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Prevalence of aerobic bacterial flora among diabetic foot ulcer cases

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Abstract

Diabetic Foot Ulcer (DFU) is debilitating complication among diabetic patients. DFU with infection is also one of the major causes of hospitalization in diabetic patients. This study investigated the microbiology of diabetic foot infections and their susceptibility to antibiotics in patients admitted at wards and intensive care unit of Rajah Muthiah Medical College and Hospital, Chidambaram. A retrospective analysis was conducted with clinical specimens taken from patients with DFI over a 5 months period from December 2014 to April 2015. A total of 50 patients, female 28 (56%) and male 22 (44%) with positive clinical specimens were identified. The most frequently isolated pathogens were Proteus spp. (40%) and the second most predominate bacteria encountered were *Staphylococcus aureus* (30%) followed by *Klebisella* spp. (16%), *Escherichia coli* (13%), *Pseudomonas* (7%), *Streptococcus* spp. (2%) and *Bacillus* spp. (2%). The antimicrobial susceptibility results showed that the gram negative organisms were highly susceptible to the most of the antibiotics used. All the *Staphylococccus aureus* isolates showed sensitive to methicillin, no MRSA strains were encountered among DFU patients in this study. Likewise, all the gram negative isolates were 100% sensitive to Imipenem. These findings have shown that there were no multiple drug resistance strains and MRSA strains.

Keywords: DFU, pathogens, *Staphylococcus aureus*, Imipenem.

Introduction

Diabetes mellitus is a metabolic disorder that affects many people throughout the world due to the lack of Insulin secretion and elevation of blood glucose level. It hinders the life of nearly 40 millions of people in India. Hence, it has been labeled as “The diabetic capital of the world”. (Wild *et al.*, 2004). Approximately one-in-four people with diabetes will develop an ulcer during their lifetime and as many as half of these ulcerations will develop an infection (Lavery *et al.*, 2007). The term “diabetic foot wound” refers to a variety of pathological conditions. Ulcers, the most frequent and characteristic type of lesions, are defined as any break in the cutaneous barrier, but they usually extend through the full thickness of the dermis (Lazarus *et al.*, 1994). The risk of ulcers in diabetic patients, increases in the presences of the following factors; diabetics mellitus of more than 10 years, poor glycemic control or other diabetics-related complications (cardiovascular, renal diabetic retinopathy).

Ischemia, neuropathy and infection are the 3 pathogenic mechanisms underlying diabetic foot complications. Infection worsens the wound condition, delays the healing mechanism and, if appropriate measures are taken on time, could have avoid from the systemic infection,

septicemia, amputation or even death. Early diagnosis is necessary to prevent the spread of infection, especially with resistant bacterial strains and immune compromised individuals. The diabetic wounds are mostly infected by pus forming microorganisms like *Enterococci* sp., *Staphylococcus aureus, Pseudomonas aeruginosa, E. coli, Klebsiella* sp. and *Proteus* sp. (Revathi *et al.,* 1998). The multidrug resistant bacteria have been reported in many diabetic foot infections (Zubair *et al.,* 2010).

It is always necessary to evaluate different microorganisms infecting the wound on a routine basis in addition to administering regular glycemic control, wound care, surgical debridement, pressure-offloading and maintaining adequate blood supply (Apelqvist *et al.,* 2011). Bacteriology of diabetic foot infections is highly complicated. It involves both aerobes and anaerobes. Many researchers have presented a picture of mixed infection with both aerobic and anaerobic bacteria. *Staphylococcus aureus* is the most common infecting organism in diabetic foot infections (Tentolouris *et al.,* 2006).

The present study was directed to screen the aerobic bacterial flora associated with diabetic foot infection and to find out antibiotic sensitivity pattern of the isolates.

Materials and Methods

Patient inclusion criteria

The diabetic patients with complaints of the foot infections admitted in wards and intensive care unit of Rajah Muthiah Medical College and Hospital, Chidambaram were selected and included in this study. The patient's with foot ulcer (purulent discharge) alone were selected and included in the present study.

