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Original Article

A STUDY ON BACTERIOLOGICAL IDENTIFICATION OF SUB-GINGIVAL PLAQUES IN PERIODONTITIS PATIENTS

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ABSTRACT

Objective: The current study's objectives were to isolate and characterize the periodontal pathogens from subgingival plaque obtained from patients with chronic periodontitis using conventional microbiological techniques.

Materials: In the Department of Microbiology at a Tertiary Care Teaching Hospital was where this study was carried out. Over the course of six months, from January to June 2019, samples were taken from subgingival pockets in patients with chronic periodontitis who visited the periodontology outpatient department at our institute of dental sciences. There were 21 cases in the research. For the purpose of preventing saliva contamination, tooth surfaces were dried using sterile gauze. Subgingival plaque samples were taken from the majority of diseased sites using a sterile periodontal Gracy curette, put in a test tube with fluid thioglycollate medium, and taken to the microbiology lab where they were processed right away using conventional microbiological procedures.

Results: E. coli grew in the majority of samples (9), followed by Pseudomonas species and Staphylococci in 7 samples. Only sample number 7 out of 21 showed no growth for Candida albicans; the others all exhibited development. Samples like Sample 1, 5, 6, 8, 10, 11, 12, 13, 15, 16, 17, 18, 19, and 20 produced isolates that were multidrug resistant.

Conclusion: Therefore, while developing a treatment plan for adult patients with periodontitis, the microbial diversity discovered in the current study should be taken into account. Variations in the quantities and species of cultivable bacteria have been found in investigations of periodontitis patients from different geographic regions, including developed and developing countries.

Keywords: Periodontitis, Yeast, Aerobes, AST, Culture

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INTRODUCTION

Periodontitis is an oral cavity disease that is characterized by ongoing inflammation of the periodontal tissues. Long-term accumulation of a lot of tooth plaque is what causes the sickness [1]. In order to prevent severe and irreparable damage to the tooth's protective and stable structures, persistent periodontitis must be diagnosed early on [2]. However, since chronic periodontitis is a disease that advances easily, very few people would seek dental care in the early stages. Periodontitis that is mild to moderately advanced can be managed with the proper mechanical removal of the biofilm and subgingival analytics. Regular monthly periodontal checkups and thorough dental hygiene are essential for controlling the severity of the disease. Given their [3] high prevalence in both the adult and paediatric populations, oral depression contaminations are a serious general medical concern generally. There are more than one billion cases of serious periodontal diseases worldwide, which are estimated to affect 14% of adults [4, 5]. The main causes of periodontal disease are poor dental hygiene and tobacco smoking [6]. Periodontitis was generally reported to start after the age of 15, and by the time they were 17 y old, 10% of Indian young men were affected [7]. To determine the prevalence of periodontal disease in the Konda Reddy clans residing in Bhadrachalam, Khammam District, a fresh report was initiated. Additionally, it was discovered that the majority of them (93.60%) used twigs to brush their teeth, while only 6.20% used a combination of a toothbrush, finger, and twig together with toothpaste and charcoal [8]. The oral cavity is home to several different types of facultative bacteria Streptococcus species, as well as Granulicatella, Gemella, and Veillonella, while most microscopic organisms are localised to certain areas [9]. The biological locality of commensal, cooperative, and harmful bacteria present in the oral cavity is known as the human oral microbiota. The majority of oral microbiota occurs as a biofilm, and it plays a crucial role in maintaining oral homeostasis, protecting the mouth cavity, and preventing the spread of infection. The present study aimed to isolate and identify the periodontal pathogens using standard microbiological techniques from subgingival plaque collected from patients suffering with Chronic Periodontitis.

MATERIALS AND METHODS

This study was conducted in the Department of Microbiology in a Tertiary Care Teaching Hospital. Samples were collected from subgingival pockets in patients with chronic periodontitis attending the Periodontology Outpatient Department at our Institute of Dental Sciences, over a period of 6 mo from January to June 2019. The study comprised of 21 cases. Clinically diagnosed cases of chronic periodontitis were included in the study and patients with history of systemic conditions such as diabetes mellitus, nutritional deficiencies, pregnant woman, antibiotic usage in the last 2 mo and patients with a history of undergoing any dental procedures in the last 2 mo were excluded.

