

Original Research Article

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Microbial Profiles of Diabetic Foot Ulcers: A Random Comparison within India

C. Meenakshisundaram^{1*}, J. Uma Rani¹, Usha Anand Rao², V. Mohan³ and R. Vasudevan⁴

¹Department of Microbiology, Sri Venkateswara Medical College and Research Centre, Pondicherry-605 102, India

²Department of Microbiology, Dr. A.L.M. Post Graduate Institute of Basic Medical Sciences, Tharamani Campus, University of Madras, Chennai-600 013, India

³Director, ⁴Surgeon, Dr. Mohan's Diabetic Specialties Research Centre, Gopalapuram, Chennai-600 028, India

*Corresponding author

ABSTRACT

The present study is an analysis of a retrospective data gathered in Chennai in 2005, in order to compare the bacteriological profiles revealed in Chennai, with the bacteriological profiles reported by other investigators from various other locations in India, as reported in the literature, with a view to incorporate the lessons learnt, for the benefit of future studies on topics of diabetic foot ulcers. The retrospective study was related to 75 patients receiving treatment for diabetic foot ulcers, in a tertiary health care centre in Chennai City, India, during a period of 5-months from May to September 2005. The patients were screened for bacterial pathogens in their wounds. A total of 104 isolates were obtained. In the microbiological analysis carried out as per standard procedures, the pathogens were identified as i) *Escherichia coli* (22.2%), ii) *Staphylococcus aureus* (17.3%), iii) *Pseudomonas aeruginosa* (17.3 %), iv) *Klebsiella* species (10.6%), v) Coagulase Negative *Staphylococcus* (CONS) species (10.6 %), vi) *Proteus* species (9.6 %), vii) *Streptococcus* species (5.8 %), viii) *Corynebacterium* species (3.8%), and ix) *Enterococcus* species (2.9%). This result was compared with similar findings of 17-other Investigators in India (8-from South India and 9-from North India), as obtained in the literature review. This random comparison has been useful to realize the reality that the pathogens found in diabetic foot ulcers could include bacteria (aerobes, anaerobes and facultative-anaerobes) and fungal organisms. This implies that there is a need to carry out the antimicrobial sensitivity tests for all categories of pathogens present in the wound. Considering the potential risk of a trivial injury in diabetic patients getting slowly and surely converted into more serious complications, it may be concluded that all classes of pathogens need to be examined, thoroughly, in the Clinical Microbiology Laboratory attached to each District Headquarters Hospital of the Government, in order to augment the efforts of Clinicians in selecting the appropriate therapies needed for the diabetic foot ulcer patients who, mostly, happen to be hailing from the rural India. No pathogen needs to be ignored or spared!

Keywords

Diabetic Foot Ulcer, Anaerobes, Aerobes, Fungal Organisms, Microbiological studies, Rural India.

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Introduction

In India's around 50.8 millions of the population were affected with diabetes in the year 2010 and the disease is progressing at an alarm ingrate, and the number is likely to increase up to 87.0 million by the year 2030, as estimated by Ramachandran *et al.*, (2010). This statistical report would describe the need for the caution to be shown by all stake-holders, including the susceptible diabetics, the care-takers, and the Government whose major role is to protect public health, in a Welfare State!

Shaw *et al.*, (2010) estimated that an increase of 69.0% could be expected in the increase of numbers of adults with diabetes in developing countries, and an increase of 20.0% in developed countries.

American Podiatric Medical Association (APMA) (2016), broadly indicated the possibility that a diabetic foot ulcer as an open sore or wound could occur in about 15.0% of patients with diabetes, mostly located at the bottom of the foot, and that, among those who develop a foot ulcer, approximately 6.0% (of them) would have to be hospitalized due to infection. However, they concluded, with an optimistic note, that the development of a diabetic foot ulcer is "preventable".

