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MOLECULAR DETECTION OF BACTERIA IN DIABETIC FOOT INFECTION : AN INVESTIGATION IN PATIENTS FROM AL-DEWANIYAH CITY, IRAQ

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Abstract

The microbial community on a host relies on its immune status and pathophysiological condition. *Diabetes mellitus* is a metabolic disorder associated with a 25% increased risk of developing foot infection. The pathophysiological differences between diabetic foot infection (DFI) may alter the microbial composition in infections. The present study aims to diagnosis of the microbes colonized in DFI in Adewaniyah city. Pus specimens were collected from 55 DFI patients to investigate the bacteria associated with foot infection. For this investigation, molecular biological approaches were performed. Common bacteria detected in both DFI were *Pseudomonas* spp. *Staphylococcus* spp. occurred only in DFI patients were also statistically significant. The present investigation implicated a complex association of the bacterial population in DFI.

Key words : Bacteria, diabetic foot, Al-Dewaniyah city, Iraq.

Introduction

Diabetes mellitus (DM) is now a major health problem all over the world and is increasing globally at an alarming rate (Tabish, 2007). It has been declared an epidemic in developing countries, including Iraq. Approximately 347 million people are suffering from *DM* worldwide, which is predicted to double by the year 2025 (Wild *et al*., 2004). The prevalence of diabetes in Iraq is increasing rapidly, leading to complications of chronic diabetes. Diabetic foot infection (DFI) is one of the most serious complications of *DM*. Diabetic patients have a 25% increased risk of developing a foot ulcer (Boulton, 2008). The primary causes of DFI are microbial agents, and their early diagnosis is essential for appropriate antimicrobial therapy (Lipsky, 1999). Once an infection has developed in DFI patients, it is difûcult to treat because of impaired microvascular circulation to the lower limb, which limits the access of phagocytic cells and antibiotics to infected sites (Lipsky *et al*., 2012). Common organisms reported in foot infection are mainly *Staphylococcus* spp. and *Enterococcus* spp. arising from the patient's own body (Armstrong and Lavery, 1998). Extensive tissue destruction and poor blood circulation are a result of infection with *Pseudomonas* spp., *Proteus* spp., and *Enterococcus* spp. bacterial groups (Armstrong and Lavery, 1998). The major predisposing factor associated with these infections is foot ulceration, which is usually related to peripheral neuropathy and peripheral vascular disease, and various immunological disturbances play a secondary role in the development of diabetic foot ulceration (Lipsky *et al*., 2004). The present study was performed to molecular diagnosis of the bacterial composition in foot lesions of DFI patients.

Materials and Methods

Morphological and biochemical isolate identification

Microscope observations and gram staining were performed on the isolates. The morphological characteristics (shape, size, edge, elevation, form and opacity) on NA plates were recorded. Biochemical tests were performed for Gram positive bacteria (Bergey *et al*., 1994).

Samples collection and characterization of Bacterial isolates

Fifty five swab samples of human *diabetic mellitus* were collected from patient of skin disease and processed for bacterial isolation by using standard methods, specimens were inoculated on blood and Mac Conkey agar plates. The streaked plates were incubated at 37 ºC for 24 hr. Bacterial colonies on blood agar plates were later Gram stained. Characterization of bacterial isolates was based on standard microbiological methods. Identification of isolates were done based on colony morphology, motility, catalase test, Urease test, Indole test (Bauer *et al*., 1966).

Bacterial genomic DNA extraction

Bacterial genomic DNA was extracted from *Staphylococcus* and *P. aeruginosa* isolates by using (Presto™ Mini gDNA Bacteria Kit. Geneaid. USA). One ml of overnight bacterial growth was added to 1.5 ml of BHI broth in microcentrifuge tubes and centrifuged at 10000 rpm for 1 minute. Then after the supernatant was discarded and bacterial cells pellets were used in genomic DNA extraction and the extraction was done according to company instruction. After that, the extracted gDNA was checked by Nanodrop spectrophotometer, then store at -20 ºC until performing PCR assay.

