Diabetic patients are more prone to the development of foot ulcers, because their underlying tissues are exposed to colonization by various pathogenic organisms. Hence, biofilm formation plays a vital role in disease progression by antibiotic resistance to the pathogen found in foot infections. The present study has demonstrated the correlation of biofilm assay with the clinical characteristics of diabetic foot infection. The clinical characteristics such as the ulcer duration, size, nature, and grade were associated with biofilm production. Our results suggest that as the size of the ulcer with poor glycemic control increased, the organism was more likely to be positive for biofilm formation. A high-degree of antibiotic resistance was exhibited by the biofilm-producing gram-positive isolates for erythromycin and gram-negative isolates for cefpodoxime. Comparisons of biofilm production using 3 different conventional methods were performed. The strong producers with the tube adherence method were able to produce biofilm using the cover slip assay method, and the weak producers in tube adherence method had difficulty in producing biofilm using the other 2 methods, indicating that the tube adherence method is the best method for assessing biofilm formation. The strong production of biofilm with the conventional method was further confirmed by scanning electron microscopy analysis, because bacteria attached as a distinct layer of biofilm. Thus, the high degree of antibiotic resistance was exhibited by biofilm producers compared with nonbiofilm producers. The tube adherence and cover slip assay were found to be the better method for biofilm evaluation.