Viswanathan *et al.*, (2006) reported that the prevalence of foot infection was higher among the rural patients, compared to the urban patients, in addition to highlighting upon the percentages of amputations or recurrences being higher with rural patients. Mohan *et al.*, (2008), also reported similar views, in addition to reiterating the fact that awareness among the diabetic patients prevailed only among the people in urban and peripheral-urban areas. Therefore, based on studies undertaken by many such investigators in India, the kind of medical

care needed in rural areas, in the case of diabetic foot infections, must be up-dated in India, in extending improved facilities of microbiological analysis and antibiotic-sensitivity-based treatment options, at every District Hospital in India (Meenakshisundaram *et al.*, (2016). It was generally concluded that the diabetic foot ulcer proved itself, at the global level, as the fourth largest killer-disease, and hence the priority.

Louie *et al.*, (1976), using the then-available laboratory techniques, established the fact that aerobic bacteria and anaerobic bacteria could co-exist in the diabetic foot ulcer, and reported that there was a necessity to select antibiotics in such a way as to cover the likelihood of a complex containing aerobic and anaerobic pathogens. Murali *et al.*, (2014) reported that *Pseudomonas aeruginosa*, as a single pathogen, could exist alone, or co-exist with many other pathogens, such as *Acinetobacterspp*, *Ciprobacterspp*, *Enterobacterspp*, Gram-negative Bacilli, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterococci spp*, Methicillin-resistant coagulase-negative staphylococci (CONS) species, Methicillin-resistant *Staphylococcus aureus* (MRSA), and Methicillin-sensitive *Staphylococcus aureus*(MSSA), in percentages of combinations varying from 12.0% to 36.0%. In other words, each pathogen had the flexibility of either prevailing alone, or existing in combination. However, each species seem to have choice of their own preference!

Thus, it is seen that the bacterial diversity in chronic wounds has been established as a reality. The inference is that the pathogens might have a synergistic effect among them, which needs to be investigated in future studies, so that the information gathered in

these lines would help evolving appropriate treatment methods and management procedures, for achieving a positive and early cure from the illness.

Dixit *et al.*, (2011) conducted a cross-sectional study involving the screening of 323 diabetic patients across India, and concluded that 87.0% of diabetic patients in India suffered from foot ulcer or blisters during the first year of on-set. Dixit *et al.*, (2014) reported that the awareness of the diabetic patients was lacking about the foot-care which must be given prominence, by healthcare personnel and policy-makers.

Abilash *et al.*, (2015) reported that 18 patients out of 100 suffered from diabetic foot ulcer caused by fungal organisms, the predominant species being i) *C. albicans*, and ii) *C. tropicalis*.

Saravanan Sanniyasi, *et al.*, (2015) reported that ulcers of duration more than about 7 or 8 months showed significant positivity in fungal pathogens, and that fungal infections were harboured in 19 patients out of 105 patients studied by them, in Chennai, as against 9 out of 103 patients reported by Bansal *et al.*, (2008).

Uckay *et al.*, (2008) summarised the highlights of 13-Investigators of India, who reported the presence of Gram-negative pathogens to be the most predominant organisms isolated from diabetic foot ulcers in 8-South Indian locations and 5-North Indian locations; whereas, the Gram-positive organisms were reported to be the most predominant pathogens found in diabetic foot ulcers in certain foreign countries, such as Spain, Portugal, Saudi Arabia and Iran, as reported by various investigators, in their respective countries.

Pathare *et al.*, (1998) studied the diabetic foot infections of 252-patients, in Mumbai,

isolating 775 clinical strains, which corresponded to 71.09% of aerobic pathogens, 28.91% of anaerobic pathogens. This could imply that the anaerobes cannot be overlooked in the evaluation and treatment requirements. Chincholikar *et al.*, (2002) reported that 28.0% of bacterial isolates collected from diabetic foot ulcers corresponded to anaerobes. Ramani *et al.*, (1991) reported that about 27.3% of the strains gathered from diabetic foot ulcers of 75-patients were identified as anaerobes. Sapico *et al.*, (1980; 1984) reported that the concomitant presence of aerobes and anaerobes could be a reality in diabetic foot infections.

