Original Article

Bacteriological Profile of Diabetic Wound Infections with Special Reference to Drug Resistance

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ABSTRACT

Aim: A prospective study was carried out on patients with diabetic foot lesions to determine their clinical characteristics, spectrum of microbial flora and asses their comparative in vitro susceptibility to the commonly used antibiotics.

Materials and Methods: A total number of 100 cases of diabetic wound infections were screened for aerobic and anaerobic isolates. 50 non-diabetic patients with wound infection were screened for aerobic and anaerobic isolates which is taken as control.

Results and Conclusion: Out of 100 wound swabs collected from diabetic wounds, 44 samples were culture positive. From 44 cultures positive samples 48 aerobic bacteria were isolated. Among the 44 culture positive samples 40 were monomicrobial, whereas rest of the 4 culture positives revealed polymicrobial isolates. The incidence of diabetic wound infections were more among males and prevalence is more among the age group 40 – 69 years (mean age of incidence is 59.69 ± 9.71 years).

In the present study Pseudomonas aeruginosa was the predominant isolate followed by Staphylococcus aureus, Escherichia coli, Klebsiellapneumoniae, and Staphylococcus epidermidis. The incidence of wound infection is more among the diabetic patients when compared to non-diabetic patients because several immune defence mechanisms are defective like decrease in leukocyte chemotaxis, phagocytosis and defective intracellular killing. In the present study Pseudomonas aeruginosa 7(20.59%) was the predominat ESBL producer, followed by Klebsiellapneumoniae 4(11.76%) and Escherichia coli 3(8.82%). Total ESBL producers in gram negative isolates was 41.18%. Metalobetalactamases production among the Pseudomonas aeruginosa was 16.67%. The results also had shown the polymicrobial nature of the diabetic wound infection.

Keywords: Diabetes, wound, MRSA, ESBL, MBL

INTRODUCTION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia. The hyperglycemia results from defects in insulin secretion, insulin action or both. The chronic hyperglycemia of diabetes is associated with specific chronic complications resulting in damage or failure of various organs, notably the eyes, kidney, nerves, heart & blood vessels. [1]

Diabetes causes a number of deleterious effects on immune defense mechanisms both cellular and humeral.Included are changes in leukocyte function, altered microvascular response, and changes in the complement cascade, cytokine network, and chemokine formation. There are harmful effects of hyperglycemia on leukocyte function such as decrease in chemotaxis, phagocytosis, adherence, and bacteriocidal activity. [2]

Optimal management of diabetic wound infection can decrease infection related morbidity, duration of hospital stay and incidence of gangrene, limb amputation. Thus this study was attempted to know the bacteriological profile and associated resistance in isolates from the diabetic patients, to formulate a local antibiotic policy in establishing an empirical treatment for diabetic wound infections.

MATERIALS AND METHODS

The present study "bacteriological profile of diabetic wound infections" was conducted in the Department of

Microbiology, ChalmedaAnand Rao Institute of Medical Sciences, Bommakal, Karimnagar for a period of one year. 100 wound swabs were collected from inpatients and out patients who are attending CAIMS General Hospital. Patients who are clinically diagnosed diabetes mellitus with wound infections are included in this study. 50 wound swabs were also collected from non-diabetic patients with wound infection, which is taken as a control group.

The wound site was first decontaminated with 70% ethyl alcohol after which the wound is washed well with sterile saline and dried. Three wound swabs are collected from the wound site under sterile precautions and transported in sterile screw caped tubes (Hi-media). One swab is for Gram's staining, the second swab for aerobic culture and the third for anaerobic culture.

The pus swabs were cultured on Blood & Mac conkey agar and incubated at 37^0C for 24 – 48 hrs. Wound swabs were inoculated on two Anaerobic Blood agar plates and incubated in GEN bags and Mc IntoshFilde's anaerobic jar with anaerobic generators. Anaerobic condition was detected by the anaerobic indicator capsule. After incubating for 48 – 72 hours plates were observed for growth.

All organisms isolated were identified according to standard microbiological methods. Antimicrobial susceptibility of bacterial isolates was performed by Kirby Bauers disk diffusion method. The isolates were tested for drug resistance by the following methods.

