CLINICO-BACTERIOLOGICAL PROFILE OF DIABETIC FOOT INFECTIONSK. Sridhar¹, E. Vandana Giridhar², K. R. L. Suryakirani³**HOW TO CITE THIS ARTICLE:**

K. Sridhar, E. Vandana Giridhar, K. R. L. Suryakirani. "Clinico-Bacteriological Profile of Diabetic Foot Infections". Journal of Evolution of Medical and Dental Sciences 2014; Vol. 3, Issue 74, December 29; Page: 15487-15491, DOI: 10.14260/jemds/2014/4090

ABSTRACT: Diabetics exhibit a great frequency and severity of infection. Recovery of multidrug resistant *Pseudomonas aeruginosa* and ESBL producing *Klebsiella* and *Escherichia coli* are of serious concern. **AIMS AND OBJECTIVES:** The aims of the study are to establish the Bacteriological profile in Wagner's grades of foot ulcers in Diabetics and foot ulcers in Non-diabetics, Antibigram of isolates. **MATERIAL AND METHODS:** A total of 265 samples were collected from two Study Groups. *Pseudomonas aeruginosa* isolates were subjected to screening of Amp – C β lactamase by disc antagonism test. *Klebsiella* species and *Escherichia coli* that were found to be resistant to first, second and third generation cephalosporins were confirmed for ESBL production by Disc Potentiation Test. **RESULTS:** More number of ulcers (111) were of Grade II in diabetics and the commonest risk factor was found to be Neuropathy (122). Nature of infection in foot ulcers was mostly polymicrobial (35.8%) in Diabetics where as it was only Monomicrobial (100%) in Non-diabetics. All the CONS isolated (59) from diabetic foot ulcers were sensitive to Linezolid and resistant to Penicillin. They were moderately resistant to Cephalosporins (68%) and Fluoroquinolones (59%). Ten of *Escherichia coli* (32.2%) and 26 of *Klebsiella* species (44.0%) were ESBL producers. 18 of *Pseudomonas* spp (27.2%) were positive for Amp C beta lactamase. **CONCLUSION:** Because of Neuropathy and the altered immune status in Diabetic patients, opportunistic and polymicrobial infections are common in Diabetic foot ulcers. Moreover these patients show Multi Drug Resistant patterns. **KEYWORDS:** Diabetic foot ulcers, wagner's, grades, MDR *Pseudomonas*.

INTRODUCTION: Diabetes mellitus comprises a group of common metabolic disorders that share the phenotype of hyperglycaemia. The term diabetic foot includes any foot lesion occurring as a result of diabetes or its complications. The presentation of diabetic foot is in the form of a non-specific ulcer. Depending on the severity of the lesion diabetic foot was classified by Wagner into six grades (0 to 5). Diabetic foot is characterized by a classical triad of neuropathy, ischemia and infection.

Out of all the infections, the foot infections are the most common cause for prolonged hospital stay among the diabetics. The bacteriology and antibiotic sensitivity pattern of various pathogens in diabetic foot infections has been undergoing changes over the period of years.

Infection may be superficial or deep. Superficial infections are usually monomicrobial while deep infections are polymicrobial.¹ Prevalence of multiple antibiotic resistant bacteria in foot infections calls for culture guided therapy.

The aims of the study are to establish the Bacteriological profile in Wagner's grades of ulcer in Diabetics and foot ulcers in Non-diabetics, Antibigram of isolates.

MATERIALS AND METHODS: A total of 265 samples were collected from two Study Groups.

Study group I consisted of 225 samples collected from patients with foot ulcers attending Surgical OPD and Medical and Surgery Wards with Diabetes Mellitus.

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Study Group II consisted of 40 samples collected from patients with foot ulcers but without Diabetes Mellitus.

SAMPLE COLLECTION: The samples were collected on the first day of admission. The sample taken was pus and exudate from the base of the ulcer after cleaning the necrotic tissue with a saline gauge. The material was collected by swabbing the base of the ulcer with two sterile swabs, one for direct smear and the other for inoculating into liquid and solid media. The specimens were processed by direct microscopy and culture. Organisms were identified by morphological, cultural and biochemical characteristics. Antibiotic susceptibility of the isolates was performed by modified Kirby – Bauer disc diffusion method.