Sample collection

Tooth surfaces were dried with sterile gauze to avoid contamination by saliva. Subgingival plaque sample was collected from most pathological site using sterile periodontal Gracy curette and placed in fluid thioglycollate medium in a test tube and brought to the microbiology laboratory and processed immediately.

Laboratory methods

In the lab, a fluid thioglycollate medium sample was added to 1 ml of trypticase soy broth and vortexed for 1 minute. Direct smears of the samples were made, and modified Fontana staining and Hucker's modification of the Gram-stain for anaerobes was used. A standard loop was used to take a loopful of the sample from the vortexed solution and inoculate it onto the plates of nutrition agar, Mac Conkey agar (MA), and brain heart infusion agar. As per the

guidelines outlined in Mackie and McCartney's Practical Medical Microbiology, all aerobic isolates were recognized and biochemically characterized. The antimicrobial susceptibility testing was done for aerobic isolates by disc diffusion method as described by Kirby and Bauer on Mueller-Hinton agar. The diffusion of the antimicrobial agent into the seeded culture media results in a gradient of the antimicrobial. AST was performed according to CLSI guidelines using Mueller-Hinton agar (MHA) plates using the concentration of antibiotics per well, recommended by the WHO experts committee on biological standardization. The plates were incubated at 37 °C for 16-18h h.

RESULTS

Majority of the samples 9 showed the growth of E. coli followed by Pseudomonas species and Staphylococci in a total of 7 samples. Only one sample showed the growth of Proteus and one sample with no growth (table 1). All the samples showed growth for *Candida albicans* except sample number 7 out of 21 samples showed no growth. The growth was seen in the MCA plates (table 2) for culture confirmation. The results of the antibiotic susceptibility pattern of isolated pathogens were depicted in table 3. The samples like Sample 1, 5, 6,8,10,11,12,13,15,16,17,18,19 and 20 yielded multidrug-resistant isolates.

Table 1: Culture identification from sub-gingival samples

Patient sample	Colony morphology	Organism isolated
Sample 1	Golden yellow colonies on nutrient agar having round, convex, opaque, and smooth-glistening surface with a diameter of about 2 mm	Staphylococci species
Sample 2	Smooth, cream or white colonies with entire edges	Enterococci species
Sample 3	Appear large, circular, low convex, grayish, white, moist, smooth, and opaque	E. coli
Sample 4	Golden yellow colonies on nutrient agar having round, convex, opaque, and smooth-glistening surface with a diameter of about 2 mm	Staphylococci species
Sample 5	Smooth, cream or white colonies with entire edges	Enterococci species
Sample 6	Golden yellow colonies on nutrient agar having round, convex, opaque, and smooth-glistening surface with a diameter of about 2 mm	Staphylococci species
Sample 7	No Growth	-
Sample 8	Appear large, circular, low convex, grayish, white, moist, smooth, and opaque	E. coli
Sample 9	Flat and smooth colonies that are between 2	Pseudomonas species
Sample 10	Golden yellow colonies on nutrient agar having round, convex, opaque, and smooth-glistening surface with a diameter of about 2 mm	Staphylococci species
Sample 11	Golden yellow colonies on nutrient agar having round, convex, opaque, and smooth-glistening surface with a diameter of about 2 mm	Staphylococci species
Sample 12	Flat and smooth colonies that are between 2	Pseudomonas species
Sample 13	Flat and smooth colonies that are between 2	Pseudomonas species
Sample 14	Golden yellow colonies on nutrient agar having round, convex, opaque, and smooth-glistening surface with a diameter of about 2 mm	Staphylococci species
Sample 15	Appear large, circular, low convex, grayish, white, moist, smooth, and opaque	E. coli
Sample 16	Appear large, circular, low convex, grayish, white, moist, smooth, and opaque	E. coli
Sample 17	Appear large, circular, low convex, grayish, white, moist, smooth, and opaque	E. coli
Sample 18	Appear large, circular, low convex, grayish, white, moist, smooth, and opaque	E. coli
Sample 19	Smooth, cream or white colonies with entire edges	Enterococci species
Sample 20	Golden yellow colonies on nutrient agar having round, convex, opaque, and smooth-glistening surface with a diameter of about 2 mm	Staphylococci species
Sample 21	Appear large, circular, low convex, grayish, white, moist, smooth, and opaque	E. coli

