IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)
e-ISSN: 2279-0853, p-ISSN: 2279-0861.Volume 17, Issue 6 Ver. 5 (June. 2018), PP 27-31
www.iosrjournals.org

Bacteriology of Diabetic Foot Ulcers with Special Reference to Antibiotic Resistance in GGH, Kakinada

Dr E. Vandana Giridhar[1] Dr C. Lakshmi Kalavathi[2]
1.Assistant Professor, Dept. Of Microbiology, ACSR, GMC, Nellore, Dr. NTRUHS, Andhra Pradesh, India
2.Assistant Professor, Dept. Of Pathology, ACSR, GMC, Nellore, Dr. NTRUHS, Andhra Pradesh, India
Corresponding Author: Dr C Lakshmi Kalavathi[2]

Abstract:
Background: *Because of the altered immune status in Diabetic patients, opportunistic and infections are common in Diabetic foot ulcers.*
Aim: *The present study was conducted to know the bacteriology and antibiogram of Diabetic foot infections in and around Kakinada and detection of ESBL producers.*
Material and Methods: *The material for the present study was collected from patients attending OPs and those admitted in the Departments of Medicine and Surgery in Government General Hospital, Kakinada over a period of 6 months. 125 samples of pus and exudates were obtained from patients with Diabetic foot ulcers.*
Results: *Male to female ratio observed was 2.57. The common age group affected is between 50-70 years. A total of 203 isolates were obtained from 125 cases. Staphylococcus aureus was commonest organism isolated constituting 32% of the total isolates. Pseudomonas aeruginosa was the second common organism isolated (18.4%). Coagulase Negative Staphylococci (16.8%), Klebsiella species(16.8%), Escherichia coli(8.8%), Proteus species(5.6%) and Acinetobacter species (1.6%) were the other organisms isolated. The antibiotic sensitivity testing showed Staphylococcus aureus being sensitive to Linezolid, Vancomycin, Tetracycline, Erythromycin and Cotrimoxazole. They were resistant to Penicillins, Fluoroquinolones and Cephalosporins. The Gram Negative bacilli were sensitive to Carbapenems, Betalactam & Betalactam inhibitors, Piperacillin & Tazobactam, Cephalosporins, Fluoroquinolones, Aminoglycosides and Cotrimoxazole in the decreasing order of susceptibility. All of them were resistant to Ampicillin. Of the 34 isolates of Klebsiella species, 44.11% were ESBL producers and of the 18 isolates of Escherichia coli,33.33% were ESBL producers.*
Conclusion: *Poly-microbial infections were common in patients with Diabetic foot ulcers. it is advisable to do Antibiotic Sensitivity testing before starting treatment.*
Keywords: *diabetic foot ulcer, polymicrobial, ESBL producers, antibiotic sensitivity, Wagner's grades*

Date of Submission: 19-05-2018 Date Of Acceptance: 11-06-2018

I. Introduction

Diabetes mellitus is a non-communicable metabolic disorder. The global prevalence of diabetes has risen from 4.7% in 1980 to 8.5% in 2014 [1] .India had 69.2 million people living with diabetes (8.7%) as per the 2015 data [2]. There has been an increase in prevalence of Type 2 Diabetes globally in all age groups.

Diabetic foot ulcers are a resource consuming, disabling morbidity that is often the first step towards lower limb amputation for which prevention is the best treatment. [3]. The International Diabetic Federation and W.H.O. chose " Diabetic Foot " as the theme for World Diabetes Day Nov 14th, 2005 with the slogan – PUT FEET FIRST; PREVENT AMPUTATIONS [4] .

The specific organisms found in diabetic foot infections differ not only from patient to patient and hospital to hospital, but also from one part of the country to another [5] .Superficial wounds are usually mono-microbial but deep wounds are poly-microbial. However, the pattern of micro-organisms depends to a great extent on microbial flora of the lower limb and other factors like metabolic disorders and hygienic conditions of the patients [6]. Although increasing antimicrobial resistance is a pertinent problem in India, there is a paucity of data on the frequency of Multi Drug Resistant Organisms' infections and the outcome of such infections among Diabetic foot ulcers in this region [7]. So, this study was performed to determine the common etiological agents of Diabetic foot infections, to detect in vitro susceptibility to routinely used antibiotics and ESBL producers in patients attending tertiary hospital.