Detection of MRSA

Detection of MRSA was done by Oxacillin disc (1µg) on the bacterial lawn culture of Staphylococcus aureus. After overnight incubation, the zone of inhibition was measured. An inhibition zone diameter less than or equal to 10mm indicates MRSA. [3]

Cefoxitin Disc Diffusion Test

All the isolates were subjected to Cefoxitin disc diffusion test using a 30 µg disc. A 0.5 McFarland standard suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 37 0C for 18 h and zone diameters were measured. An inhibition zone diameter of = 19 mm was reported as Oxacillin resistant and =20 mm was considered as Oxacillin sensitive4.

Detection of ESBL Production

Screening for ESBL

This was done as part of the routine susceptibility testing, according to criteria recommended by the NCCLS. Two discs, Ceftazidime (30µg) and Cefotaxime (30µg), were used. An inhibition zone of < 22 mm for Ceftazidime and < 27 mm for Cefotaxime indicated that the strain probably produced ESBL. [5]

Phenotypic confirmatory test for ESBL Production :

The suspension for inoculum was prepared from 4-5 isolated colonies and turbidity was compared with 0.5 McFarland standard. Sterile cotton swab soaked in this suspension was used to make lawn culture on Mueller Hinton agar plates. Ceftazidime (30 µg) and Ceftazidime + Clavulunic acid (20 µg + 10 µg) were placed at the distance of 20mm from center to center.

Plates were incubated at 37°C overnight. A > 5mm increase in zone diameter for the antimicrobial tested in combination with Clavulanic acid versus its zone when tested alone confirmed ESBL production. [6]

Detection of Metallo Betalactamases

Imipenem resistant Pseudomonas aeruginosa isolates were further screened for MBL production by Double disc synergy test.

Imipenem-Ethylene Diamine Tetra Acetic Acid (EDTA) Double Disc Synergy Test

Test organisms were inoculated on the plates with Muller Hinton agar. An Imipenem (10 microgram) disc was placed 20mm centre to centre from blank disc containing 10 microL of 0.5M EDTA (750 microgram). Enhancement of the zone of inhibition in the area between Imipenem and the EDTA discs in comparison with the zone of inhibition on the far side of the drug was interpreted as positive. [7]

Ethics approval

The study protocol was reviewed and approved by the Institutional Ethics committee, at Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, 2015, and written informed consent form was obtained from all participants.

RESULTS

Among the 100 patients with diabetic wound infection 68(68%) were male and 32(32%) were female. The incidence of diabetic wound was more among the age group between 40 – 69 years with a mean age of about 59.69 ± 9.71 years.

Out of 100 wound swabs collected from diabetic wounds, 44 samples were culture positive. From 44 culture positive samples 48 aerobic bacteria were isolated. Among the 44 culture positive samples 40 were monomicrobial, whereas rest of the 4 culture positives revealed polymicrobial isolates.

Table-1 : Aerobic Gram Positive and Gram Negative Bacteria Isolated in Diabetic Wound Infection

S.No	Gram positive cocci	Percentage
1	Staphylococcus aureus	10 (20.83%)
2	Staphylococcus epidermidis Gram negative bacilli	4 (8.33%)
3	Pseudomonas aeruginosa	18(37.50%)
4	Escherichia coli	9 (18.75%)
5	Klebsiellapneumoniae	7 (14.58%)
	TOTAL ISOLATES	48

Pseudomonas aeruginosa 18(37.50%) was the predominant isolate followed by Staphylococcus aureus 10(20.83%), Escherichia coli 9(18.75%), Klebsiellapneumoniae 7(14.58%) and Staphylococcus epidermidis 4(8.33%). No Anaerobic bacteria were isolated in the present study.

In the present study Pseudomonas aeruginosa isolates were resistant to Ciprofloxacin (88.89%), Gentamycin (83.33%), Ceftriaxone (77.78%), Ceftazidime (72.22%), Carbenicillin (66.67%) and Amikacin (61.11%). Pseudomonas aeruginosa isolates were sensitive to Imipenem (72.22%), Piperacillin / tazobactam (61.11%) and Cefotaxime (44.44%).