Table 2: Growth on MacConkey agar

Patient sample	Colonies	Organisms isolated
Sample 1	Pink colour colonies-Lactose Fermenter	E. coli
Sample 2	Pink colour colonies-Lactose Fermenter	E. coli
Sample 3	Pink colour colonies-Lactose Fermenter	Klebsiella species
Sample 4	Pink colour colonies-Lactose Fermenter	E. coli
Sample 5	Lactose Fermenter	E. coli
Sample 6	Colourless colonies, Non-Lactose Fermenter	Pseudomonas species
Sample 7	No Colonies	No Growth
Sample 8	Pink colour colonies-Lactose Fermenter	E. coli
Sample 9	Pink colour colonies-Lactose Fermenter	Klebsiella species
Sample 10	Pink colour colonies-Lactose Fermenter	E. coli
Sample 11	Colourless colonies, Non-Lactose Fermenter	Pseudomonas species
Sample 12	Colourless colonies, Non-Lactose Fermenter	Pseudomonas species
Sample 13	Colourless colonies, Non-Lactose Fermenter	Proteus species
Sample 14	Colourless colonies, Non-Lactose Fermenter	Pseudomonas species
Sample 15	Pink colour colonies-Lactose Fermenter	E. coli
Sample 16	Pink colour colonies-Lactose Fermenter	Klebsiella species
Sample 17	Pink colour colonies-Lactose Fermenter	E. coli
Sample 18	Colourless colonies, Non-Lactose Fermenter	Pseudomonas species
Sample 19	Colourless colonies, Non-Lactose Fermenter	Pseudomonas species
Sample 20	Pink colour colonies-Lactose Fermenter	E. coli
Sample 21	Colourless colonies, Non-Lactose Fermenter	Pseudomonas species

