



Original Research Article

Characterization of *Pseudomonas aeruginosa* and its Association with Diabetic Foot Ulcer Isolated from a Tertiary Care Hospital in Tamilnadu, India

C.Meenakshisundaram^{1*}, Usha Anand Rao², P.Rajendran³,
V.Mohan⁴ and R.Vasudevan⁵

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

²Former Professor, Department of Microbiology, Dr. A.L.M. Post Graduate Institute of Basic Medical Sciences, Tharamani Campus, University of Madras, Chennai-600 113, India

³Presently, Professor of Microbiology, Madha Medical College and Hospital, Chennai-600 112, India

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

⁵Consultant Surgeon, Dr. Mohan's Diabetic Specialties Research Centre, Gopalapuram, Chennai-600 028, India

*Corresponding author

ABSTRACT

Keywords

Pseudomonas aeruginosa,
Diabetic foot ulcer,
Escherichia coli

75 diabetic foot ulcer cases were screened for bacterial pathogens. Out of the 104 isolates, majority of them were *Escherichia coli* (25), followed by coagulase positive *Staphylococcus aureus* (18), *Pseudomonas aeruginosa* (18), *Klebsiella* species (11) and others. Out of the 18 *Pseudomonas aeruginosa* isolates from diabetic foot ulcer cases, the majority (9) were in the Wagner's Grade three. About 66% of *Pseudomonas aeruginosa* were resistant to Cefotaxime, followed by Ceftriaxone (44%), and Ciprofloxacin (44%). The increasing incidence of *Pseudomonas aeruginosa* and its antimicrobial resistance in diabetic foot ulcer is a matter of great concern.

Introduction

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia, resulting from defective insulin secretion, insulin action or both. The prevalence of diabetes is 6.3 % in the general population, 8.7% among persons 20 years of age, and older. Approximately, 15–25% of persons with diabetes will develop a

DFU in their lifetimes, as a general case (Grossi *et al.*, 1991).

S. aureus was the most commonly isolated organism, ranging from 23% to 76% of ulcer cases (Diamantopaulos *et al.*, 1998, 2013; Wheat, 1986; Wheat *et al.*, 1986a,b).

High prevalence of common pathogens with antibiotic resistance, and their ever-increasing threat to public health causes a great concern to medical practitioners. This kind of resistance could occur through many varied mechanisms (Beach and Champney, 2014).

The chronic hyperglycemia of diabetes associated with long term damage, dysfunction and failure of various organs, especially the eyes, kidney, nerves, heart and blood vessels. Traditionally type 2 diabetes is common in individuals over the age of 40. It is often associated with obesity, decreased physical activity and heredity (Zimmet *et al.*, 1990, 2001; Eriksson *et al.*, 1991). Recent data from several countries show that type 2 diabetes increasingly becoming a problem among adolescents, and even among children (Glaser *et al.*, 1997). Diabetic foot ulcer (DFU) is usually multi-factorial, and the bacterial infection would complicate the spectrum of the disease. Infections are usually exogenous, from soil, air and commensals of the skin itself.

The aim of the present research study is to isolate and identify bacterial pathogens from diabetic foot ulcer cases, in samples collected from a reputed diabetic care center in Chennai City, Tamilnadu, India and to analyze the most common drug resistance pattern for *Pseudomonas aeruginosa*.

Material and Methods

75 pus samples in swab were collected from chronic foot ulcer cases attending a private diabetic center in Chennai City, during the period from May 2005 to September 2005, and were transferred to the laboratory, in Carey-Blair transport medium, to analyze for bacterial growth by standard procedures (Mackie and McCartney). Ethical clearance was obtained from the Institutional Ethical

Committee, and the necessary signed-consent - letter was obtained from all participants, after explaining the purpose of the study, and the benefit intended for the patients. All the isolates were identified and the antibiotic susceptibility test was performed, according to the method of Kirby-Bauer (1989). The diabetic foot ulcer was graded according to Wagner's classification.

Results and Discussion

Out of the 75 DFU cases, 104 bacterial isolates were obtained. Majority of CFU cases (22.2 %) showed *Escherichia coli* in ulcer, followed by *Pseudomonas aeruginosa* (17.3%), and *Staphylococcus aureus* (17.3%), and other organisms (Table 1). Since *P. aeruginosa* is generally a multi-drug resistant organism (antibiogram), the same was analyzed against routinely-used antibiotics.

Cefotaxime was found to be resistant for 66% of strains (Table 2). However, the Imipenem and piperacillin are highly sensitive for *Pseudomonas*. Moreover, out of 18 *Pseudomonas aeruginosa* cases, 8.6 % were under Wagner's Grade III ulcer (Table 3).

In the present DFU study, *Escherichia coli* and *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the predominant infections. Hutchinson and McGuckin (1985, 1990, 1995) had reported that *Staphylococcus aureus* prevailed in 23% to 76% of DFU cases. The organism is both pathogenic and evasive in nature. It is beyond the scope of this study to find out MRSA strains. Similarly, the isolation of anaerobic micro-organisms is also not done, although it is being proposed for future studies. *Escherichia coli* is also a well known pathogen in DFU cases. However, the *Pseudomonas aeruginosa* is the major

concern in DFU, as it is usually multidrug resistant, in effect. In the present study, 18 isolates were *Pseudomonas* spp. Giovanni bonfiglio (1998) from Italy reported 19.5% *Pseudomonas* in DFU; Sapio *et al.* (1994a, b, 2009) from California, united states had reported a prevalence of 6.3% *Pseudomonas* in DFU cases. This shows that the bacterial infections in DFU cases could vary according to the environment, as well as, to the individual immune status. The present

study also showed the anticipated antibiogram result, where in, except for Imipenam and Piperacillin, almost all other drugs (Table 2) were more than 30% resistant to the *Pseudomonas* isolates. Majority of the cases of *Pseudomonas* infection were in the category of Grade III of Wagner’s classification of DFU. This also corroborates with the many previous reports on diabetic foot ulcer.