Polymerase chain reaction (PCR) (*Staphylococcus*)

PCR was performed for detection of staphylococcus bacterium based on amplification of 16SrRNA gene. The 16SrRNA gene primes were designed in this study using NCBI-GenBank deposited sequence *Staphylococcus* sp. partial 16S rRNA gene, isolate BA-141 (GenBank: HF947327.1) by using primer3 plus design online. The primers were used to amplify a 410bp using 16S rRNA-F primer (ATGGATCCGCGCCGTATTAG) and 16S rRNA-R primer (AATGAC CCTCCACGGTTGAG) were provided by (Macrogen company, Korea). Then PCR master mix was prepared by using (AccuPower® PCR PreMix kit. Bioneer. Korea). The PCR premix tube contains freeze-dried pellet of (Taq DNA polymerase 1U, dNTPs 250µM, Tris-HCl (pH 9.0) 10mM, KCl 30mM, $MgCl_2$ 1.5mM, stabilizer, and tracking dye) and the PCR master mix reaction was prepared according to kit instructions in 20µl total volume by added 2µl of purified genomic DNA and 1µl of 10pmole of forward primer and 1µl of 10pmole of reverse primer, then complete the PCR premix tube by deionizer PCR water into 20µl and briefly mixed by Exispin vortex centrifuge (Bioneer. Korea). The reaction was performed in a thermocycler (T100 Thermal cycler. Biorad/ USA) by set up the following thermocycler conditions; initial denaturation temperature of 95°C for 5 min; followed by 30 cycles at denaturation 95°C for 30 s, annealing 60°C for 30 s and extension 72°C for 1min and then final extension at 72°C for 10 min. The PCR products were examined by electrophoresis in a 1% agarose gel, stained with ethidium bromide and visualized under UV transilluminator.

Polymerase chain reaction (PCR) (*P. aeruginosa*)

PCR was performed for detection *P. aeruginosa* based on amplification of 16SrRNA gene. The 16SrRNA gene primes were designed in this study using NCBI-GenBank deposited sequence *P. aeruginosa* partial 16S rRNA gene, isolate PY11 (GenBank: AJ271411.1) by using primer3 plus design online. The primers were used to amplify a 410bp using 16S rRNA-F primer (TGCCTGGTAGTGGGGGATAA) and 16S rRNA-R primer (GGATGCAGTTCCCAGGTTGA) were provided by Macrogen Company, Korea. Then same steps where performed as those of *Staphylococcus* isolates.

Results

In the present study, specifically active members of the human *diabetic mellitus* microbiota were identified by comparing rDNA.

Detection by PCR

Preliminary results show that two types of bacteria are found in the armpit samples of humans are *Staphylococcus* and *P. aeruginosa*, which have been diagnosed according to the standard methods as well as PCR technique. The two isolates were confirmed through the interaction of polymerization series and using special primers in each type of germs as well as through the use of electrophoresis method for each sample.

Detection of *Staphylococcus* by PCR

PCR was performed for detection staphylococcus bacterium based on amplification of 16SrRNA gene using 16S rRNA-F primer (ATGGATCCGCGCCGTATTAG) and 16S rRNA-R primer (AATGAC CCTCCACGGTTGAG). As detected by agarose gel electrophoresis image that shown the PCR product of 16S rRNA gene in Staphylococcus sp., where M: Marker (1500-100bp), lane (1-5 and 7-9) positive PCR amplification at (410bp) PCR product size (fig. 1).

Detection of *P. aeruginosa* by PCR

PCR was performed for detection ***P. aeruginosa*** based on amplification of 16SrRNA gene using 16S rRNA-F primer (TGCCTGGTAGTGGGGGATAA) and 16S rRNA-R primer (GGATGCAGTTCCCAGGTTGA). As detected by

