiotics like Penicillin derivatives and preserving vancomycin, Linezolid, in combination of metronidazole (for anaerobic organisms).In serious complications, modern techniques include I.V. infusion of linezolid and metronidazole followed by oral treatment.

Table.1 Showing total number of isolates & positive cultures

Total No: of samples	Gram positive isolates (N=120)	Gram negative isolates (N=120)
150	45	75

Table.2 Showing % of Gram negative isolates

Sl No	Name of the pathogen	Total number	%
1	*Pseudomonas aeruginosa*	25	33.3
2	*Escherichia coli*	15	20
3	*Klebsiella species*	12	16
4	*Citrobacter species*	10	13.5
5	*Acinetobacter species*	08	10.6
6	*Proteus species*	05	6.6

Table.3 Sensitivity pattern of Gram negative isolates

Sl No	Organism	Antibiotic	% (S)
1	Pseudomonas aeruginosa	Polymyxin-B Colistin Imipenem	100
2	Citrobacter species	1. Colistin 2. Pip+Taz 3. Amikacin 4. Amoxicillin+clavulanic acid 5. Imipenem	100

Table.4 Showing Resistance Pattern of Gram Negative Isolates

SlNo	Organism	Antibiotic	% (R)
1	*Ps. aeruginosa*	Gentamicin Cefixime Ceftazidime Ciprofloxacin	60 32 28 24
2	*Escherichia coli*	Gentamicin Aztreonam Ceftriaxone Cefixime	53.1 46.6 66.6 66.6
3	*Klebsiella species*	Gentamicin Cef+sulbactam Aztreonam	66.6 50 33.3
4	*Citrobacter spp*	Gentamicin Pip+ Tazo Aztreonam	50 40 30
5	*Acinetobacter spp*	Pip+Tazo Amoxy+Clav Cef+sulbactam	50 37.5 37.5
6	*Proteus species*	Aztreonam	40

Table.5 Showing % ESBL Producers

Total Number of Gram negative isolates	% of ESBL producers	Name of the isolate	%
75	66.6 (50)	*Klebsiella spp* *Citrobacter spp* *Escherichia coli*	50 30 20

Table.6 Sensitivity Pattern of Gram Positive Cocci (n=45)

Gram Positive Isolate	Antibiotic	% (S)
Staphylococcus aureus	Cefoxitin Amoxy +Clav Vancomycin Sparfloxacin Pip +Tazo	100 66.6 100 50 33.3
Enterococcus	Vancomycin Sparfloxacin Linezolid	60 40 40

Diabetic Foot Ulcers

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