RESEARCH STUDY

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A Comparative Study on Treatment of Diabetic Foot Infection by Acetic Acid, Rifocin and Probiotics

Article Information

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Abstract

Introduction: Diabetic foot infections are one of the most severe complications of diabetes. This study was aimed to determine the common bacterial isolates of diabetic foot infections and the in vitro antibiotic susceptibility then treatment.
Methods: A swab was taken from the foot ulcer, and the aerobic bacteria were isolated and identified by cultural, microscopic and biochemical test, then by api-20E system. After that their antibiotic susceptibility pattern was determined. Then local and systemic treatment was used to treat the diabetic foot patients.
Results: Bacterial isolates belonging to twelve species were obtained from diabetic foot patients. Gram (-) bacteria were the predominant pathogens in the diabetic foot infections, high percentage recorded by *Klebsiella pneumoniae* (25.71%). Polymicrobial infection was observed in 72% patients. Imipenem was the most affected antibiotic in susceptibility test, except for *Acinetobacter spp*. that resist for all antibiotic used, followed by amikacin and ciprofloxacin. Local treatment gave more inhibitory effect on diabetic foot infections than the systemic treatment.
Conclusion: High prevalence of multi-drug resistant pathogens was observed. Gram (-) bacteria especially *Klebsiella pneumoniae* was the predominant pathogens in the diabetic foot infections, and *Staphylococcus aureus* was the most common of Gram (+) bacteria. Local treatment was the best for treatment of diabetic foot infection patients.

Introduction

Foot ulceration or infection is one of the leading causes of human mortality and morbidity. It represents a severe complication of diabetes and the most common cause of diabetes associated hospital admissions (Lavery *et al.*, 2007)[(1).]Diabetic foot is characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, leading to development of gangrene and even necessitating limb amputation (Anandi *et al.*, 2004 [(2)] ; Khanolkar *et al.*, 2008 [(3)].

Diabetic ulcers have 15 to 46 times higher risk of limb amputation than foot ulcers due to other causes (Alavi *et al.*, 2007)[(4).] It is predicted that the number of people with diabetes will rise from an estimated 171 million in 2000 to 366 million in 2030 (Wild *et al.*, 2004) [(5).] Diabetic foot infections are often polymicrobial in nature (Gadepalli *et al.*, 2006 [(6)] ; Alavi *et al.*, 2007[(4)] **)**. The increasing association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers is the most problem faced by the physician or the surgeon in treating diabetic ulcers without resorting to amputation (Yoga *et al.*, 2006) [(7).]

Initial therapy of diabetic foot infections is frequently empiric because reliable culture data is lacking. There is variability in prevalence of common bacterial pathogens isolated between Gram (+) and Gram (-) bacteria, as shown (Viswanathan *et al.*, 2002)[(8).] So, this study was performed to determine the common etiological agents of diabetic foot infections and their in vitro susceptibility to routinely used antibiotics. The treatment of patients with diabetic foot infections by local and systemic agents were also studied.

Methods:

Processing of specimens: A swab from the ulcer of diabetic foot patients was obtained. The specimens were taken immediately to the microbiology laboratory and processed without any delay. The specimens were inoculated on blood agar and MacConkey agar for isolation of aerobic bacteria. After 24 hours incubation at 37°C, the bacterial isolates were identified based on standard bacteriological methods.

Antibiotic susceptibility testing: Antibiotic susceptibility testing was performed by Kirby Bauer's disc diffusion method according to National Community for Clinical Laboratory Standards, NCCLs, (2002)[(29).] Amoxicillin-clavulanic acid, piperacillin, tetracycline, ciprofloxacin, gentamicin, amikacin, Cefotaxime, erythromycin, netilmicin, vancomycin, Ampicillin, Aztreonam, Chloramphenicol, Ticarcillin-Clavulanic acid, Ticarcillin and imipenem were tested for bacterial isolates .

Treatment of the patients: Treatment of the patients was carried out at the Hospital . At the beginning, ٦٧ patients were divided into two groups (A ,B), each contained 30 patients, while the other 7 patients have no bacterial growth therefore they did not treated. Systematic (oral) treatment was used for group A, and a local treatment for group B. In the systemic treatment, a swab was taken from each patient for identification of pathogenic isolates and testing their susceptibility for antibiotics to chose the most affected one. The chosen antibiotic was administrated to the patient for 3 weeks, then a second swab was taken from each patient to determine effect of the systemic treatment on the diabetic foot infection. In the local treatment, after taken a swab from the patients and identifying the causative bacterial pathogen, each patient was supplied with the therapeutic agents, and asked to use it. Another swab was taken from the diabetic foot ulcer, after one week, to detect the effect agents on the infecting bacteria. This procedure was repeated for four consecutive weeks.

