



COMPARISON OF PERIODONTOPATHOGENS AND DETECTION OF TETRACYCLINE RESISTANCE GENES BY PCR

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ABSTRACT

Periodontal disease is the result of an imbalance in the microbial ecology of the oral cavity, and it also depends on host susceptibility.[1] Studies have shown a close association between periodontitis and the presence of a specific group of microorganisms, mainly *Porphyromonasgingivalis*, *Aggregatibacter*, *Actinomycetemcomitans*, *Treponema denticola* and *Tannerella forsythia*. Thus, this study is to compare the periodontopathogens and to detect the tetracycline resistance genes by PCR among patients with different periodontal conditions. In our study, *T. forsythia* was the most frequently detected pathogen in 5/5 (100%) chronic periodontitis and 4/5 (80%) aggressive periodontitis patients. *A. actinomycetemcomitans* was the least detected pathogen as it detected only one sample of chronic periodontitis case. Upon subjected them to tetracycline resistance gene (TetM gene), all were negative. *T. forsythia* and *A. actinomycetemcomitans* were the most frequently and least detected periodontic pathogens respectively. Tetracycline resistance was not observed in our isolates. This study reveals the knowledge on the prevalence of periopathogens and tetracycline resistance gene in our region.

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INTRODUCTION

Periodontal disease is the result of an imbalance in the microbial ecology of the oral cavity, and it also depends on host susceptibility.[1] Studies have shown a close association between periodontitis and the presence of a specific group of microorganisms, mainly *Porphyromonasgingivalis*, *Aggregatibacter*, *Actinomycetemcomitans*, *Treponema denticola* and *Tannerella forsythia* [1,2]. Recent studies have reported periodontal pathogens resistant to different antibiotics, with percentages that vary according to the populations studied, this could be due to the lack of control over antibiotic use and to poor patient treatment compliance in certain countries [3]. Tetracycline and some of its derivatives have been widely used as adjuvants in the treatment of periodontitis, both topically and systemically. [4,5]. Thus, this study is to compare the periodontopathogens and to detect the tetracycline resistance genes by PCR among patients with different periodontal conditions.

MATERIALS AND METHODS

Five subgingival samples from each periodontic cases which includes chronic periodontitis and aggressive periodontitis as well as healthy population were subjected to 16s rDNA

analysis for *P. gingivalis*, *T. denticola*, *T. forsythia*, *A. actinomycetemcomitans*, *P. intermedia* and *Eikenellacorrodens* followed by the detection of tetracycline resistance genes (TetM) by PCR as per the following cyclic condition. Detection of the gene was carried out using primer as depicted in table 1. Bacterial DNA was extracted by boiling lysis method. 1 µL of DNA extract was used as template for PCR reaction. The reaction mixture contained 2mM of MgCl₂ 0.2mM dNTP mix and 0.5µM of can gene with IU of Taq polymerase (New England Biolabs) in a 1x PCR buffered reaction. A positive control of *S. aureus* with *cna* gene was also included in this study. PCR amplification was carried out using thermal cycler (Eppendorf) with the following cycling condition. Initial denaturation at 97°C for 1 min and 35 cycles for 30s, 54°C for 1min and 74°C for 1min, followed by a final extension of 10 min at 72°C. PCR products were resolved in 1.5% agarose gel. A 100bp ladder was including in all the gel analysis.[6]

Five subgingival samples from each periodontic cases which includes chronic periodontitis and aggressive periodontitis as well as healthy population were subjected to 16s rDNA analysis for *P. gingivalis*, *T. denticola*, *T. forsythia*, *A. actinomycetemcomitans*, *P. intermedia* and *Eikenellacorrodens* followed by the detection of tetracycline resistance genes (TetM) by PCR as described elsewhere. The amplicons were resolved in 4% agarose gel electrophoresis and findings were compared.

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Table 1 Gene sequencing of *bla_{NDM-1}* gene

Primer	Primer sequence	Product size
<i>tetM</i>	GCG TAC AAG CAC AGA CTC GT AGC CAT AGC GTA TCC CCT CC	1142bp

RESULTS

In our study, *T. forsythia* was the most frequently detected pathogen in 5/5 (100%) chronic periodontitis and 4/5 (80%) aggressive periodontitis patients. *A. actinomycetemcomitans* was the least detected pathogen as it detected only one sample of chronic periodontitis case. Upon subjected them to tetracycline resistance gene (*TetM* gene), all were negative.

DISCUSSION

In this cross-sectional study, we used PCR to determine the prevalence of nine periodontopathogens in the subgingival microbiota of patients diagnosed as healthy gingivitis, CP and AgP. Study conducted by Collins and coworkers in 2016 have demonstrated that, 63.6% of *P. micra*, 54.5% of *T. forsythia*, 45.4% *F. nucleatum* and *E. corrodens* and followed by other periopathogens isolated from healthy population. Whereas, in case of chronic periodontitis cases, *T. forsythia* (96.7%) scored the first predominant pathogen and in case of aggressive periodontitis cases, Both *F. nucleatum* and *T. denticola* were the most identified bacteria. [6]

In contrast to their study, we found *T. forsythia* was the most frequently detected pathogen in 5/5 (100%) chronic periodontitis and 4/5 (80%) aggressive periodontitis patients. *A. actinomycetemcomitans* was the least detected pathogen as it detected only one sample of chronic periodontitis case.

CONCLUSION

T. forsythia and *A. actinomycetemcomitans* were the most frequently and least detected periodontic pathogens respectively. Tetracycline resistance was not observed in our isolates. This study reveals the knowledge on the prevalence of periopathogens and tetracycline resistance gene in our region.

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