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Frequency and Antimicrobial Susceptibility Pattern of *Porphyromonas gingivalis* Isolated from Patients with Periodontitis

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ABSTRACT

Porphyromonas gingivalis is one of the most predominant organisms associated with periodontitis. It possesses multiple virulence factors that make it one of the most potent pathogen found in the oral cavity. **Objectives:** The aim of this study was to evaluate the occurrence of *P. gingivalis* in periodontitis patients and to explore its susceptibility against various antimicrobial drugs. **Method:** 100 cases of periodontitis were selected. Strains of *P. gingivalis* were isolated and identified by their morphology, biochemical features, hemagglutination and lack of fluorescence. Susceptibility pattern was evaluated by using E-test. **Results:** 44 strains of *P. gingivalis* were isolated from 100 cases. All the strains were sensitive to amoxicillin/clavulanic acid, clindamycin, erythromycin and metronidazole with MIC_{90} 0.19, 0.023, 0.50 and 0.047 respectively. Only 30% strains were sensitive to amoxicillin and tetracycline with MIC_{90} 32 and 64 respectively. MIC range for ciprofloxacin was 0.19-1.5 (MIC_{90} 1.00) while MIC range for doxycycline was 2-32 (MIC_{90} 32). **Conclusion:** *P. gingivalis* is a frequent pathogen of periodontitis. It is susceptible to most of the antimicrobial drugs but reistance is developing against few drugs such as amoxicillin and tetracycline.

Keywords: *Porphyromonas gingivalis;* Periodontitis; Occurrence; Antimicrobial drugs; Susceptibility; E-test.

INTRODUCTION

Periodontitis is one of the major challenges to the dentist as a large portion of population is inflicted with it. It is the disease of periodontium characterized by inflammation of the gums, resorption of the alveolar bone and degeneration of periodontal membrane. Being a progressive disease, moderate disease eventually affects the majority of the persons and advanced disease is seen nearly in half of the individuals above 65 years of age[1]. This prevalence rises to 93% in the Pakistani population of this age group[2].

The etiology of periodontitis is multi-factorial with an essential role of bacteria and their products. The complex flora involved in periodontal infections mainly comprises obligate and facultative anaerobes[3]. It was concluded by mutual consensus that three organisms are the most predominant etiological agents for periodontitis. *Porphyromonas gingivalis* and *Tannerella forsythia* are primarily associated with chronic periodontitis whereas *Actinobacillus actinomycetemcomitans* is more frequent in aggressive periodontitis[4,5]. *P. gingivalis* is a gram negative, black pigmented, non motile, non fermentative, anaerobic bacillus[6]. It possesses multiple virulence factors in the form of different proteases and powerful hemolytic enzymes that make it one of the most potent pathogens found in the oral cavity[7].

Despite the complex microbiological nature of periodontal diseases, these are curable and preventable entities with mechanical therapy and chemotherapy[8]. Chemotherapy involves

antimicrobial agents along with anti-inflammatory drugs. Antimicrobial agents are mainly indicated in aggressive, progressive, necrotizing or spreading periodontal lesions. The significance of antibiotics increases many times in immunocompromised persons and patients with systemic signs and symptoms of infection[9].

The guideline for the administration of antimicrobial drugs for any infectious disease is based on the susceptibility pattern of the drugs. During 1980, a number of studies were conducted by different groups of scientists to evaluate the susceptibility pattern of various pigmented oral pathogens. Gentamicin was found to be ineffective while penicillin, clindamycin, erythromycin, metronidazole, and tetracycline showed excellent inhibitory activity. The results for vancomycin, spiramycin, and chloramphenicol were of intermediate nature[10,11]. At that time no case was reported for β-lactamase production[12].

The overuse of selective drugs including some irrationality has led to the emergence of bacterial resistance[13]. van Winkelhoff et al.[14] and Herrera et al.[15] demonstrated the variability in the susceptibility pattern of periodontal pathogens and β-lactamase production in different populations. Clinical isolates of *P. gingivalis* are sensitive to most of the routinely used antibiotics. However the information available in this respect is considered not to be enough.

AIMS AND OBJECTIVES

The aim of this study was to evaluate the occurrence of *P. gingivalis* in periodontitis patients and to explore its susceptibility against various antimicrobial drugs.

MATERIALS AND METHODS

Study Sample

Total of 100 adult subjects were selected as purposive non probability sample from cases suffering from periodontitis. All the patients were evaluated for any co-morbidity, current medication, recent dental/periodontal treatment and history of chewing pan, betel nuts or smoking so that the effects of confounders can be eliminated. Patients with at least 4mm of periodontal attachment loss at more than three sites were considered for the study (adapted from Armitage[16] and Cruz et al.[17]) Two reference strains of *P. gingivalis* (ATCC 33277) and *Bacteroides fragilis* (ATCC 25285) were also included in every cycle of culture and susceptibility testing to monitor the consistency of the procedure.