Specimen collection

About 50 samples were collected from DFU patients by means of wound swabs. The collected wound swab samples were immediately placed in sterile test tubes and further processed in the laboratory.

Isolation of organisms

The pus samples were inoculated onto Blood and MacConkey agar plates and incubated at 37°C for 18–24 hrs to isolate aerobic bacteria. Bacterial species were diagnosed according to colony morphology and color on the media, the results of confirmatory biochemical tests (Indole, Methyl red, Voges–Proskauer, Simmons' Citrate, Semisolid Mannitol and Oxidase test, Coagulase, Catalase, Novobiocin sensitivity test) according to Morello *et al.* (2006).

Antibiotic sensitivity test

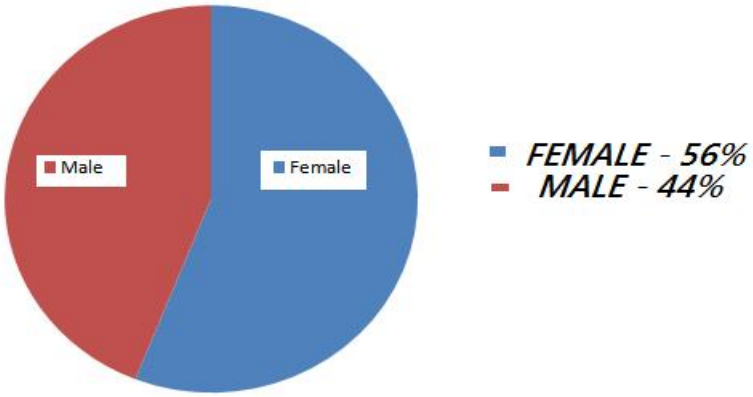
Antibiotic susceptibility testing was carried out using the Kirby-Bauer disc diffusion technique on Muller-Hinton agar and commercial antibiotic discs were used for antimicrobial testing (Bauer *et al.,* 1996). The antibiotic discs used were Piperacillin G, Ampicillin (10 µg), Tetracycline (30 µg), Gentamycin (10 µg), Chloramphenicol (30 µg), Cotrimoxazole, Ceftazidime, Methicillin, Amoxicillin, Ciprofloxacin (10 µg), Amikacin (30 µg) and Imipenem (10 µg). The antibiotic disc impregnated culture plates were incubated at 37°C for overnight. The diameter of the zone of inhibition was measured and recorded as resistant or susceptible according to the National Committee for Clinical Laboratory Standards (NCCLS) interpretative criteria (2000).

Results and Discussion

The present study was carried out for a period from December 2014 to April 2015 in the Division of Microbiology, Faculty of Science, Annamalai University. Totally 50 pus samples were collected from the patients admitted in wards of Rajah Muthiah Medical College and Hospital, Chidambaram. About 28 (56%) pus samples were collected from females and 22 (44%) samples from male gender (Fig-1).

According to the results of Smith (2014), wound swab techniques yielded a better percentage of recovery rate of anaerobes (55.6%), gram positive (52.4%) and all species (51.6%) when compared with tissue culture. He concluded that the swab-based culture method for chronic wounds is a reasonable one which is being currently used in UK, hence wound swabs were used in the present investigation.

Figure-1 Number of subjects included in the study



About 60 isolates were obtained from wound swabs of infected patients. The gram staining revealed 19 (32%) isolates belonged to gram positive cocci, one isolate (2%) belonged to gram positive rod and remaining 40 (67%) isolates belonged to gram negative rods. Most of the samples were polymicrobial in nature, they were consisted of 2–3 microbial agents. The most predominant flora isolated from DFU patients were *Proteus* spp. (40%) and the second most predominant bacteria encountered were *Staphylococcus aureus* (30%). This was followed by *Klebsiella* spp. (16%), *E. coli* (13%), *Pseudomonas* (7%), *Streptococcus* spp. (2%) and *Bacillus* spp. (2%) respectively (Fig-2). Iyanar *et al*. (2010) conducted a study to isolate and identify the antibiotic susceptibility pattern of the bacteria from diabetic foot infections from the patients of Kancheepuram District, Tamil Nadu, India. According to them, *Pseudomonas aeruginosa* was the predominant bacteria (48.3%) it was followed by *Staphylococcus aureus* (38%) and other bacteria. In recent years, there has been increase in the fungal infection of UT as like bacterial infection among diabetic patients because of higher glucose level in the urine (Ponmudi *et al*., 2015).