Isolates of *Pseudomonas aeruginosa* were subjected to screening of Amp – C β lactamase by disc antagonism test. The test isolate was spread over Muller-Hinton Agar plate. Cefotaxime (30 μ g) and ceftazidime (30 μ g) discs were placed 14 mm apart from center to center. The isolates that showed blunting of cefotaxime zone of inhibition adjacent to ceftazidime were considered screen positive for Amp-C β lactamase. The test was compared with control strain ATCC 27853 of *Pseudomonas aeruginosa*.²

Klebsiella species and *Escherichia coli* found to be resistant to first, second and third generation cephalosporins by Kirby Bauer Disc Diffusion Method were selected for ESBL confirmation by Disk Potentiation Test. A non - ESBL producing organism (*Escherichia coli* ATCC 25922) was used as a negative control and an ESBL producing organism (*Klebsiella pneumoniae* ATCC 700603) was used as a positive control. Confirmation was done by Disk Potentiation Test (CLSI guidelines) In this test, a pair of discs containing Cephalosporin alone and another containing Cephalosporin along with Clavulanic acid was placed on opposite sides of the same inoculated plate. The test organism was regarded as an ESBL producer, when the zone of inhibition around the combination disc was at least 5mm larger than that of the disc containing only cephalosporin. Kits used for confirmation: KIT I - Cefotaxime disc - 30mcg and Cefotaxime / Clavulanic Acid - 30mcg / 10mcg. KIT III - Ceftazidime disc - 30mcg and Ceftazidime / Clavulanic acid - 30mcg/10mcg.

RESULTS: Out of 225 cases of Study group I, 172 were from rural areas, 53 from urban. 157 were males and 68 were females. The cases were over a wide range of age from 45-65yrs. Onset of ulcers was spontaneous in 142 cases, and by injury in 83. More number of ulcers (111) were of Grade II and the commonest risk factor was found to be Neuropathy (122). Nature of infection in foot ulcers was mostly polymicrobial (35.8%) in Diabetics where as it was only Monomicrobial (100%) in Non-diabetics.

Isolates	I	II	III	IV	V	Total (%)
Staph. aureus	47	52	10	2	1	112(31.9)
CONS	17	31	11	0	0	59(16.8)
Pseudomonas spp	12	35	14	3	2	66(18.8)
Klebsiella spp	12	28	17	2	0	59(16.8)
Escherichia coli	9	15	7	0	0	31(8.9)
Proteus spp	2	3	5	7	2	19(5.4)
Acinetobacter	0	2	2	1	0	5(1.4)
Total	99	166	66	15	5	351(100)

Table 1: Study Group I-Distribution of Isolates in Wagner's Grades of ulcers

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Organisms isolated	Diabetic Foot Ulcers (n=351)		Non diabetic Foot Ulcers (n=40) No. of	
	No. of Isolates	%	Isolates	%
Staphylococcus aureus	112	31.9	15	37.5
Coagulase Negative Staphylococci	59	16.8	0	0
Pseudomonas aeruginosa	66	18.8	11	27.5
Klebsiella species	59	16.8	8	20.0
Escherichia coli	31	8.9	3	7.5
Proteus species	19	5.4	3	7.5
Acinetobacter species	5	1.4	0	0

TABLE 2: STUDY GROUPS I & II-Comparative table for isolates

Analysis of antibiogram of isolates revealed that all the CONS isolated (59) from diabetic foot ulcers were sensitive to Linezolid and resistant to Penicillin. They were moderately resistant to Cephalosporins (68%) and Fluoroquinolones (59%). Ten of *Escherichia coli* (32.2%) and 26 of *Klebsiella* species (44.0%) were ESBL producers. 18 of *Pseudomonas* spp (27.2%) were positive for Amp C beta lactamase.