The antibiotic sensitivity results of Staphylococcus aureus isolates showed higher level of resistance to Erythromycin (90%), Ampicillin (80%), Ciprofloxacin (70%) and Amoxicillin / clavulanic acid (60%) and Gentamycin (40%). They were sensitive to Vancomycin (100%), Linezolid (80%), Piperacillin/ tazobactam (70%) and Cefotaxime (70%).

In the present study Escherichia coli were resistant to Amoxicillin / clavulanic acid (77.78%), Ciprofloxacin (55.56%), Gentamycin (44.44%) and Cefotaxime (33.33%) and sensitive to Imipenem (77.78%), Piperacillin / tazobactam (77.78%), Ceftazidime (77.78%) and Cefepime (66.67%).

Table-2 :Antibiotic Susceptibility Pattern of Gram Negative Isolates

Sl No.	Antibiotics	Number of sensitive organisms			Number of resistant organisms		
		Kleb	Pseudo	E.coli	Kleb	Pseudo	E.coli
1	Amoxicillin / clavulanic acid	3(42.86%)	-	2(22.22%)	4(57.14%)	-	7(77.78%)
2	Amikacin	5(71.43%)	7(38.89%)	6(66.67%)	2(28.57%)	11(61.11%)	3(33.33%)
3	Ciprofloxacin	3(42.86%)	2(11.11%)	4(44.44%)	4(57.14%)	16(88.89%)	5(55.56%)
4	Cefotaxime	4(57.14%)	8(44.44%)	6(66.67%)	3(42.86%)	10(55.56%)	3(33.33%)
5	Ceftazidime	4(57.14%)	5(27.78%)	7(77.78%)	3(42.86%)	13(72.22%)	2(22.22%)
6	Ceftriaxone	4(57.14%)	4(22.22%)	6(66.67%)	3(42.86%)	14(77.78%)	3(33.33%)
7	Cefepime	6(85.71%)	5(27.78%)	6(66.67%)	1(14.29%)	13(72.22%)	3(33.33%)
8	Gentamycin	2(28.57%)	3(16.67%)	5(55.56%)	5(71.43%)	15(83.33%)	4(44.44%)
9	Imipenem	7(100%)	13(72.22%)	7(77.78%)	0(0%)	5(27.78%)	2(22.22%)
10	Piperacillin / tazobactam	6(85.71%)	11(61.11%)	7(77.78%)	1(14.29%)	7(38.89%)	2(22.22%)
11	Carbenicillin	-	6(33.33%)	-	-	12(66.67%)	-

In the present study Klebsiellapneumoniae were resistant to Amoxicillin / clavulanic acid (57.14%), Ciprofloxacin (57.14%), Gentamycin (71.43%) and Cefotaxime (42.86%) and sensitive to Imipenem (100%), Piperacillin / tazobactam (85.71%), Ceftazidime (57.14%) and Cefepime (85.71%).

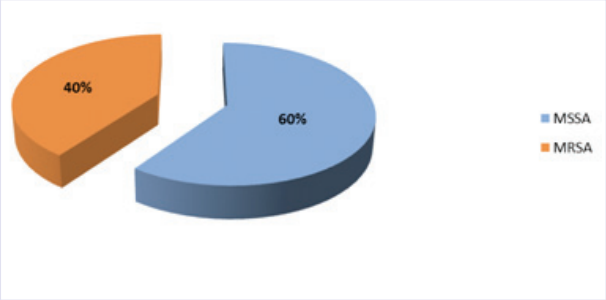

Figure- 1 : Incidence of Mrsa

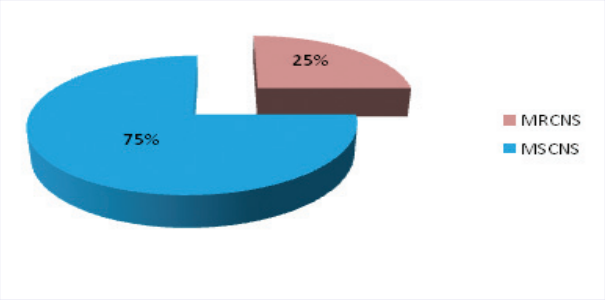

Figure 2 : Incidence of Mrcns (Staphylococcus Eiderimidis)