Antibiotic sensitivity pattern of the isolates revealed that the gram negative organisms (*E. coli*, *Proteus and Klebsiella*) were highly susceptible to the most of the antibiotic used. Interestingly, all the *Staphylococccus aureus* isolates showed sensitive to methicillin, this showed no MRSA strains were encountered among DFU patients in this study. Likewise, all the gram negative isolates were 100% sensitive to Imipenem (Tables-1 and 2). These findings have shown that there were no multiple drug resistance strains and MRSA strains encountered in the present investigation. The study results of Sajjad Raja and Nishi Singh (2007) confirmed that Imipenem was equally effective against gram negative and gram positive organisms.

In contrast to them, Banashankari *et al*. (2012) determined the bacterial spectrum in diabetic foot lesions and analyzed the antibiotic susceptibility pattern of the bacterial isolates. The results revealed that the frequent isolates were gram negative aerobes 66% followed by gram positive aerobes (32%). *Proteus* spp. was dominant bacterial flora and it was followed by *Staphylococcus aureus* and most of the *Staphylococcus aureus* (70%) were sensitive to methicillin. Our study showed similar results, stating that the most predominant flora was *Proteus* spp. (40%) followed by *Staphylococcus aureus* (30%). But our results were contradictory in one aspect, since all the *Staphylococcus aureus* strains were sensitive to methicillin (100%). Karthik *et al*. (2015) determined the bacterial isolates from diabetic males and females with the complications of UTI admitted in RMMCH, Annamalai University and found that most of the isolates were highly sensitive to Ciprofloxacin, Nalidixic acid and Ofloxacin and poorly effective to Amikacin and Gentamycin.

Tiwari *et al*. (2009) analyzed 62 diabetic foot infection patients, among them 43.5% had mono-microbial infections, 35.5% had poly-microbial infections and 21% had sterile culture. According to their results, *E. coli* was the common isolate and was sensitive to Piperacillin/Tazobactam. Goldstein *et al.* (1995) studied the relative

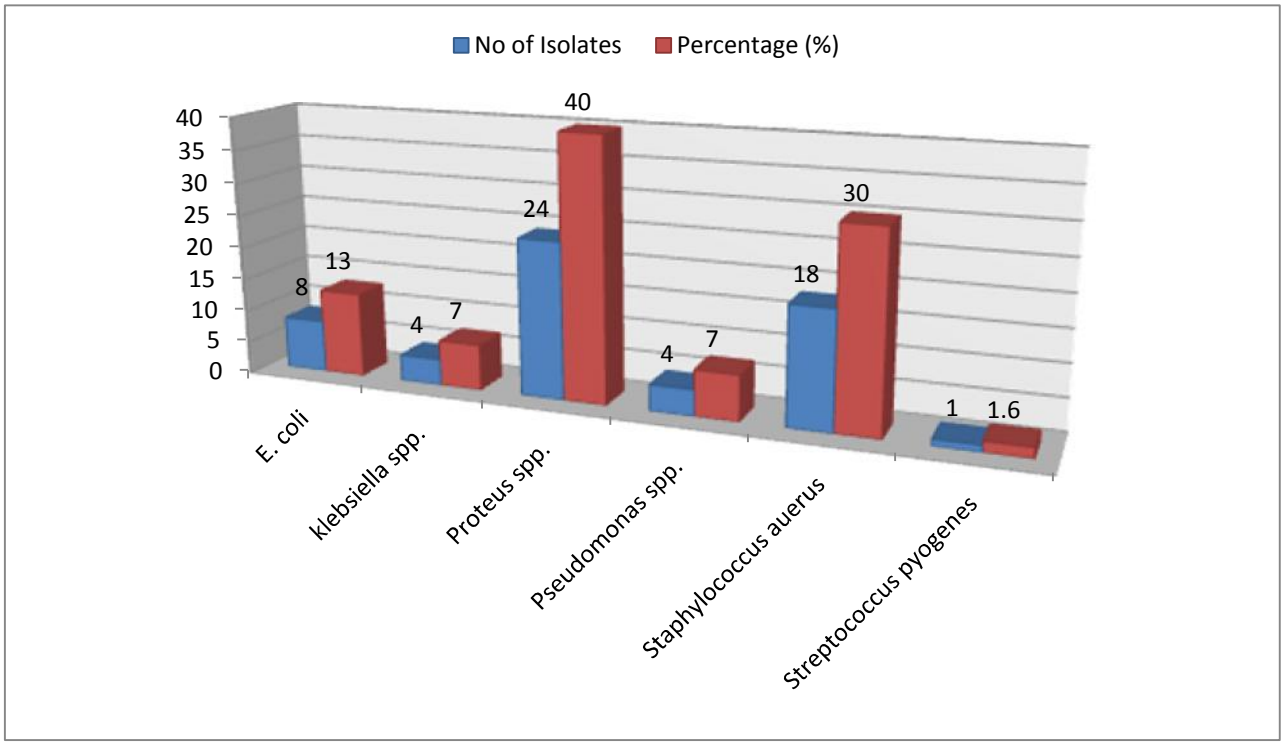
Table-1 Antibiotic sensitivity pattern for bacteria isolated from diabetic foot ulcers