II. Materials And Methods

Study group consisted of 125 samples collected from patients with foot ulcers attending to Surgical OPs and Medical and Surgery Wards with Diabetes mellitus in the Government General Hospital, Kakinada

over a period of six months from May to October 2014. Age, sex, socioeconomic status, duration and type of Diabetes, details of ulcer, examination findings, relevant investigation reports and treatment details were recorded on a predesigned proforma.

Collection of Samples was done using standard microbiological techniques. The pus and exudates from the base and margin of the ulcer were collected with two sterile swabs under strict aseptic conditions. One swab was used to make a direct smear and Grams staining of the smear was done by following standard procedures. The second swab was inoculated onto Nutrient Agar, Blood Agar, and Mac Conkey Agar. The inoculated plates were incubated overnight (16 - 18 hours) after which they were examined for bacterial growth. In case of bacterial growth, the bacteria were further identified using standard biochemical tests.

Antibiotic Susceptibility Testing was done by modified Kirby Bauer Disc Diffusion Method following CLSI guidelines. Kits used for confirmation of ESBL producers by Disc Potentiation Test -KIT I -- Cefotaxime disc -- 30mcg and Cefotaxime / Clavulinic Acid -- 30mcg / 10mcg. KIT III -- Ceftazidime disc -- 30mcg and Ceftazidime / Clavulinic acid -- 30mcg/10mcg.

III. Results

125 samples of pus and exudates were obtained from patients who were Diabetic with foot ulcers.

Table 1: Urban/ Rural Distribution (n = 125)

Locality	N (%)
Urban	27(21.6%)
Rural	98 (78.4%)

Cases were more from rural areas (98) than urban areas (27).

Table 2: Age Distribution (n = 125)

Age Distribution	N(%)
< 40 Years	2 (1.6%)
41-50	20 (16%)
51-60	43(34.4%)
61-70	41(32.8%)
71-80	14(11.2%)
>80	5(4%)

Diabetic foot ulcers were more common in the age groups 50-60 years and 60-70 years.

Table 3: Gender Distribution (n = 125)

Sex	N (%)
Male Patients	90(72%)
Female Patients	35 (28%)

The male to female ratio was 2.57 in our study.

Table 4: Nature of Bacterial isolates (n = 125)

Nature of isolates	N (%)
Poly microbial	78 (62.4%)
Mono microbial	47 (37.6%)

Table 5: Distribution of isolates in various Wagner's grades. (n = 203)

Chi-square = 46.23 , df = 30 , P-value < 0.03. statistically not significant

Organisms	Grade 0	Grade1	Grade2	Grade3	Grade4	Grade5	Total (%)
Staphylococcus aureus	0	27	30	6	1	1	65 (32%)
Coagulase Negative Staphylococci	0	9	17	6	0	0	34 (16.8%)
Pseudomonas aeruginosa	0	7	20	8	2	1	38 (18.4%)
Klebsiella species	0	7	16	10	1	0	34 (16.4%)

Escherichia coli	0	5	9	4	0	0	18 (8.8%)
Proteus species	0	1	2	3	4	1	11 (5.6%)
Acineto bacter species	0	0	1	1	1	0	3 (1.6%)
Total	0	56	95	38	9	3	203

Staphylococcus aureus, Pseudomonas aeruginosa and Coagulase Negative Staphylococci were more predominantly isolated in Grade 2 Diabetic foot ulcers.

Table 6: Antibiotic Susceptibility pattern of Gram positive Cocci

Drugs	Staphylococcus aureus (n = 65) sensitive	resistant	Coagulase Negative Staphylococci (n = 34) sensitive	resistant
Penicillin	0	65(100%)	0	34(100%)
Erythromycin	34 (52.3%)	31(47.7%)	19 (55.9%)	15(54.1%)
Cotrimoxazole	31 (47.7%)	34(52.3%)	19 (55.9%)	15(54.1%)
Tetracycline	42 (64.6)	23(35.4%)	24 (70.6%)	10(29.4%)
Ciprofloxacin	23 (35.4%)	42(64.6%)	14 (41.2%)	20(58.8%)
Vancomycin	60 (92.3%)	5(7.7%)	33 (97.1%)	1(2.9%)
Linezolid	64 (98.5%)	1(1.5%)	34 (100%)	0(100%)
Cefoxitin	52 (80%)	13(20%)	28(82.4%)	6(17.6%)
Cephalosporins	20 (30.8%)	45(69.2%)	11 (32.4%)	23(67.6%)