Table 3: Antibiotic susceptibility of periodontal pathogens

Sample	Antibiotic symbol	Antibiotic	Zone of inhibition	Senstive/Resistant S/R
Sample 1	C	Chloramphenicol	No zone	R
	TEI	Teicoplanin	No zone	R
	LZ	Linezolid	No zone	R
	NIT	Nitrofurantoin	No zone	R
	CPM	Cefepime	No zone	R
Sample 2	HLG	Gentamicin	30 mm	S
	AK	Amikacin	25 mm	S
	IMP	Imipenam	30 mm	S
	COT	Co-Trimoxazole	30 mm	S
	AMP	Ampicillin	20 mm	S
	MRP	Meropenem	20 mm	S
	AMC	Amoxicillin	30 mm	S
Sample 3	AMC	Amoxicillin	25 mm	S
	AMP	Ampicillin	20 mm	S
	IMP	Imipenam	30 mm	S
	AK	Amikacin	25 mm	S
	MRP	Meropenem	25 mm	S
	HLG	Gentamicin	25 mm	S
	COT	Co-Trimoxazole	15 mm	S
Sample 4	AK	Amikacin	20 mm	S
	HLG	Gentamicin	25 mm	S
	COT	Co-Trimoxazole	25 mm	S
	AMP	Ampicillin	25 mm	S
	MRP	Meropenem	25 mm	S
	IMP	Imipenam	30 mm	S
	AMC	Ampicillin	20 mm	S
Sample 5	IMP	Imipenam	25 mm	S
	MRP	Meropenem	15 mm	R
	COT	Co-Trimoxazole	10 mm	R
	HLG	Gentamicin	20 mm	S
	AMC	Amoxicillin	15 mm	R
	AMP	Ampicillin	10 mm	R
	AK	Amikacin	25 mm	S
Sample 6	C	Chlorophenicol	20 mm	S
	TEI	Teicoplanin	15 mm	R
	LZ	Linezolid	30 mm	S
	NIT	Nitrofurantoin	15 mm	R
	CPM	Cefepime	5 mm	R
Sample 8	AMP	Ampicillin	05 mm	R
	AMC	Amoxicillin	10 mm	R
	AK	Amikacin	15 mm	S
	MRP	Meropenem	10 mm	R
	HLG	Gentamicin	20 mm	S
	COT	Co-Trimoxazole	15 mm	S
	IMP	Imipenam	25 mm	S
Sample 9	AMP	Ampicillin	22 mm	S
	AMC	Amoxicillin	26 mm	S
	AK	Amikacin	15 mm	S
	HLG	Gentamicin	20 mm	S
	COT	Co-Trimoxazole	25 mm	S
	IMP	Imipenam	25 mm	S
	MRP	Meropenem	23 mm	S
Sample 10	AMP	Ampicillin	10 mm	R
	AMC	Amoxicillin	14 mm	R
	MRP	Meropenem	10 mm	R
	AK	Amikacin	15 mm	S
	HLG	Gentamicin0	28 mm	S
	COT	Co-Trimoxazole	25 mm	S
	IMP	Imipenam	25 mm	S
Sample 11	AMP	Ampicillin	08 mm	R
	AMC	Amoxicillin	10 mm	R
	MRP	Meropenem	10 mm	R
	AK	Amikacin	25 mm	S
	HLG	Gentamicin	24 mm	S
	COT	Co-Trimoxazole	15 mm	R
	IMP	Imipenam	30 mm	S
Sample 12	AMP	Ampicillin	05 mm	R
	AMC	Amoxicillin	08 mm	R
	MRP	Meropenem	08 mm	R
	AK	Amikacin	10 mm	R
	HLG	Gentamicin	15 mm	R

Sample	Antibiotic symbol	Antibiotic	Zone of inhibition	Senstive/Resistant S/R
	COT	Co-Trimoxazole	05 mm	R
	IMP	Imipenam	30 mm	S
Sample 13	AMP	Ampicillin	10 mm	R
	AMC	Amoxicillin	20 mm	S
	AK	Amikacin	10 mm	R
	MRP	Meropenem	10 mm	R
	HLG	Gentamicin	26 mm	S
	COT	Co-Trimoxazole	08 mm	R
	IMP	Imipenam	25 mm	S
Sample 14	AMP	Ampicillin	15 mm	R
	AMC	Amoxicillin	27 mm	S
	MRP	Meropenem	20 mm	S
	AK	Amikacin	20 mm	S
	HLG	Gentamicin	25 mm	S
	COT	Co-Trimoxazole	25 mm	S
	IMP	Imipenam	25 mm	S
Sample 15	AMP	Ampicillin	13 mm	R
	AMC	Amoxicillin	25 mm	S
	MRP	Meropenem	20 mm	R
	AK	Amikacin	24 mm	S
	HLG	Gentamicin	18 mm	S
	COT	Co-Trimoxazole	15 mm	R
	IMP	Imipenam	30 mm	S
Sample 16	AMP	Ampicillin	15 mm	R
	AMC	Amoxicillin	20 mm	S
	MRP	Meropenem	18 mm	R
	AK	Amikacin	17 mm	R
	HLG	Gentamicin	13 mm	R
	COT	Co-Trimoxazole	19 mm	R
	IMP	Imipenam	30 mm	S
Sample 17	AMP	Ampicillin	10 mm	R
	AMC	Amoxicillin	10 mm	R
	MRP	Meropenem	18 mm	R
	AK	Amikacin	18 mm	R
	HLG	Gentamicin	30 mm	S
	COT	Co-Trimoxazole	20 mm	S
	IMP	Imipenam	27 mm	S
Sample 18	AMP	Ampicillin	08 mm	R
	AMC	Amoxicillin	18 mm	R
	MRP	Meropenem	10 mm	R
	AK	Amikacin	15 mm	R
	HLG	Gentamicin	10 mm	R
	COT	Co-Trimoxazole	15 mm	R
	IMP	Imipenam	25 mm	S
Sample 19	AMP	Ampicillin	08 mm	R
	AMC	Amoxicillin	10 mm	R
	MRP	Meropenem	10 mm	R
	AK	Amikacin	15 mm	R
	HLG	Gentamicin	20 mm	S
	COT	Co-Trimoxazole	10 mm	R
	IMP	Imipenam	15 mm	R
Sample 20	AMP	Ampicillin	08 mm	R
	AMC	Amoxicillin	17 mm	R
	MRP	Meropenem	15 mm	R
	AK	Amikacin	15 mm	R
	HLG	Gentamicin	30 mm	S
	COT	Co-Trimoxazole	08 mm	R
	IMP	Imipenam	17 mm	R
Sample 21	AMP	Ampicillin	25 mm	S
	AMC	Amoxicillin	10 mm	R
	MRP	Meropenem	25 mm	S
	AK	Amikacin	20 mm	S
	HLG	Gentamicin	25 mm	S
	COT	Co-Trimoxazole	10 mm	R
	IMP	Imipenam	24 mm	S