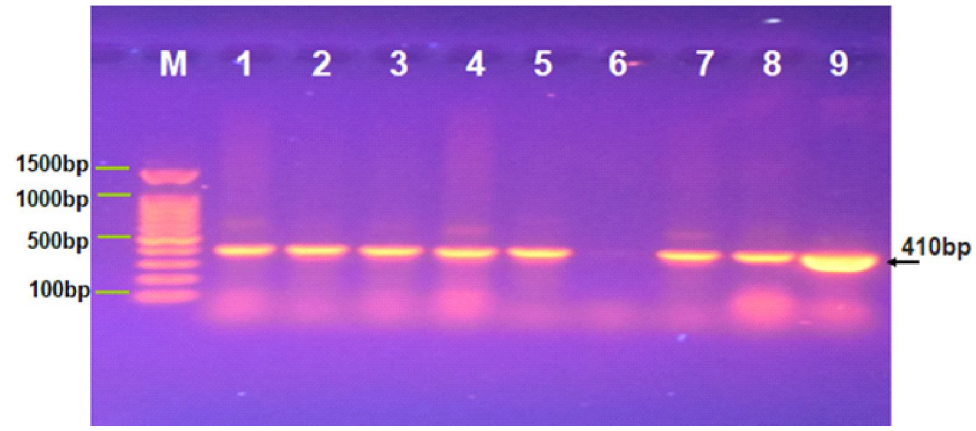

Fig. 1 : Agarose gel electrophoresis image shows the PCR product of 16S rRNA gene in *Staphylococcus* sp. isolates. Where, M : Marker (1500-100bp), lane (1-5 and 7-9) positive PCR amplification at (410bp) PCR product size.

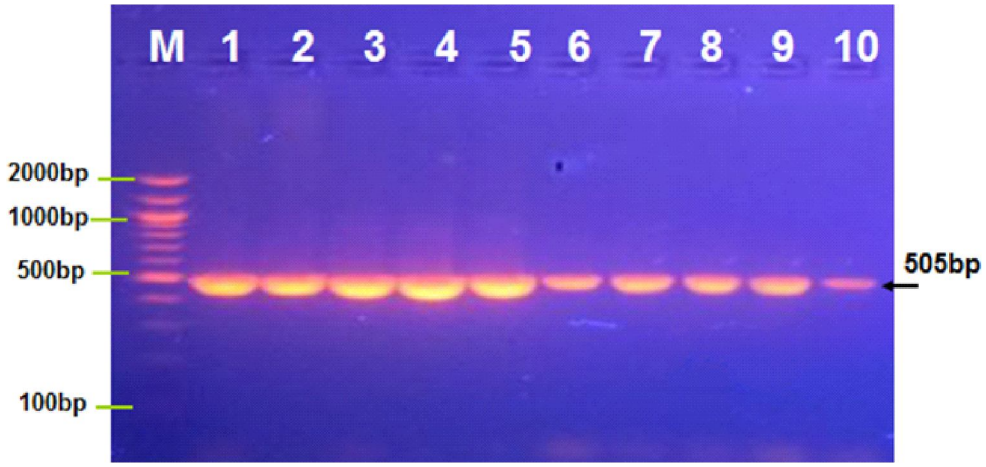

Fig. 2 : Agarose gel electrophoresis of PCR assay show positive amplification of 16S rRNA gene that used for specific detection of *P. aeruginosa* isolates. Where, Lane (M) DNA marker (2000-100bp), Lane (1-10) positive *P. aeruginosa* isolates at 505bp PCR product size.

agarose gel electrophoresis image that shown the PCR product of 16S rRNA gene in *P. aeruginosa*, where M: Marker (2000-100bp), lane (2-5 and 7) positive PCR amplification at 505bp PCR product size (fig. 2).

Discussion

The pathophysiological and metabolic differences in DFI patients indicates that there may be a difference in bacterial population compositions. To address this hypothesis, in this investigation, we analyzed bacteria in pus samples from 55 DFI patients. In our study, we determined that diabetic patients older than 40 years of age were more susceptible to foot infection. The average age of DFI-patients was 58 years and these patients were mostly male. Our study revealed that most of the DFI patients were from rural areas (lower middle income family), displayed culture-positive results; however, the bacterial population compositions were distinctly different in DFI patient pus samples patients. Among the isolates, *Pseudomonas* spp., and *Staphylococcus* spp. organisms were predominant in both patients. These results are compatible with the findings of previous studies (Shankar *et al*., 2005; Gadepalli *et al*., 2006). Our study demonstrated that most DFI patient infections were polymicrobial in nature were signiûcant in DFI patients we have diagnosis by PCR Technique and we fixed of this bacteria by this technique this result are high significant to two type of bacteria *Pseudomonas* spp., and *Staphylococcus* spp. However, common genera found in both DFI *Pseudomonas* spp., and *Staphylococcus* spp. (Murali *et al*., 2014; Ozer *et al*., 2010). These polymicrobial infections are responsible for chronic wounds and more complex infections. Through phenotypic and genotypic characterization, it was observed that there were ûve strains of *Pseudomonas* spp. obtained from DFI, including *P. aeruginosa* and *Staphylococcus* species isolates in DFI patients (Hefni *et al*., 2013). DFI patients have chronic non-healing ulcers due to several underlying factors such as poor glycemic control, peripheral neuropathy, poor blood supply