The diversity of micro organism in wounds is influenced by factors such as wound type, depth, location, the level of tissue perfusion, and the immune-competence of the host. In diabetic patients, pre-existing conditions provides nourishment for unhindered bacterial growth.

Gjodsbol *et al.*, (2006) reported that chronic venous leg ulcers accommodate *Staphylococcus aureus* (in 93.5 % of ulcers), *Enterococcus faecalis* (in 71.7 % of ulcers), *Pseudomonas aeruginosa* (in 52.2% of ulcers), Coagulase negative *Staphylococcus* (in 45.7% of ulcers), *Proteus* species (in 41.3% of ulcers), and anaerobic bacteria (in 39.1% of ulcers).

Bowler and Davies (1999) reported that open-wound pathogens are aerobic microbial organisms such as *Staphylococcus* species and *Streptococcus* species, and that anaerobic microorganisms, such as *Peptostreptococcus* species, *Prevotella* species, *Porphyromonas* species, *Bacteroides* species, etc., play a potential role in clinical manifestation of chronic wound infections. These anaerobes may even act synergistically to invade tissues,

without penetrating deep into the wound-compartment. For this reason, the anaerobes cannot be ignored, in the treatment procedures, and hence, in the microbiological testing routines.

Lipsky *et al.*, (1997) described that aerobic Gram-positive cocci are the major pathogens in diabetic foot infections, and that aerobic Gram-negative bacilli or anaerobes were present in chronic wounds.

The rate of anaerobic infections in diabetic foot ulcers could vary from 5.0% to 95.0%, as reported by Amalia *et al.*, (2002). Based on such informations, Pednekar *et al.*, (2015) reported that the emergence of resistance against the commonly-used antimicrobial agents (AMAs) was a reality, and therefore, it must be made “mandatory” to record the antimicrobial resistance patterns of anaerobic microbial organisms, concurrently, while recording the antimicrobial resistance patterns of the aerobes, so that the procedures related to the treatment and management of diabetic foot ulcers could get simplified/streamlined.

If the diabetic foot infection, in patients, are not cured by administering antibiotic therapy against the isolated bacterial species, based on bacterial sensitivity tests, then the possibility of the presence of anaerobic bacteria or fungal organisms can be suspected. Redkar *et al.*, (2000) identified bacterial organisms from non-healing diabetic foot wound by using 16S rDNA-sequencing and isolated the “missing” organisms.

Shahi *et al.*, (2013) reported the occurrence of multiple antibiotic resistance phenotype and Class1-integron in bacteria isolated from diabetic foot ulcers, involving Gene Cassettes, and Beta-lactamase genes, for many Multi Drug Resistant Organisms

encountered in diabetic foot infections in India, by labeling the bacterial species with accession numbers based on 16S rRNA sequence, and identifying the various susceptible antibiotic agents for each pathogen. Such data, if generated in each geographical zone in India, and made available for the guidance of clinicians practicing in various parts of India, it would be of immense benefit to those who suffer from diabetic foot infections.

The present study was conducted with the following aim and objective: To compare the data relating to the various pathogens in diabetic foot ulcers, as identified in the retrospective study, undertaken in Chennai, during 2005, with similar results reported by other investigators in different locations of India. And also to improve upon the understanding of the shortcomings, if any, in the retrospective study, so that suitable modifications in future studies, can be incorporated, with regard to revised approaches in methodologies, as contributions from other investigators.

Materials and Methods

Pus swabs were collected from 75 patients attending Dr. V. Mohan's Diabetes Specialties Center, Gopalapuram, Chennai-600 028 (South India), during a period of 5-months, from May to September 2005. These pus samples were transported to the Laboratory in Carry-Blair transport medium (Hi-Media, Mumbai, India). All the isolates were identified, adopting the standard procedures spelled out in NCCLS of 2002 (Meenakshisundaram *et al.*, 2015).