Table7: Antibiotic susceptibility pattern of Aerobic Gram negative Bacilli

Drugs	Pseudomonas aeruginosa(n=38) Sensitive	Klebsiella species(n=34)	Escherichia coli(n=18)	Proteus species(n=11)	Acinetobacter species(n=3)
Ampicillin	0	0	0	0	0
Aminoglycosides	17 (44.7%)	16 (47.1%)	14 (77.8%)	7 (63.6%)	1(33.3%)
Fluoroquinolines	21 (55.3%)	13 (38.2%)	16 (88.9%)	8 (72.7%)	1 (33.3%)
Cotrimoxazole	6 (15.8%)	19 (55.9%)	5 (27.8%)	2 (18.2%)	0
Cephalosporins	23 (79%)	19 (55.9%)	12 (66.7%)	4 (36.4%)	2 (66.7%)
Carbapenems	38 (100%)	24 (70.6%)	18 (100%)	11 (100%)	3 (100%)
Beta lactams + Beta lactam inhibitors	25 (65.8%)	19 (55.9%)	18 (100%)	10 (90.9%)	3 (100%)
Piperacillin+ Tazobactum	35 (92.1%)	22 (64.7%)	17 (94.4%)	10 (90.9%)	2 (66.7%)

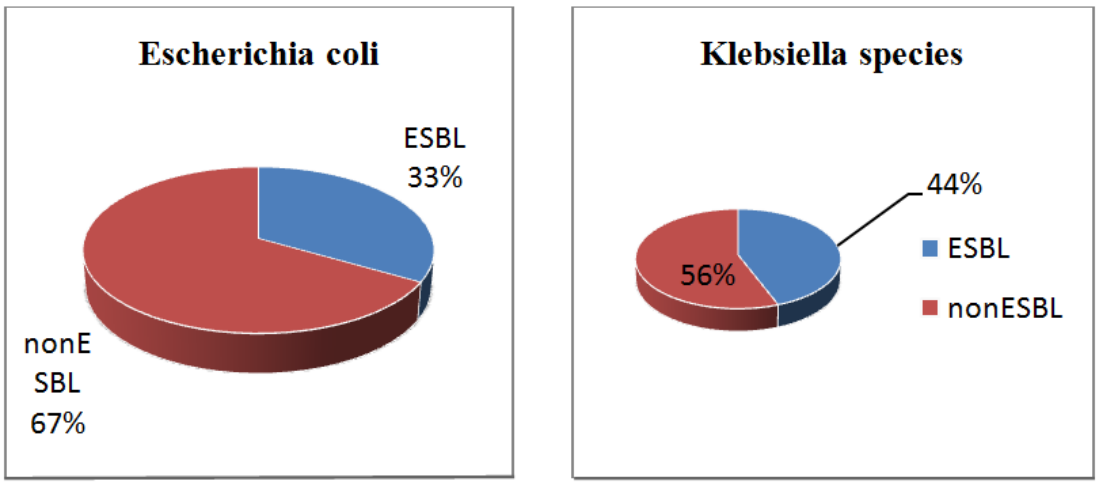

Fig 1: ESBL producers

IV. Discussion

In our study, out of 125 patients, 98 (78.4%) hailed from rural areas and the rest 27 (21.6%) hailed from urban areas. This is because most of the people work in the fields or in small scale industries in and around Kakinada. They usually work barefoot and so are more prone to injuries of the foot which may go unnoticed till they ulcerate and cause inconvenience. Ulcers were more common in ages 51-60 yrs -43 (34%) and 61-70 yrs - 41 (32.8%). In a similar study conducted by Rool-ul-Muquim, Samson Griffith et al [3], ulcers were more common in the ages between 41-50 yrs – 47(47%) and 51-60yrs – 32(32%). This shows that there is a delay in the onset of foot ulcers. This could possibly be due to an improvement in the immune status of the patients along

with early diagnosis and good control of diabetes and its complications. Out of 125 samples, 90(72%) were from males and 35 (28%) were from females. The male to female ratio was 2.57. In a similar study conducted by C. Anandi et al [8], 65% of the patients were males and 35% were females. The male female ratio was 2.03. This is correlating with my study. This indicates that male diabetic patients are more prone to foot ulcers than females. This is probably due to their occupational exposure which makes them more prone for foot injuries.