S. No	Antibiotic used	*Staphylococcus aureus* (N=18)	*Streptococcus pyogenes* (N=1)	*Bacillus* spp. (N=1)
1	Amikacin	16(89%)	1(100%)	1(100%)
2	Ampicillin	8(44%)	–	1(100%)
3	Chloramphenicol	12(67%)	1(100%)	1(100%)
4	Ciprofloxacin	18(100%)	1(100%)	1(100%)
5	Cotrimoxazole	15(83%)	1(100%)	1(100%)
6	Gentamycin	10(55%)	–	1(100%)
7	Tetracycline	17(94%)	1(100%)	1(100%)
8	Methicillin	18(100%)	–	–

Table-2 Antibiotic sensitivity of various gram negative bacteria isolates from diabetic foot ulcers

S. NO	Antibiotic used	*Klebsiella* spp. (N=4)	*Proteus* spp. (N=24)	*Pseudomonas* spp. (N=4)	*E. coli* (N=8)
1	Amikacin	3(75%)	8(33%)	4(100%)	8(100%)
2	Chloramphenicol	–	17(71%)	4(100%)	6(75%)
3	Ciprofloxacin	4(100%)	24(100%)	4(100%)	8(100%)
4	Cotrimoxazole	1(25%)	4(17%)	2(50%)	3(37%)
5	Gentamycin	3(75%)	15(62%)	4(100%)	7(87%)
6	Tetracycline	1(25%)	4(17%)	4(100%)	8(100%)
7	Piperacillin	4(100%)	12(50%)	4(100%)	7(87%)
8	Amoxicillin	4(100%)	16(66%)	3(75%)	6(75%)
9	Ceftazidime	4(100%)	20(83%)	4(100%)	8(100%)
10	Imipenem	4(100%)	24(100%)	4(100%)	8(100%)

Figure-2 Prevalence of bacterial flora among DFU patients



frequency of bacterial isolates in foot infections and assessed their susceptibility to oral antibiotic like Sparfloxacin, Levofloxacin, and others. In the study, *Staphylococcus aureus* was the common isolates (76%) including MRSA (20%), *Streptococci, Enterococci, Enterobacteriaceae* were also encounted. Sparfloxacin and Levofloxacin were the active against ≥88% of isolates. According to Gadepalli *et al.* (2006), 72% of patients were positive for MDROs. ESBL production and methicillin resistance was also observed in 44.7% and 56.0% of bacterial isolates. In conclusion, this type of study is necessary to setup appropriate antibiotic therapy thereby reducing the risk of amputation procedures due to DFU.