Results and Discussion

The percentages of prevalence of different bacterial species isolated from the 104-samples are presented in Table-1, and

depicted in Figure-1. Among a total of 75 patients, selected in the study group, the wound categories were found to correspond to Wagner's Grades 2,3,4 and 5, pertaining to diabetic foot ulcer.

This data of the retrospective study were compared with the findings of other investigators in India (8-scholars from South India, and 9-scholars from North India). The 16-locations covered in this comparison are: Chennai, Chidambaram, Karaikal, Salem, Bengaluru, Thiruvananthapuram, Kochi (Cochin), Calicut (Kozhikodu), Manipal (9-cities representing South India), and, Mumbai, Ahmedabad, Chandigarh, New Delhi, Lucknow, Allahabad, Aligarh and Kolkata (8-cities representing North India). The details are shown in Table-2.

The prevalence of the various (nine) species of bacteria isolated in the retrospective study of 2005, in Chennai (as listed in Table-1), generally, agree with the results reported by many other Investigators in India (listed in Table-2), in so far as the presence of specific pathogen in the wounds of patients at each site is concerned, although the percentages of prevalence of each bacterial species varied from place to place, obviously due to the differences in the "statuses" of the wounds encountered, in terms of Wagner Grades, in each place.

The percentage of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*, reported as 17.3% , 17.3%, and 22.2%, respectively, in Chennai were found to be prevalent, in varying percentages, in all the other 8-South Indian cities, and in all the 8-North Indian cities, reviewed.

The percentage of *Enterococcus* species reported to be present in Chennai at 2.9% , was found to be present , in varying percentages, in the 7-other South Indian

cities, and in 6-North Indian cities, reviewed.

The Coagulase Negative *Staphylococcus* (CONS) species, reported to be present in Chennai at 10.6%, was found to be present, in varying percentages, in the 3-other South Indian cities and in 4-North Indian cities reviewed.

The *Klebsiella* species reported to be present in Chennai at 10.6%, was found to be present in varying percentages, in 7-other South Indian cities, and in the 6-North Indian cities, reviewed.

The *Proteus* species reported to be present in Chennai at 9.6%, was found to be present, in varying percentages, in 6-other South Indian cities, and in all the 8-North Indian cities, reviewed.

The *Streptococcus* species reported to be present in Chennai at 5.8%, was found to be present, in varying percentages, in the 7-other South Indian cities, and in 4-North Indian cities.

In the case of *Corynebacterium* species which was recorded as 3.8% in the retrospective study in Chennai, were found in only 1-other place in South India, namely, Calicut (Kozhikodu), in South India; and were present in varying percentages in 2-North Indian cities, namely, Mumbai and Aligarh.

Anaerobes which were not reported in the retrospective study in Chennai, were found prevalent in 1-South Indian city (Chidambaram), and in 3- North Indian cities (Mumbai, New Delhi and Lucknow).

Fungal organisms, which were not reported in the retrospective study in Chennai, were found prevalent in 1-South Indian city

(namely, Kochi), and in 3-North Indian cities (namely, Mumbai, Chandigarh, and Kolkata).

The random comparison of microbiological data relating to diabetic foot ulcer, as listed in Table-2, describes the nationwide situation, prevailing over a period of 17-years,(by mere coincidence), from the year 1998 to 2015. This exercise of comparison

of data has been found useful in ascertaining the importance of evaluating the wholesome microbiological diversity, comprising of aerobes, anaerobes and fungal organisms, so as to help ascertaining the importance of evaluating the antimicrobial sensitivity tests for all these various classes of pathogens, for arriving at a set of comprehensive/optimum treatment strategies/options.