to the extremities and polymicrobial infections in their feet (Jeffcoate and Harding, 2003).

Conclusion

This investigation demonstrates a complex association of the bacterial population in DFI patients with different antimicrobial resistance patterns. An altered microbial composition was observed in DFI patients. This study is expected to generate valuable information, which will be helpful in the management and prevention of foot infections in our population and will help clinicians to select and develop appropriate drugs.

References

Armstrong, D. and L. A. Lavery (1998). Diabetic foot ulcers: prevention, diagnosis and classification. *Am Farm Physician*., **57** : 1325-32.

Bauer, A. W., W. M. Kirby, J. C. Sherris and M.Turck (1994). Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*., **45(4)** : 493-496.

Bergey, D. H. and John G. H. (1994). Bergey's Manual of Determinative Bacteriology. Baltimore: Williams and Wilkins; p. 9.

Boulton, A. J. (2008). The diabetic foot: grand overview, epidemiology and pathogenesis. *Diabetes/Metab Res Rev*., **24(S1)** : S3—6.

Gadepalli, R., B. Dhawan, V. Sreenivas, A. Kapil, A. Ammini and R. Chaudhry (2006). A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. *Diabetes Care*, **29(8)** : 1727-1732.

Hefni, A.A.-H., A.-M.R. Ibrahim, K. M. Attia, M. M. Moawad, A. F. El-Ramah, M. M. Shahin, M. Al-Molla and L. A. Al-Satar (2013). Bacteriological Study of Diabetic Foot Infection in Egypt.

Jeffcoate, W. J. and K. G. Harding (2003). Diabetic foot ulcers. *The Lancet*, **361(9368)** : 1545-1551.

Lipsky, B. A. (1999). Evidence-based antibiotic therapy of diabetic foot infections. *FEMS Immunol Med Microbiol*., **26(3-4)** : 267—76.

Lipsky, B. A., A. R. Berendt, H. G. Deery, J. M. Embil, W. S. Joseph, A. W. Karchmer, J. L. LeFrock, D. P. Lew, J. T. Mader and C. Norden (2004). Diagnosis and treatment of diabetic foot infections. *Clin Infect Dis*., **39(7)** : 885-910.

Lipsky, B. A., A. R. Berendt, P. B. Cornia, J. C. Pile, E. J. Peters, D. G. Armstrong, H. G. Deery, J. M. Embil, W. S. Joseph and A. W. Karchmer (2012). Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. *Clin Infect Dis*., **54(12)** : e132—73.

Murali, T. S., S. Kavitha, J. Spoorthi, D. V. Bhat, A. S. Bharath Prasad, Z. Upton, L. Ramachandra, R. V. Acharya and K. Satyamoorthy (2014). Characteristics of microbial drug resistance and its correlates in chronic diabetic foot ulcer infections. *J Med Microbiol*., http://dx.doi.org/10.1099/jmm.0.076034-0.

Ozer, B., A. Kalaci, E. Semerci, N. Duran, S. Davul and A. Yanat (2010). Infections and aerobic bacterial pathogens in diabetic foot. *Afr J Microbiol Res*., **4(20)** : 2153-2160.

Shankar, E., V. Mohan, G. Premalatha, R. Srinivasan and A. Usha (2005). Bacterial etiology of diabetic foot infections in South India. *Eur J Intern Med*., **16(8)** : 567-570.

Tabish, S. A. (2007). Is diabetes becoming the biggest epidemic of the twenty-first century? *Int J Health Sci*., **1(2)** : V.

Wild, S., G. Roglic, A. Green, R. Sicree and H. King (2004). Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*, **27(5)** : 1047—53.