Based on Wagner's classification, the isolates were Staphylococcus aureus (32%), Pseudomonas aeruginosa (18.4%), Coagulase Negative Staphylococci (16.8%), Klebsiella species (16.8%), Escherichia coli (8.8%), Proteus species (5.6%) and Acinetobacter species (1.6%).

In the present study, Polymicrobial infections were common in Diabetic foot ulcer because of the altered immune status in diabetic patients which makes them more prone to infections.

Staphylococcus aureus was the primary isolate and showed resistance to Penicillin, Fluoroquinolones and Cephalosporins and sensitivity to Linezolid, Vancomycin, Tetracycline, Erythromycin and Cotrimoxazole in decreasing order of sensitivity. In a study by Benu Dhawan et al [7], Staphylococcus aureus showed resistance to Penicillin and Fluoroquinolones which is correlating with the present study. This study also showed resistance to Erythromycin but the present study showed sensitivity to Erythromycin.

Coagulase Negative Staphylococci were isolated and showed sensitivity to Linezolid, Vancomycin, Tetracycline, Erythromycin and Cotrimoxazole and resistance to Penicillin, Fluoroquinolones and Cephalosporins.

Pseudomonas aeruginosa which is the second common isolate showed susceptibility to Carbapenems, Piperacillin & Tazobactum, Beta lactam & Betalactam inhibitors and Fluoroquinolones. They showed resistance to Aminoglycosides, Cephalosporins and Cotrimoxazole which is correlating with a similar study by Benu Dhawan et al [7].

Klebsiella species which was the 3rd common isolate showed sensitivity to Carbapenems, Piperacillin & Tazobactam, Betalactam & Betalactam inhibitors, Cotrimoxazole and Aminoiglycosides. They showed resistance to Fluoroquinolones and Cephalosporins correlating with similar study by Sivaraman Umadevi et al [9] .

Escherichia coli which was the next common isolate and showed sensitivity to Carbapenems, Betalactam&Betalactam inhibitors, Piperacillin&Tazobactum, Fluoroquinolones, Aminoglycosides and Cephalosporins in the decreasing order. They were resistant to Ampicillin and Cotrimoxazole correlating with a similar study by Dipali A Chincholkar et al [6].

Proteus species was found to be sensitive to Carbapenems, Piperacillin & Tazobactam, Betalactams & Betalactam inhibitors, Fluoroquinolones and Aminoglycosides. They were found to be resistant to Ampicillin, Cotrimoxazole and Cephalosporins correlating with similar studies by J. Vimalin Hema et al [10], and Sivaraman Umadevi et al [9] .

Acinetobacter species were found to be sensitive to Carbapenems, Betalactam&Betalactam inhibitors, Piperacillin&Tazobactum and Cephalosporins. They were resistant to Ampicillins, Aminoglycosides and Fluoroquinolones correlating with a similar study by Sivaraman Umadevi et al [9]. But in this study, the organisms were also sensitive to Fluoroquinolones which contradicts with the findings of the present study.

ESBL production is commonly seen with the Enterobacteriaceae family and more in Klebsiella species and Escherichia coli. So, ESBL detection was done for these two organisms. In the present study, out of a total of 34 Klebsiella species isolates, ESBL production was seen in 14 isolates(44.11%). Out of a total of 18 Escherichia coli isolates, ESBL production was seen in 6 isolates(33.33%). In a study by Sivaraman Uma Devi et al [9] Pondicherry, India, ESBL production in Klebsiella species was 60% and in Escherichia coli it was 56%.

V. Conclusion

Poly-microbial infections were more common in patients with Diabetic foot ulcers (78 – 62.4%) due to altered immune status. Isolates showed multi drug resistance pattern. Appropriate selection of antibiotics based on the antibiograms of the isolates from the lesions is most critical for the proper management of these infections. This also helps in preventing complications like gangrene and limb amputations.

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Dr E. Vandana Giridhar, Dr C. Lakshmi Kalavathi "Bacteriology of Diabetic Foot Ulcers with Special Reference to Antibiotic Resistance in GGH, Kakinada."IOSR Journal of Dental and Medical Sciences (IOSR-JDMS), vol. 17, no. 6, 2018, pp 27-31.