DISCUSSION: Male to female ratio of 2.30 in our study is comparable with Viswanathan et al³ Chennai in 2002 - 1.95, Vijaya et al⁴ Bengaluru in 2000 – 2.63, Ekta Bansal et al⁵ Chandigarh in 2008 - 3.68. C. Anandiet al⁶ in 2004, was 2.03. This is correlating with the present 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.

Though the present study included patients from 16-78 years, maximum number of cases were in the age group 45-65yrs. Syed Md Alavi⁷ from Karachi, Pakistan Reported same in 2007. In a similar study conducted by Rool-ul-Muquim, Samson Griffith et al⁸, 2005, ulcers were more common in the ages between 41-50 yrs and 51-60yrs. This shows that there is a delay in the onset of foot ulcers.

Peripheral neuropathy is commonly associated with diabetic infections and is one of the risk factor for the development of foot ulcers. 54% in the present study was same as Given by Ekta Bangal et al⁵ Chandigarh in 2008, Ravisekhar et al⁹ Delhi in 2006. And higher than Shankeret al,¹⁰ Chennai (56%) in 2005.

The number of isolates per sample was 1.56 in our study, which was close to Viswanath et al,³ Chennai in 2002 1.42 and Ekta Bansal et al,⁵ Chandigarh in 2008 – 1.52, whereas Diane M Citron et al¹¹ USA, in 2004 reported as 2.7 and NA Pathare¹², Mumbai in 1998 as 3.07.

In the present study, (As per Table -I) Grade 2 diabetic ulcers were more prone to infections with opportunistic organisms like Coagulase Negative Staphylococci and also susceptibility to infections by a wide range of micro-organisms like *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella* species and these observations are significant (p value is 0.0001).

In study group I, as per Table II, the primary isolate was *Staphylococcus aureus* 112 (31.9%) followed by *Pseudomonas aeruginosa* 66(18.8%), Coagulase Negative Staphylococci 59(16.8%), *Klebsiella* species 59(16.8%), *Escherichia coli* 31(8.8%), *Proteus* species 19(5.4%) and *Acinetobacter*

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species 5(1.4%). The observations of the present study are correlating with a study conducted by Dipali A Chincholkar et al,¹³ differing with a study of C. Anandi et al.

In the present study *Pseudomonas aeruginosa* isolates were resistant to more than 50% of drugs. Out of the 66 *Pseudomonas* isolates screened for Amp-C β lactamase 18 were screen positive. (27.2%).

ESBL production is commonly seen with the Enterobacteriaceae family and more in *Klebsiella* species and *Escherichia coli*. 44% in *Klebsiella* and 32.2% in *Escherichia coli* of the present study is far less than reported by Christopher L. Emery et al,¹⁴ 1997, BenuDhawan et al,¹⁵ 2006, P N Sridhar Rao et al,¹⁶ 2008, Helen Derbyshire et al,¹⁷ 2009 and Sivaraman Uma Devi et al,¹⁸ 2010.

Polymicrobial infections were common in Diabetic foot ulcers. Only Monomicrobial infections were common in the Non-diabetic foot ulcers. Polymicrobial infections were more common in all diabetic foot ulcers and were proved by many studies at different periods of time.

It is observed that Coagulase Negative Staphylococci and *Acinetobacter* species were isolated only in patients with Diabetic foot ulcers. The p - value for the same is >0.05. There is an increased incidence of Coagulase Negative Staphylococcus in Diabetic patients because of their altered immune status which in turn leads to an increase in opportunistic infections by the colonizing organisms.

CONCLUSION: Because of Neuropathy and the altered immune status in Diabetic patients, opportunistic and polymicrobial infections are common in Diabetic foot ulcers. Moreover these patients show Multi Drug Resistant patterns. So, it is advisable to do Antibiotic Sensitivity Testing before starting treatment for such patients. It also helps in preventing further complications like gangrene formation and limb amputations.