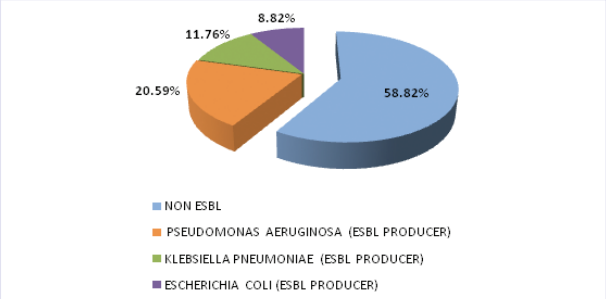

Figure 3 : Incidence of ESBL among gram negative isolates (Pseudomonas Aeruginosa, Escherichia Coli, Klebsiella Pneumoniae)

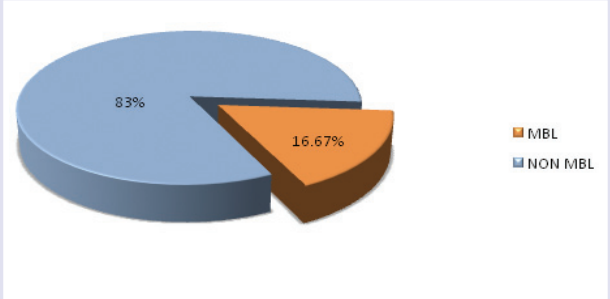

Figure 4 : Incidence of MBL in Pseudomonas Aeruginosa

Table -3 : Drug Susceptibility Pattern In Gran Positive Cocci

Sl No.	Antibiotics	Number of sensitive organisms %		Number of resistant organisms %	
		Staphylococcus aureus	Staphylococcus epidermitis	Staphylococcus aureus	Staphylococcus epidermitis
1	Ampicillin	2(20%)	2(50%)	8(80%)	2(50%)
2	Amoxicillin / clavulanic acid	4(40%)	2(50%)	6(60%)	2(50%)
3	Amikacin	5(50%)	4(100%)	5(50%)	0(0%)
4	Ciprofloxacin	3(30%)	3(75%)	7(70%)	1(25%)
5	Cefotaxime	7(70%)	3(75%)	3(30%)	1(25%)
6	Erythromycin	1(10%)	2(50%)	9(90%)	2(50%)
7	Gentamycin	6(60%)	2(50%)	4(40%)	2(50%)
8	Linezolid	8(80%)	4(100%)	2(20%)	0(0%)
9	Piperacillin / tazobactam	7(70%)	4(100%)	3(30%)	0(0%)
10	Vancomycin	10(100%)	4(100%)	0(0%)	0(0%)
11	Oxacillin	6(60%)	3(75%)	4(40%)	1(25%)

Incidence of methicillin resistance among gram positive isolates was found to be 35.71%. In the present study the incidence of Methicillin resisistant among Staphylococcus aureus isolates was 40%. Methicillin resistance coagulase negative Staphylococcus (Staphylococcus epidermidis) was 25%.

Pseudomonas aeruginosa 7(20.59%) was found to be the predominat ESBL producer, followed by Klebsiellapneumoniae 4(11.76%) and Escherichia coli 3(8.82%). Total ESBL producers in gram negative isolates was 41.18%. Metalobetalactamases production among the Pseudomonas aeruginosa was 16.67%.

From the total 50 samples of control group (non diabetic) 11 aerobic bacteria were isolated, and the incidence of wound infections in the control group was 22% less than the test group. Staphylococcus aureus 5(45.45%) was found to be the predominant isolate followed by Pseudomonas aeruginosa 2(18.18%), Escherichia coli 2(18.18%), Klebsiellapneumoniae 1(9.09%) and Staphylococcus epidermidis 1(9.09%). No Anaerobic bacteria were isolated in the control group. Unlike the diabetic patients all the isolates were monomicrobial.