References

Apelqvist, J., Elgzyri, T., Larsson, J., Londahl, M., Nyberg, P. *et al*. (2011). "Factors related to outcome of neuroischemic/ischemic foot ulcer in diabetic patients." *Journal of Vascular Surgery* **53**(6): 1582–1588.

Banashankari, G.S., Rudresh, H.K. and Harsha, A.H. (2012). "Prevalence of gram negative bacteria in diabetic foot-A clinico-microbiological study. Al Ameen." *J. Med. Sci*. **5**(3): 224–232.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. (1996). "Antibiotic susceptibility testing by standardized single disc method." *Am. J. Clin Pathol*. **44**: 493–496.

Gadepalli, R., Dhawan, B., Sreenivas, V., Kapil, A., Ammini, A.C. and Chaudhry, R.A. (2006). "Clinicomicrobiological study of diabetic foot ulcers in an Indian tertiary care hospital." *Diabetes Care* **29**: 1727–1732.

Goldstein, D.B., Linares, A.R., Cavalli-Sforza, L.L. and Feldman, M.W. (1995). "An evaluation of genetic distances for use with microsatellite loci." *Genetics* **139**: 463–471.

Iyanar, K., Vijaykumar, G. and Khan, A.K.F. (2010). "Correlation and path analysis in multicut fodder sorghum." *Elect. J. Plant Breed* **1**(4): 1006–1009.

Karthik, P., Sivagurunathan, P.*, Uma, C. and Bhuvaneswari, M. (2015). "Incidence of bacterial Uropathogens among diabetic patients." *American Journal of Biological and Pharmaceutical Research* **2**(1): 47–50.

Lavery, A.C., Wiebe, P.H., Stanton, T.K., Lawson, G.L., Benfield, M.C. and Copley, N. (2007). "Determining dominant scatterers of sound in mixed zooplankton populations." *J. Acoust. Soc. Am* **122**(6): 3304–3326.

Lazarus, G.S., Cooper, D.M., Knighton, D.R., Margolis, D.J., Pecoraro, R.E., Rodeheaver, G. and Robson, M.C. (1994). "Definitions and guidelines for assessment of wounds and evaluation of healing." *Arch. Dermatol.*, **130**: 489–493.

Morello, R., Bertin, T.K., Chen, Y., Hicks, J., Tonachini, L., Monticone, M., Castagnola, P., Rauch, F., Glorieux, F.H., Vranka, J., *et al*. (2006). "CRTAP is required for prolyl 3-hydroxylation and mutations cause recessive osteogenesis imperfecta." *Cell* **127**: 291–304.

Ponmudi, R., Uma, C., Sivagurunathan, P. and Aruljothi, S. (2015). "Occurrence and antifungal susceptibility pattern of candiduria isolates among diabetic patients." *International Journal of Pharmacy Review & Research* **5**(4): 345–349.

Revathi, G., Puria, J. and Jaid, K. (1998). "Bacteriology of Burns." *Burns* **24**: 347–349.

Sajjad Raja, N. and Nishi Singh, N. (2007). "Antimicrobial susceptibility pattern of clinical isolates of *Pseudomonas aeroginosa* in a tertiary care hospital." *J. Microbiol. Immunol. Infect*. **40**: 45–49.

Smith, M .E., Robinowitz, N., Chaulk, P. and Johnson, K., (2014). "Comparision of chronic wound culture techniques: Swab versus curetted tissue for microbial recovery". .*Br J Community Nurs.* **19**(90): S22-S26.

Tentolouris, N., Liatis, S. and Katsilambros, N. (2006). "Sympathetic system activity in obesity and metabolic syndrome." *Annals of the New York Academy of Sciences* **1083**: 129–152.

Tiwari, V.M., Wahr, J. and Swenson, S. (2009). "Dwindling groundwater resources in northern India, from satellite gravity observations." *Geophys. Res. Lett*. 36: L18401. doi:10.1029/2009GL039401.

Wild, S., Roglic, G., Green, A., *et al*. (2004). "Global prevalence of diabetes." *Diabetes Care* **27**: 1047–1053.

Zubair, M., Malik, A. and Ahmad, J. (2010). "Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in North India." *Biology and Medicine* **2**(4): 22–34.

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