REFERENCES:

1. A. Ramachandra Socio Economic Issue in diabetic Foot, Asian Journal of diabetology Volume 7 No.3 2005, July.
2. Mackie and MC Cartney's - practical medical microbiology 14th Edition - Churchill, and Living Stone publication.
3. Viswanadhan V, Jasmine J J, Snehalatha C, Ramachandran A. Prevalence of pathogens in diabetic foot infections in South Indian type 2 diabetic patients 2002.
4. Vijaya D, Lakshmikanth, Sheshadri. Bacteriology of diabetic foot infection. Biomedicine.2000; 20 (3): 176-9.
5. Bansal E, Garg A, Bhatia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Indian J Pathol Microbiol 2008; 51: 204-8.
6. Anandi C, Alaguraja D, Natarajan V, Ramanathan M, Subramaniam CS, Thulasiram M, Sumithra S. Bacteriology of diabetic foot lesions. Indian J Med Microbiol. 2004; 22; 175-8.
7. Seyed Mohammad Alavi, Azar D Khosravi, Abdulah Sarami, Ahmad D, Effat AM. Bacteriologic study of diabetic foot ulcer. Pak J Med Sci. Oct-Dec 2007; 23 (5): 681-684.
8. Rool-ul-Muquim, Samson Griffith, Mukhtar Ahmed. Evaluation and Management of Diabetic foot ulcers according to Wagner's classification – a study of 100 cases. Review in Evaluation and Management of Diabetic Foot 2005.
9. Ravisekhar G, BenuDhawan, Srinivas V, Arti K, Ammini AC, Rama chaudhry. A clinico - microbiological study of diabetic foot ulcer in an Indian tertiary care hospital. Diabetes Care Aug 2006; 29 (8): 1727-32.

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10. Shankar EM, Mohan X, Premalatha G, Srinivasan RS, Usha AR. Bacterial etiology of diabetic foot ulcers in South India, the diabetic capital of India. Department of microbiology. Dr. ACM PG institute of basic medical sciences, University of Madras, Taramani campus, Chennai, India, 2005.
11. Diane M Citron, Ellie JC Goldstein, Vreni Merriam C, Benjamin A Lipsky, and Murray A Abramson. Bacteriology of moderate to severe diabetic foot infections and invitro activity of antimicrobial agents. Journal of Clinical Microbiology, September 2007; Vol 45, No 9: 2819-2828.
12. Pathare NA, Ball A, Talvalkar GV, Antani DU. Diabetic foot infections: a study of micro-organisms associated with the different Wagner grades. Indian J Pathol Microbiol. 1998 Oct; 41 (4): 437-41.
13. Dipali A Chincholikar and Ram Prasad B Pal. Study of Fungal and Bacterial infections of Diabetic Foot. Indian Journal of Pathology and Microbiology 45 (1): 15-22, 2002.
14. Christopher L Emery and Lisa A Weymouth. Detection & Clinical Significance of Extended Spectrum Beta lactamases in a Tertiary Care Medical Centre. Journal of Clinical Microbiology, August 1997 p - 2061-2067.
15. Benu Dhawan, Ravi shekar.G, Srinivas.V, Ammini. AC, Aarti Kapil, Ramachaudary. A Clinicomicrobiological study of diabetic foot ulcer in Indian tertiary care hospital. Diabetes care Aug 2006; 29 [8:] 1727-32.
16. P. N. Sridhar Rao, K.G. Basavarajappa, G. Leela Krishna. Detection of Extended Spectrum Beta lactamases from clinical isolates in Davngere. Indian Journal of Pathology and Microbiology, 51 (4), October - December 2008.
17. Helen Derbyshire Trevor Winstanley, Gemma Kay, Katie Evans, Carmal Vaughan, Umadevi Kavuri. A Simple Disc diffusion method for detecting Amp C& ESBLs in clinical isolates in Enterobacteriaceae. Journal of Antimicrobial Chemotherapy, 2009, 63, 497-501.
18. Sivaraman Uma Devi, Sailesh Kumar, G. Kandha Kumari, Noyal Maria Joseph, Joshy M Easow, Srinivasan Srirangarajan, Sruthi Raj, Selvaraj Stephen. Indian Journal of Medical Microbiology, 2010.

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Date of Submission: 06/12/2014.
Date of Peer Review: 08/12/2014.
Date of Acceptance: 19/12/2014.
Date of Publishing: 26/12/2014.