Antibiogram of the control group revealed that isolates were sensitive to multiple drugs unlike the diabetic patients. None of the isolate from the control group was MRSA, ESBL and MBL producer.

As diabetic mellitus is a metabolic disorder the isolates from the wound infections were multidrug resistant, some of them are MRSA and ESBL, MBL producers.

DISCUSSION

VenkataNagaraju E et al [8] reported 40% incidence of Pseudomonas aeruginosa similar to present study. Lower incidence of Pseudomonas aeruginosa were reported by Priyadarshini Shanmugam et al [9] 16%.

Farwa Rizvi et al [10] 22% and Yoga et al [11] 20% reported Staphylococcus aureus. There results are similar to our observation. Higher incidence of Staphylococcus aureus was reported by Maryam Aminiet al [19] 34.6%. Ravisekhargadepalli et al [13] reported 13.7% low incidence of Staphylococcus aureus compared to present study.

Maryam Amini et al [12] 20.2%, Shao-Hua Wang et al [14] 20% of Escherichia coli was reported. Their results were similar to our observation. Lower incidence of Escherichia coli isolates were reported by Priyadarshini Shanmu-gam et al [9] 14.6%. Higher incidence of Escherichia coli was reported by Girish et al [15] 22.22% than the present findings.

Rajalakshmi V et al [16] 15% reported similar incidence of Klebsiellapneumoniae compared to present study. Low incidence of Klebsiellapneumoniae was reported by Priyadarshini Shanmugam et al 98% than the present study. Incidence of Klebsiella pneumoniae reported by Sivaramanumadevi et al [17] 20.5% was high compared to the present study.

Girish et al [15] reported 11.1% of Staphylococcus epidermidis which was nearly similar to the present study. Shao Hua Wang et al [14] 3.8% reported lower incidence of Staphylococcus epidermidis.

The incidence of polymicrobial microbial study by Yoga R et al116% was similar to present findings. Higher incidence of polymicrobial isolates were reported in studies done by Ravisekhargadepalli et al [13] 70% compared to present study. Low polymicrobial incidence was reported by Farwa Rizvi et al [10] 2% compared to the present study.

In the present study the incidence of Methicillin resisistant Staphylococcus aureus was 40% which was similar to the study results of Kavita et al [18] 41.5%. Higher incidence of MRSA was reported by Maryam Amini et al [12] 66.7%. Lower incidence of MRSA was reported by Priyadarshini Shanmugam et al [9] 33.33%.

In the present study 25% Methicillin resistance coagulase negative Staphylococcus (Staphylococcus epidermidis) was reported. Higher incidence of MRCNS was reported by VinitaRawat et al [20] 50%.

In the present study Pseudomonas aeruginosa 7(20.59%) was the predominat ESBL producer, followed by Klebsiellapneumoniae 4(11.76%) and Escherichia coli 3(8.82%). Total ESBL producers in gram negative isolates was 41.18%.

Priyadarshini Shanmugam et al [19] 37.5% and Vinita Rawat et al [20] 40% reported similar ESBL producing gram negative isolates SivaramanUmadevi et al [17] 56% reported higher incidence of ESBL producers.

In the present study Metalobetalactamases production among the Pseudomonas aeruginosa was 16.67%. Higher incidence of MBL producers was reported by Priyadarshini Shanmugam et al [9] 37%,Vinita Rawat et al [20] 37.5%.

CONCLUSION

The incidence of wound infection is more among the diabetic patients when compared to non diabetic patients. The organisms associated with diabetic wound infections were resistant to multiple drugs.

The prevalence of Multidrug resistance was alarmingly high in the diabetic patients. A detailed knowledge of the susceptibility to antimicrobial agents is necessary to facilitate the development of effective strategies to combat the growing problem of resistance especially MRSA, ESBL and MBL strains in diabetic wound infections.

By the present study causative organism was isolated with authentic antibiogram which helps judicious use of antimicrobials in the diabetic wound infections to control chronic strain and limit the acquisition of additional resistance genes in the existing strains.

CONFLICT OF INTEREST: None.

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