Ethical Consideration and Sampling

Permission was taken from the Ethical Review Committee of the institute. An informed verbal and written consent was obtained from each patient for the examination of periodontal status and using their subgingival plaque sample for the study. All the teeth were included for the examination at six different sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual positions). PAL and PPD were measured in millimeters using William's periodontal probe. Three deepest periodontal pockets were selected (at least one pocket in each jaw). After removal of the supra gingival plaque with a sterile curette, tooth surfaces were dried and isolated with cotton rolls. Subgingival plaque samples were taken with individual sterile paper points of number 45. Paper points were inserted deep into the selected pocket and left in place for 15 seconds. These papers were immediately placed in anaerobic basal broth (Oxoid, United Kingdom).

Microbiological Techniques

The samples were processed within 4 hours and inoculated on selective as well as non-selective medium. Anaerobic Basal Agar (Oxoid, United Kingdom) was used as non-selective medium. 5% defibrinated horse blood was added to promote the growth of fastidious anaerobes. Same media with 5% defibrinated horse blood was made selective by adding GN-Anaerobic selective supplement (Oxoid, United Kingdom). After inoculation, media plates were incubated under anaerobic conditions for 5 to 7 days. The colonies from pure growth were identified up to species level by colonial morphology, Gram staining and Rapid ID 32A (bio Merieux, France). The identification of *P. gingivalis* was further confirmed and differentiated from other members of Porphyromonas genus on the basis of florescence[18] and hemagglutination[19]. In contrast to all other

species in the genus Porphyromonas, strains of *P. gingivalis* do not give florescence under UV light (365 nm) using Woodlight lamp (Crossmedico, Germany).

The guideline for the methodology of hemagglutination test was taken from a study conducted by Haraldsson et al.[20] 2% suspension of sheep RBCs and McFarland 3 suspension of bacterial cells were prepared in separate phosphate buffered saline (PBS). An equal volume of both suspensions were added to microtitre tubes with V-shaped bottom. Each tube was doubly diluted with PBS up to 0.125% concentration of RBCs. The tubes were incubated at 4ºC for 4 hours. Strains of *P. gingivalis* gave positive result at all the dilutions regarding hemagglutination.

Drug Susceptibility Testing

E-test (AB Biodisk, Sweden) was used to evaluate the susceptibility and minimum inhibitory concentration (MIC) in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines. Following antibiotics were used: amoxicillin, amoxicillin/clavulanic acid, tetracycline, doxycycline, ciprofloxacin, erythromycin, clindamycin and metronidazole. Bacterial suspension of McFarland turbidity 1 was prepared in anaerobic basal broth. Completely dry anaerobic basal agar plates containing 5% defibrinated horse blood were inoculated with this suspension. E-test strip was applied on the individual plates according to the instructions given in the manual. The inoculated plates along with E-test strips were then incubated under anaerobic conditions for 48 hours. MIC was recorded by the value on the strip where edge of the inhibition ellipse intersects the side of the strip.

Data Analysis

The data was entered and analyzed using SPSS 16.0. Mean±SD is given for normally distributed quantitative variables. Frequencies and percentages are given for quantitative variables. Two independent sample t-test and one way ANOVA were applied to observe group mean differences.

Pearson chi square and Fisher's exact test were applied to observe association between quantitative variables. A p value of less than 0.05 was considered significant.

RESULTS

Out of 100 cases of periodontitis, 44% were harboring *P. gingivalis* in their subgingival plaque sample. The MIC values (range, mean, MIC_{90}) of all the antibiotics are given in Table 1. MICs against the reference strains were also determined and mentioned in this table. The values for the reference strains exactly corresponded to the recommended values of CLSI.

The sensitivity results of amoxicillin and tetracycline against *P. gingivalis* were also compared with each other. Table 2 shows the significant relation between the susceptibility pattern of amoxicillin and tetracycline with a p value 0.028. 54% (7 out of 13) strains, amongst the penicillin sensitive group were also sensitive to tetracycline. Similarly, 69% (18 out of 26) strains, amongst amoxicillin resistant group were also resistant to tetracycline.

DISCUSSION

Porphyromonas gingivalis is the species that is strongly associated with destructive periodontal infections[22]. In the current study, 44% (n=44) of the cases were possessing *P. gingivalis* in their periodontal pockets. Different studies have shown diverse data for the prevalence of *P. gingivalis* in periodontitis[23,24] but all the scientists were unified at the point that *P. gingivalis* is more prevalent in periodontitis patients as compared to healthy persons with odds ratio reaching up to 12.3[22]. As the prevalence of severe forms of periodontitis shows racial differences[25], the difference in occurrence of *P. gingivalis* could be due to this factor.