DISCUSSION

Chronic periodontitis is an uncontrollable infection that irritates the mouth and destroys the bond between the gums and teeth's supporting tissue. Around 14% of people globally are estimated to be affected by severe chronic periodontitis. Less frequent dental visits and cigarette use are the two main causes of chronic periodontitis. In the entire world, 51% of people had periodontal disease, and 46.6% had gum disease. 26.2% of people had direct periodontitis, whereas 19% had severe periodontitis. Males predominate more than females. Heteogeneity is another possibility. In India, 17.3% of the population suffers from chronic periodontitis.

Periodontitis' aetiology has been linked to a number of bacterial species or groups of species [10-12]. A particular bacterial strain is linked to adult periodontitis, and several complexes have been identified in subgingival plaque samples [13-15]. Adult patients with periodontal disease were examined clinically, epidemiologically, and microbiologically by Daniluk *et al.* [16], who discovered that no Candida yeasts were recovered from these individuals. Aerobic and anaerobic bacteria cultures from subgingival and supragingival plaque samples were found to be present in 19 out of 21 patients. Forty-two bacterial strains in all, 24 (57.1%) of which belonged to 7 anaerobic species and 18 (42.9%) to 12 aerobic species, were recovered from subgingival plaques. Streptococci and Staphylococci made up the majority of the isolated aerobic organisms. However, E. coli, Staphylococci, and Pseudomonas were the most frequently isolated species in the current investigation. Candida growth was seen in the majority of the samples. C. albicans, however, may play a part in the formation of periodontal microbial plaque and its adherence to the periodontal tissues, according to Jarvensivu *et al.* [17]. Additionally, according to these authors' findings, fungal components could not be seen in the epithelium, whereas hyphal germination begins in the gingival pocket [17]. Pseudomonas aeruginosa and periodontal pathogens in the oral cavity and lungs of cystic fibrosis patients were examined by Caldas *et al.* in 2015. In this study, 16 P. aeruginosa strains were identified [18]. The results presented in the study were somewhat correlated from the previous studies and most of the predominant organisms isolated were Pseudomonas, Staphylococci and E. coli.

CONCLUSION

In conclusion, the microbial diversity discovered in the present study should be taken into account in the periodontitis treatment plan for adult patients. Variations in the quantities and species of cultivable bacteria have been found in investigations of periodontitis patients from different geographic regions, including developed and developing countries. For oral infections, there are many different microbiological diagnostic techniques available, and the treatment of these infections depends on the relationship between the doctor and the microbiologist.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Declared none

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