**TITLE:** DETECTION AND QUANTIFICATION OF *PSEUDOMONAS AERUGINOSA* FROM LEG CHRONIC WOUND CLINICAL SAMPLES BY REAL TIME PCR

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**ABSTRACT:**
Chronic wounds are a public health problem, since they represent a significant impact in patient's quality of life and great costs to the public system. *Pseudomonas aeruginosa* is an opportunistic pathogen commonly isolated from wounds. Since bacterial presence and load are factors that interfere with the cicatrization process and the following outcome. The development of a fast and sensitive method that allows those items quantification is important for differentiation between colonization and infection, affecting decision making and guidance of the treatment course. This study aimed to determine and quantify the presence of *P. aeruginosa* in eighty clinical samples from leg chronic wounds from patients treated in a university hospital in Rio de Janeiro state, and compare the results with the ones found through culture identification method. DNA extraction from all samples and ATCC strain was conducted with the use of the commercial Promega Kit; real time PCR was carried out with StepOne Real Time PCR System (Applied Biosystems) and utilizing SYBR Green PCR Master Mix. The standard curve was developed with the use of six known concentrations ($10^5$ – 1 copies of DNA/mL, of genomic DNA of *P. aeruginosa* ATCC 25783. After analyzing the melt curve, amplification plot and standard curve the detection limit observed was 10 copies/mL. From 80 samples tested, 30 had under 10 copies/mL, less than the established limit. The other 50 samples were considered positive for *P. aeruginosa.* Twenty-three were classified in the range of 10 - 100 copies, from which 10 samples were negative for culture growth. Twelve in the 100 - 1000, with two samples negative for culture growth. Nine in the 1.000 - 10.000, from which three samples were negative for culture growth. Five in the 10.000 - 100.000, with three samples that were negative for culture growth; and 1 above 100.000, that were also positive for culture growth. The results showed that the exclusive use of a culture identification method would very likely underestimate the bacterial load of the wound, thus possibly interfering with the treatment choice and healing process.

**Keywords:** chronic wound**,** *Pseudomonas aeruginosa*, real time PCR, molecular diagnosis

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