Results:

From the 67 patients with diabetic foot, 64% were male and 36% were female. The age ranged from 28-75 yrs. On the other hand, the duration of diabetes mellitus was between 4 to 35 yrs, while that of infection was from 1 week to 20 yrs. A total of 105 bacteria were isolated from these patients. The bacteria isolated from the diabetic foot ulcers are summarized in table 1. One type of pathogenic bacteria was detected in 17 (28%) of 60 infected patients, while 43 (72%) of the patients were infected with more than one types (Polymicrobial infection) ; 41(69%) of them with two types and 2(3%) with three types of pathogens. On the other hand, no any bacterial type was detected in the rest (7, 10.45%) of the patients. Gram (-) bacteria were the predominant pathogens in the diabetic foot infections.

Species of bacteria	No.	%
Klebsiella pneumoniae	27	25.71
Pseudomonas aeruginosa	20	19.04
Staphylococcus aureus	18	17.14
Escherichia coli	13	12.38
Proteus mirabilis	12	11.47
Citrobacter freundii	5	4.76
Acinetobacter baumannii	2	1.90
Enterobacter cloacae	2	1.90
Morganella morganii	2	1.90
Pseudomonas fluorescence	2	1.90
Aeromonas hydrophila	1	0.95
Serratia marcescens	1	0.95
Total	105	100

Results of the antibiotic susceptibility testing declared that**,** with exception of *Acinetobacter baumannii*, all bacterial isolates were completely sensitive to imipenem. Susceptibility of most bacterial isolates to the amikacin was also reported in this study, From the Enterobacteriaceae isolates, *E. coli* showed the highest resistance to the antibiotics used in the study. Adversely, *E. coli* appeared sensitive to only imipenem and amikacin.

Pseudomonas aeruginosa resisted penicillin, cephalosporin and chloramphenicol, but sensitive to aminoglycosids and carbapenem. *Aeromonas hydrophila* showed resistance to penicillin group and sensitive to aminoglycosids. While *S. aureus* found to be sensitive to amikacin, gentamycin and ciprofloxacin, and resistant to erythromycin and ampicillin. Resistance of all isolates to the penicillin group and cephalosporin group also found in this study.

Discussion:

Diabetic foot ulcer is chronic and non-healing due to several factors such as neuropathy, high plantar pressures and peripheral arterial disease (Frykberg *et al.,* 2000) (9) . Such chronic long-standing ulcers are more prone for infection which delays the wound healing process In this study, Gram (-) bacteria were the predominant pathogens in the diabetic foot infections, Similar findings were also recorded by various studies such as (Shankar *et al.,* 2005 (10) ; Gadepalli *et al.,* 2006 (6) ; Alavi *et al.,* 2007(4) ; Raga, 2007 (11) ; Ekta *et al.,* 2008 (12)). But in the studies of Mantey *et al.,* (2000) (13), Dang *et al.,* (2003) (14) and Diane *et al.,* (2007) (15), Gram (+) bacteria was found to be the predominant organisms in the diabetic foot infections. One type of pathogenic bacteria was detected in 17 (28%) of 60 infected patients, while 43 (72%) of the patients were infected with more than one types (Polymicrobial infection). Polymicrobial infection was also observed by several other studies such as (Wight-Pascoe *et al.,* 2001(16) ; Anandi *et al.,* 2004(2) ; Altrichter *et al.,* 2005 (17) ; Shankar *et al.,* 2005 (10) ; Alavi *et al.,* 2007(4)). Adversely, Viswanathan *et al.,* (2002) (8) and Raga (2007) (11) detected only one type of bacteria in the patients of DFI. Results declared that**,** all bacterial isolates were completely sensitive to imipenem. In this regard, Livermore *et al.* (2001) (18) found that imipenem have strong activity against most Enterobacteriaceae bacteria. Susceptibility of most bacterial isolates to the amikacin was reported in this study, which is closed to that found by Paterson *et al.* (1999) (19) who reported high sensitivity to amikacin among bacterial isolates of their study. Umadevi *et al.* (2011) (20) also detected that the members

of Enterobacteriaceae were found to be susceptible to amikacin.