Table.1 Percentage prevalence of bacterial species in diabetic foot ulcers (Number of isolates=n=104)

S.no	Organism	No. of isolates(%)
1.	<i>Staphylococcus aureus</i>	18 (17.3%)
2.	<i>Pseudomonas aeruginosa</i>	18 (17.3%)
3.	Coagulase Negative <i>Staphylococcus</i> (CONS)spp.	11 (10.6%)
4.	<i>Escherichia coli</i>	23 (22.2%)
5.	<i>Klebsiella</i> spp.	11 (10.6)
6.	<i>Proteus</i> spp.	10 (9.6%)
7.	<i>Streptococcus</i> spp.	6 (5.8%)
8.	<i>Corynebacterium</i> spp.	4 (3.8%)
9.	<i>Enterococcus</i> spp.	3 (2.9%)

Fig.1 Prevalence of bacterial species in the Diabetic Foot Ulcer-wound

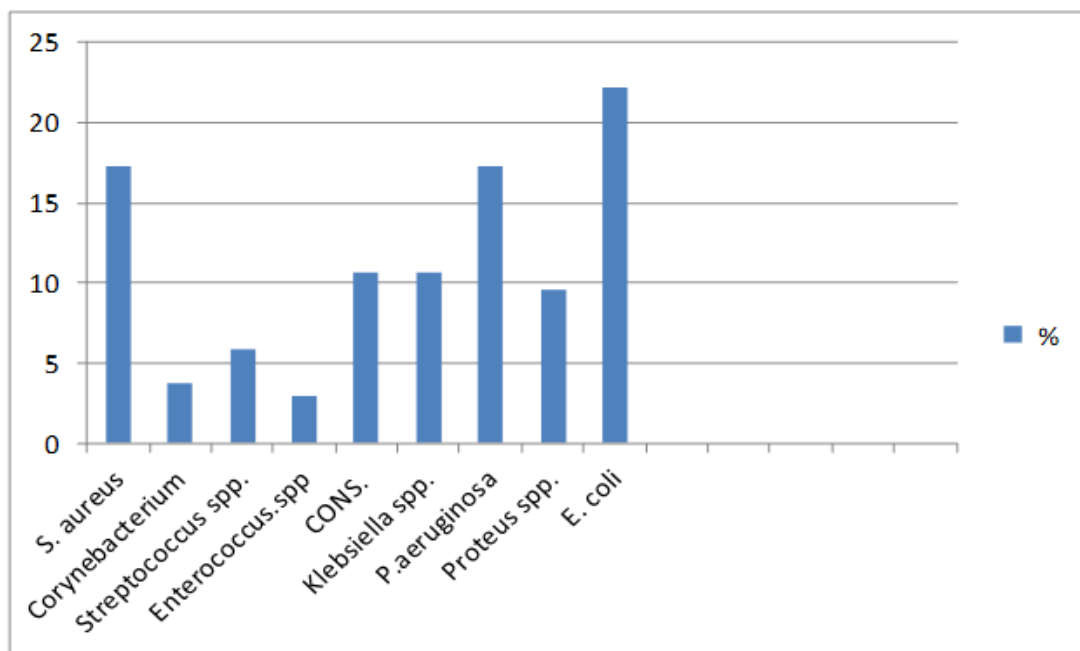


Table.2 Percentage Distribution of Pathogens in Diabetic Foot Ulcers

S. No	Investi-gators(Year)	City, State(patients)	STA	ENC	Co NS	STC	ESC	PTB	PSA	KLB	COR	Other Spp.
1 (S.I)	Meenakshi-sundaram C, <i>et al.</i> ,2015	Chennai TN(75)	17.3	2.9	10.6	5.8	22.2	9.6	17.3	10.6	3.8	..
2. (S.I)	AnandiC , <i>et al.</i> , 2004	Chidambaram TN(107)	13.6	7.3	27.7	16.9	11.3	13.6		Anaer, ## ENB
3. (S.I)	Kavitha Y, <i>et al.</i> ,2014	Karaikal Pondy(56)	32.3	6.1	6.1	3.1	4.6	15.3	12	15.3		Prov.spp; Morg.morg.
4. (S.I)	Sugandhi P, <i>et al.</i> , 2014	Salem, TN (60)	25.0	2.0		1.0	4.0		25.0	4.0		Misc. ###
5. (S.I)	Sajila NM, <i>et al.</i> , 2015	Bengaluru, Karnataka (290)	20.9	14.0	1.1	0.6	5.2		22.6	16.3		Acin NFGNB ENB; CTB
6. (S.I)	Mathew SM, <i>et al.</i> , (2014)	Calicut Kerala (76)	31.5	...		6.2	9.1	27.3	13.6	..	16.3	CTB; EBA; Misc; ***
7. (S.I)	Nair SR, <i>et al.</i> , 2015	Thiruvan-anthapuram, Kerala(250)	33.7	6.0		5.3	12.7	11.6	18.7	7.7		ENB; A.baum; CTB; St.epid.