Table.1 Bacterial Isolates from DFU cases from Chennai, Tamilnadu
(n = number of cases=75) ; Number of isolates=104

Name of Bacterial Isolates	Number (%)
<i>Escherichia coli</i>	23 (22.2)
<i>Staphylococcus aureus</i> (Coagulase Positive)	18 (17.3)
<i>Pseudomonas aeruginosa</i>	18 (17.3)
<i>Klebsiella</i> spp	11 (10.6)
Coagulase negative <i>Staphylococcus</i> (CONS)	11 (10.6)
<i>Proteus</i> spp	10 (9.6)
<i>Streptococcus</i> spp	6 (5.8)
<i>Corynebacterium</i> spp	4 (3.8)
<i>Enterococcus</i>	3 (2.9)

Table.2 Resistant pattern of *Pseudomonas aeruginosa* from DFU cases
(n=18)

Antimicrobial agent	Resistant	
	No.	(%)
Cefotaxime	10	66
Ceftriaxone	8	44
Ciprofloxacin	8	44
Ampicillin	7	38
Gentamicin	6	33
Ceftazidime	6	33
Co-amoxyclov	4	22
Piperacillin	2	11
Imipenem	1	5.5

Table.3 Wagner’s Grade of DFU in *Pseudomonas aeruginosa* infection

Wagner’s Grade	Number	%
Grade III	9	8.6
Grade IV	4	3.8
Graded II	3	2.9
Grade V	2	1.9

References

- Beach, J.M., Champney, W.S. 2014. An examination of the Inhibitory Effects of three antibiotics in combination on Ribofame biosynthesis in *Streptococcus aureus*, *Arch. Microbiol.*, 196: 249–260.
- Beach, J.M., Champney, W.S. 2014. *In vitro* activity of rifampicin against clinical isolates of *Escherichia coli* and other. *J. Microbiol. Immunol. Infect.*,
- Diamantopoulos, E.J., Haritos, G., Yfandi, M., Grigoriadou, Marga, G. 2013. Wound repair and regeneration. 2(2).
- Diamantopoulos, *et al.* 1998. Management and outcome of severe diabetic foot infections. *Exp. Clin. Endocrinal Diabetes*, 106: 346–52.
- Eriksson, *et al.* 1991. Prevalence of known diabetes in an urban Indian Environment. *Q. J. Med.*, 81: 1021–1030.
- Giovanni bonfiglio, 1998. Of gram-positive coccal and cocco-bacillary vancomycin resistant bacteria. *J. Microb. Methods*,
- Glaser, N.S., *et al.* 1997. Global prevalence of diabetic estimates for the year 2000, and projections for 2030. *Pediatr. Clin. North. Am.*, 44(2): 302–327.
- Grossi, S.G. 1991. Joseph Cordts, DiEdwardo, *et al.*, 1991. Study on the microbiology of DFU, been provided, without Diabetes. *Mol. Med. Microbiol.*, Vol. 3.
- Hutschinson, J.J., McGuckin, M. 1985. Occlusive dressings: A microbiologic and tissue infections. *J. Clin. Microbiol.*, 22(1): 80–83.
- Hutschinson, J.J., McGuckin, M. 1990. Wound bioburden and infection-related complications in diabetic.
- Hutschinson, J.J., McGuckin, M. 1995. Occlusive chronic wound care: A clinical source book for health care.
- Kirby Baur, 1989. In: John C. Sherris, (Ed.) Anti-microbial susceptibility testing, a personal perspective. *Clinics in Laboratory Medicine*, 9(2): 191.
- Sapio, M., *et al.* 1994. *J. Invest. Dermatol. Keratin: Gene Mutat.*
- Sapio, M., *et.al.* 1994. *Mol. Microbiol.*, 12(2): 253–265.
- Sapio, M., *et.al.* 2009. Clinical microbiology and infection. The official publication of the European society of Clinical and Vaccine Immunology. *CVI*, 16: 147.
- Wagner, A., Reike, H., Angel Kort, B. 2001. Highly resistant pathogens in patients with diabetic foot syndrome, with special reference to methicilin-resistant *Staphylococcus aureus* infections. *Dtsch Med. Wochenschr*, 126: 1353–1356
- Wheat, *et al.* 1986a. Diabetic foot ulcers. *J. Am. Paediatr. Med. Assoc.*, (AHCPR Publication no.....) Deep foot infections in patients with diabetes

- and foot ulcer,an entity with different characteristics...
- Wheat, L.J. 1986. Diagnostic strategies in osteromyelitis, *AMA Pusblishing Group J.*, 146(10): 1935–1940.
- Wheat, L.J., Allen, S.D., Henry, M., *et al.* Diabetic foot infections: bacteriologic analysis. *Arch. Intern. Med.*, 146: 1935–1940.
www.nature.com.Archive.
www.ncbi.nlm.gov/pmc/PMC377723/ Occlusive dressings: A microbiologic and clinical review”, 18(4): 257–268.
- Zimmet, et al. 2001. Rise in obesity, worldwide. *Genetics & Diabetes: WHO*.
- Zimmet, F.Z., *et al.* 1990. High prevalence of NIDDM and impaired glucose tolerance in Indian Creole, and Chinese mauritians, mauritius non communicable study group. *Diabetes*, 39(3): 3906.