Clinicians often treat clinically infected diabetic foot ulcers without information from cultures of the wound. The results of wound cultures may also be affected by previous antibiotic therapy. Thus, we aimed to study the microbial isolates, and antimicrobial sensitivity of previously treated patients with a clinically infected DFU. 293 consecutive patients with clinically infected DFU on prior antimicrobial treatment within the immediate past few days for a duration greater than one week were evaluated for microbial etiology, antibiotic sensitivity and final outcomes. Appropriate tissue samples i.e. purulent drainage, soft-tissue and/or bone were obtained for aerobic/anaerobic cultures and antimicrobial sensitivities. 71 patients with missing prior antibiotic data were excluded. 313 tissue samples obtained from 222 patients isolated 317 causative organisms. Most of the culture results from tissue specimens were mono-microbial (93.2%) compared to 37% in our previous cohort of 60 patients. Pseudomonas aeruginosa was the most common organism isolated on culture of bone (26.9%) or soft tissue (23.2%) specimen, respectively. Only 23% and 64% of P. aeruginosa isolates and 5.6% and 44% of Acinetobacter sp. were sensitive to quinolones and cephalosporins, respectively. Clinically infected DFU recently treated with antibiotics have predominant monomicrobial and multi drug-resistant infection. Quinolones as an empirical antibiotic choice may not be appropriate in this setting.