8. (S.I)	Chellan G, <i>et al.</i> ,2010	Kochi Kerala(518)	12.2	14.1	5.8	1.5	7.7	3.8	10.8	7.9		NFGNB; CTB; Fungal*; Misc.
9. (S.I)	Sekhar SM, <i>et al.</i> , 2014	Manipal, Karnataka (108)	32.0	8.0	---	4.0 BHS	8.0	20.0	24.0	12.0		Acin; CTB; BHS

S. No	Investigators(Year)	City, State(patients)	STA	ENC	Co NS	STC	ESC	PTB	PSA	KLB	COR	Other Spp.
10. (N.I)	Chincholikar DA, <i>et al.</i> ,2002	Mumbai Maharashtra(105)	31.2	10.0	15.6	6.3?	19.4	8.1	..	Anaer; Fungal; Serr.marce; ENB; CTB; +++
11. (N.I)	Pathare NA, <i>et al.</i> ,1998	Mumbai (252)	19.2 Stp. Spp	4.3	..	15.6	8.9	16.7	5.4 Pse. Spp	14.0	0.005	Anaer; Fungal; Morg.spp; ETB; CTB; Acin; Prov.spp; Serr.spp; Moraxella spp.
12. (NI)	Manisha J, <i>et al.</i> , 2012	Ahmedabad Gujarat(125)	12.7		4.5		16.6	4.4	30.6	20.3		CTB; Morg.Morg; Prov.rett; Prov.stu.
13.. (N.I)	Bansal E, <i>et al.</i> , 2008	Chandigarh Haryana ((103)	19.0	5.0			18.0	11.0	22.0	21.0		Fungal; Acin; CTB
14. (N.I)	Gadepalli R, <i>et al.</i> , (200)	New Delhi (80)	13.7	11.5	6.6	12.0	12.6	9.8			Anaer; Acin; Micro-coc; ENB; CTB.
15. (N.I)	Ramkant P, <i>et al.</i> ,2011	Lucknow U.P, (447)	13.8	9.5	5.0	3.0	16.1	8.8	15.8	6.7		Anaer; Acin; Sterile strains; ENB; CTB.

S. No	Investi-gators(Year)	City, State(patients)	STA	ENC	Co NS	STC	ESC	PTB	PSA	KLB	COR	Other Spp.
16. (N.I)	VermaSK, <i>et al.</i> , 2015	Allahabad U.P.(60)	31.5	14.0	7.0	21.0	15.8	Pepto; Cl.bot; S.typhi.
17. (NI)	Zubair M, <i>et al.</i> ,2011	Aligarh U.P.(60)	28.0	4.0	2.6	6.6 BHS	26.6	2.6	10.6	11.9	2.6	Acin; ENB.
18. (N.I)	Chakraborty P, <i>et al.</i> , 2015	Kolkata Bangla(90)	23.8	6.9	33.6	4.9	9.9	14.8		Fungal