Resistance of all *isolates* to the penicillin group and cephalosporin group also found in this study, and this may be related to isolates-possessing of β- lactamase enzymes (Levinson and Jawetz, 2000)[21] . Another reason for the resistance is production of the extended spectrum ß-lactamase (ESBL) or other enzymes, such as AmpC ß-lactamases, capable of hydrolyzing the extended-spectrum cephalosporins (Rice *et al*., 2003)[22] .From the Enterobacteriaceae isolates, *E. coli* showed the highest resistance to the antibiotics used in the study. This may be because *E. coli* is easily acquires the resistance factor from environment and easily resisted penicillin derivatives drug like ampicillin (Wazait *et al*., 2003)[23].

Results declared that *Acinetobacter baumannii* were completely resistant to all 16 antibiotics which may be related to factors, such as Beta-lactamase (Higgins *et al*., 2013) [24]. Biofilm formation is a second factor where A. *baumannii* is able to form biofilms for its survival (Espinal *et al*., 2012)[25]. In addition, adherence of *A. baumannii* to epithelial cells of the outer membrane also involves in survival of bacteria (Choi *et al*., 2008)[26].

After comparing the two ways of applying the pharmaceutical agents, local treatment, especially rifoc in, was found to be the superior for treatment of diabetic foot infection. It is effective especially when accompanied by appropriate wound care as a therapeutic alternative to a broad-spectrum oral antibiotic agent. In addition, local treatment appears to be safe and may avoid the opportunity of resistant bacteria that can develop after oral systemic antibiotic therapy (Lipsky *et al.*, 2008) [27]. Local treatment has also the advantages of avoiding systemic adverse effects, providing increased target site concentration and allowing the use of agents not available for systemic therapy (Lio and Kaye 2004) [28]. They added that this is another reason made topical treatment be the best for treatment of diabetic foot infection. An acceptable topical anti-infective agent would need to demonstrate activity against the spectrum of bacteria that are known to cause DFI, and it would need to avoid serious adverse effects, interference with wound healing, or induction of drug resistance.

References:

1. Lavery, L. ; Armstrong, D. and Murdoch, D. (2007). Validation of the Infectious Diseases Society of America's diabetic foot infection classification system. *Clin Infect Dis*. 44 (4): 562-565.
2. Anandi, C. ; Alaguraja, D. ; Natarajan, V. ; Ramanathan, M. ; Subramaniam, C. and Thulasiram, M. (2004). Bacteriology of diabetic foot lesions. *Indian J Med Microbiol*. 22: 175-8.
3. Khanolkar, M. ; Bain, S. and Stephens, J. (2008). The diabetic foot. *QJM*. 101: 685-95.
4. Alavi, S. ; Khosravi, A. ; Sarami, A. ; Dashtebozorg, A. and Montazeri, E. (2007).
5. Wild, S. ; Roglic, G. ; Green, A. ; Sicree, R. and King, H. (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care.* 27(5): 1047-53.
6. Gadepalli, R. ; Dhawan, B. ; Sreenivas, V. ; Kapil, A. ; Ammini, A. and Chaudhry, R. (2006). clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. *Diabetes Care*. 29: 1727-32.
7. Yoga, R. ; Khairul, A. ; Sunita, K. and Suresh, C. (2006). Bacteriology of diabetic foot lesions. *Med J Malaysia*.61:14-6.
8. Viswanathan, V. ; Jasmine, J. ; Snehalatha, C. and Ramachandran, A. (2002). Prevalence of pathogens in diabetic foot infection in South Indian type 2 diabetic patients. *J. Assoc. Physicians India*, 50: 1013-1016.
9. Frykberg, R. ; Armstrong, D. and Giurini, J. (2000). Diabetic foot disorders: a clinical practice guideline. For the American College of Foot and Ankle Surgeons and the American College of Foot and Ankle Orthopedics and Medicine. *J Foot Ankle Surg*. 45 (5): 1-60.
10. Shankar, E. ; Mohan, V. ; Premalatha, G. ; Srinivasan, R. and Usha, A. (2005). Bacterial etiology of diabetic foot infections in South India. *Eur J Intern Med*. 16: 567-70.
11. Raga, S. (2007). Microbiology of diabetic foot infections in a teaching hospital in Malaysia: a retrospective study of 194 cases. *J Microbiol Immunol Infect. 40: 39-44.*
12. Ekta, B. ; Ashish, G. ; Sanjeev, A. ; Attri, K. and Jagdish C. (2008).Spectrum of microbial flora of diabetic foot ulcers. *Indian journal of pathology and microbiology.* 51(2).
13. Mantey, I. ; Hill, R. ; Foster, A. , Wilson, S. ; Wade, J. and Edmonds, M. (2000). Infection of foot ulcers with Staphylococcus aureus associated with increased mortality in diabetic patients. *Commun Dis Public Health*. 3: 288-90.
14. Dang, C. ; Prasad, Y. ; Boulton, A. and Jude, E. (2003). Methicillin-resistant *Staphylococcus aureus* in the diabetic foot clinic: a worsening problem. *Diabet Med*. 20: 159-61.
15. Diane, M. ; Citron, C. ; Ellie, J. ; Goldstein, K. ; Vreni Merriam, C. ; Lipsky, A. and Murray A. (2007). Abramson Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. *J. CL. Microbiol.* 2819-2828.
16. Wight-Pascoe, R. ; Roye-Green, K. and Bodonaik, N. (2001). The medical management of diabetes mellitus with particular reference to the lower extremity. The Jamaican experience. *West Indian Med. J.* 50: 46-9.
17. Altrichter, L. ; Legout, L. ; Assal, M. ; Rohner, P. ; Hoffmeyer, P. and Bernard, L. (2005). Sever streptococcus agalactiae infection of the diabetic foot. *Press Med;* 34: 491-4.
18. Livermore, D. ; Oakton, K. ; Carter, M. and Warner, M. (2001). Activity of Etrapenem (MK-0862) versus *Enterobacteriaceae* with potent lactamases. *Antimicrob. Agents Chemother.* 45 (2):3l-7.
19. Paterson, D. and Yu, V. (1999). Extended-spectrum β-lactamases: a call for improved detection and control. *Clin Infect Dis*. 29: 1419-1422.
20. Umadevi, S. ; Shailesh, K. ; Noyal, M. ; Joshy, M. ; Kandhakumari, G. ; Sreenivasan, S. ; Sruthi, R. and Selvaraj S. (2011). Microbiological Study of Diabetic Foot Infections. *Indian Journal of Medical Specialties*
21. Levinson, W. and Jawetz, E. (2000). Medical Microbiology and Immunology. (4th ed.). *Appleton and Lange.*
22. Rice, L. ; Sahm, D. and Bonomo, R.(2003). Mechanisms of resistance to antibacterial agents. In: Murray, P. ; Baron, E. ; Pfaller, M. ; Jorgensen, J. and Yolken, R. editors. Manual of

clinical microbiology. 8th ed. Washington, D.C. ASM press: 1074-101.
23. Wazait, H. ; Patel, H. ; Veer, V. ; Kelsey, M. and van der Meulen, J. (2003). Catheter Associated Urinary Tract Infections: Prevalence of Uropathogens and pattern of antimicrobial resistance in a UK Hospital. *Britain Journal of Urology.* 91: 806-809.
24. Higgins, P. ; Pérez-Llarena, F. ; Zander, E. ; Fernández, A. ; Bou, G. and Seifert, H. (2013). OXA-235, a novel Class D Beta-Lactamase Involved in Resistance to Carbapenems in *Acinetobacter baumannii*. *Antimicrobial agents and chemotherapy*.
25. Espinal, P. ; Martí, S. and Vila, J. (2012). Effect of biofilm formation on the survival of *Acinetobacter baumannii* on dry surfaces. *The Journal of hospital infection* 80 (1): 56–60.
26. Choi, C. ; Lee, J. ; Lee, Y. ; Park, T. ; Lee, J. (2008). *Acinetobacter baumannii* invades epithelial cells and outer membrane protein A mediates interactions with epithelial cells. *BMC microbiology*. 8: 216.
27. Lipsky, B. ; Holroyd, K. and Zasloff, M. (2008). Topical versus systemic antimicrobial therapy for treating mildly infected diabetic foot ulcers: a randomized, controlled, double-blinded, multicenter trial of pexiganan cream. *Clin Infect Dis.* 47(12):1537-45.
28. Lio, P. and Kaye, E. (*2004*). Topical antibacterial agents. *Infect Dis Clin North Am.*18:717-33.
29. National Committee for Clinical Laboratory Standers (NCCLs). (2002). The Performance Standard for Antimicrobial Susceptibility Testing Method. (7th ed.). 22(1). USA.