All the strains were sensitive to amoxicillin/ clavulanic acid, clindamycin, erythromycin and metronidazole. These findings are comparable with the results of other studies conducted to date[26,27]. Mean MIC values for these antibiotics were far less than the cut off values for their susceptibility which indicates the highly susceptible nature of *P. gingivalis* against these four antibiotics. It was further supported by the narrow MIC ranges and

Table 1: Susceptibilities and MICs of antibiotics against *P. gingivalis* and reference strains

Antibiotics	Breakpoints against anaerobic bacteria (µg/ml)			MIC Range (µg/ml)	Mean MIC±SD (µg/ml)	MIC_{90}* (µg/ml)	Sensitive (%)	MIC (µg/ml) *P. gingivalis* ATCC 33277	MIC (µg/ml) *B. fragilis* ATCC 25285
	S	I	R						
Amoxicillin	≤4	8	≥16	1.00-48	15.79±12.042	32	29.54	0.50	48
Amoxicillin plus clavulanic acid	≤4	8	≥16	0.023-0.38	0.09±0.079	0.19	100	0.023	0.38
Tetracycline	≤4	8	≥16	1.5-64	24.24±21.189	64	29.54	0.125	0.38
Clindamycin	≤2	4	≥8	<0.016-0.032	0.02±0.005	0.023	100	0.016	1.5
Erythromycin	≤4	8	≥16	0.023-0.75	0.21±0.208	0.50	100	0.032	8
Metronidazole	≤8	16	≥32	<0.016-0.064	0.03±0.011	0.047	100	0.032	0.25
Ciprofloxacin	NA**	NA	NA	0.19-1.5	0.65±0.391	1	NA	0.19	3
Doxycycline	NA	NA	NA	2-32	15.61±11.229	32	NA	4	0.25

Breakpoints corresponds to susceptibility values recommended by CLSI [21].
* Minimum concentration required to kill 90% of the strains
** Not Available: Breakpoints are not given for these drugs against anaerobes [21].
S = sensitive, I = intermediate, R = resistant

Table 2: Comparison of sensitivity results of amoxicillin and tetracycline against *P. gingivalis*

			Tetracycline			Total
			Sensitive	Intermediate	Resistant	
Amoxicillin	Sensitive	Count	7	1	5	13
		% of Total	15.91	2.27	11.36	29.55
	Intermediate	Count	3	0	2	5
		% of Total	6.82	0.00	4.55	11.36
	Resistant	Count	3	5	18	26
		% of Total	6.82	11.36	40.91	59.09
	Total	Count	13	6	25	44
		% of Total	29.55	13.64	56.82	100%
Fisher's Exact Test= 9.612, p value= 0.028*						

*Significant p value<0.05

minimal differences between mean MIC and MIC_{90} for these antibiotics.

High level of resistance was noticed against amoxicillin and tetracycline. Although the findings of this study were in contrast to many studies such as Kulik et al.[26], Andres et al.[28] and Pajukanta et al.[29]. However, results were supported by the findings of other investigations[14,30]. Only 30% strains of *P. gingivalis* were sensitive to amoxicillin and tetracycline. Their mean MIC values and MIC_{90} were distinctly greater than the cut off values for their susceptibility. MIC ranges for these drugs were also very wide conforming the variability in their susceptibility pattern. On statistical analysis, a significant association was determined between the susceptibility results of *P. gingivalis* against amoxicillin and tetracycline. It suggests the same mechanism involved in the emergence of resistance against these drugs. Genes for resistance against amoxicillin and tetracycline are primarily located on

the plasmids[31]. The process of conjugation, which would provide an effective way to transfer resistance determinants, has been recently noticed in *P. gingivalis*[32]. This transfer of plasmid and chromosomal DNA through conjugation is assumed to be the cause of resistance.

Breakpoints for the susceptibilities against anaerobic bacteria for the remaining two drugs; ciprofloxacin and doxycycline are not available in the CLSI manual[21]. Therefore, it was impossible to interpret the susceptibility pattern against these drugs. The determined values of mean MIC and MIC_{90} are comparable to the findings of other investigations[28]. The narrow MIC range of ciprofloxacin along with lesser mean MIC and MIC_{90} values suggest a uniform susceptibility pattern against *P. gingivalis*. On the other hand, the relatively wider MIC range for doxycycline along with larger mean MIC and MIC_{90} values hint towards a variable susceptibility pattern against *P. gingivalis.*

Since no published investigation is available, even of the baseline data for periodontal microbiological characteristics in Pakistan, this study was justified. It is suggested that more such studies should be conducted involving the larger sample size with more periodontal pathogens and antimicrobial agents to make the antimicrobial therapy more rationale.

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