Anaer: Anaerobes; **Acin:** *Acinetobacter spp*; **A.baum:** *Acinetobacter baumannii*; **Bac:** *Bacillus spp*; **BHS:** *Beta haemolytic Streptococcus* : *C.div* : *Citrobacter diversus*; **CONS** :*Coagulase Negative Staphylococcus spp*; **CTB:** *Citrobacter spp*; **Cl.bot** :*Clostridium botulinum*; **COR:** *Corynebacterium spp*; **Diph.spp:** *Diphtheroid spp*; **EBA:** *Enterobacter aerogenes.*; **ENB:** *Enterobacter spp*; **ENC:** *Enterococcus spp*; **ESC:** *Escherichia coli*; (**MDR-E.coli:** *Multi Drug Resistant E.coli*); **Fungal:***Fungal organisms*; **GNC:** *Gram-Negative cocci spp*; **KLB:** *Klebsiella spp*; **LB:** *Lacto bacillus spp*; **Morg.morg:** *Morganella morganii*; **Morg.spp:***Morganella spp*; **MRSA:** *Methicillin Resistant Staphylococcus aureus*; **MSSA:** *Methicillin Resistant Staphylococcus aureus*; **NFGNB:** *Non Fermenting Gram-Negative Bacilli*; **Pepto:** *Peptococcus spp*; **Pse.spp:** *Pseudomonas spp*. **PTB:** *Proteus spp*; **PSA:** *Pseudomonas aeruginosa*; **Prov.rett:** *Providencia rettgeri*; **Prov.stu:** *Providencia stuarti*; **Prov.spp:** *Providencia spp*; **Serr.spp:** *Serratiaspp*; **Serr.merce:** *Serratia mercenscens*; **S.typhi:** *Salmonella typhimurium*; **STA:** *Staphylococcus aureus*; **STP spp:** *Staphylococcus spp*; **St.epid:** *Streptococcus epidermidis*; **STC:** *Streptococcus spp*; (**OST:** *Other Streptococcus spp*); **TN** : *Tamil Nadu (State)*; **U.P** : *Uttar Pradesh (State)*; **Pondy** : *Pondicherry (Puduchery : State)*.# *Bansal E,et.al,2008: reported Candida spp.*## *Anandi C, et.al, 2004, reported Anaerobes in Wagner Grades 2, 3, 4 and 5-only.*### *Sugandhi, et al.,2014, reported Staphylococcus saprophyticus, and Staphylococcus epidermidis. Micrococcus spp; Pleisomonas spp; Bacillus spp; Salmonella spp; and Vibrio spp.**** *Mathew, et al.,2014, reported Salmonella typhi; Lactobacillus spp; Staphylococcus epidermidis; Bacillus spp; ++ Chellan G, et.al,2010,reported MDR-E.coli; Diphtheroides spp; Serratiaspp; Morganella morganii; Citrobacter diversus; ++ Chincholikar, etal., 2002, reported Enterobacter aerogenes; Serratia marcescens;*

Facilities for the application of advanced techniques such as rDNA PCR, ERIC PCR, 16S rDNA sequencing ,etc, have become feasible, in the modern times of today, to evaluate the infection-status and the bacterial diversity of the isolates in diabetic foot ulcer-wounds (Singh *et al.*, 2009; Redkar *et al.*, 2000; Dowd *et al.*, 2008). These facilities must be made available in all Government Hospitals located in major commercial cities of India, in order to catch up with the facilities available in advanced countries, considering the widely published-caution that the Indian population affected with diabetic foot infections would reach alarming proportions by the year 2030.

In conclusion, a comprehensive analysis of the findings of the various investigators in India will be helpful to arrive at a consensus that the microbiological analysis of clinical isolates must cover the aerobic bacteria, anaerobic bacteria and fungal species, and also include evaluation of the antibiotic sensitivity patterns of these organisms, in order that an early and timely cure can be obtained. The collection of microbiological data becomes necessary at every stage of treatment, in order to form the basis for selecting any specific therapeutic option, and therefore the Clinical Microbiology Laboratory must be upgraded with modern facilities, with respect to equipments and staffing pattern.

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