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STUDY OF MICROBIAL FLORA IN ORODENTAL INFECTIONS

Microbiology

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ABSTRACT

- Orodental infections are preceded by the formation of microbial plaque which has complex nature in terms of microbial inhabitants.
- Our aim is to isolate the organisms causing these infections and to determine their antibiograms.

KEYWORDS

Orodental Infections, Antibiogram

INTRODUCTION:

- Orodental infections mainly include dental caries, gingivitis and periodontitis.
- Dental caries is an infectious microbial disease that results in localized dissolution and destruction of the calcified tissues of the teeth.
- Gingivitis is inflammation of gingiva.
- Periodontitis is gingivitis associated with bone loss and formation of periodontal pockets.

The present study was undertaken as there were no studies in our area.

AIMS AND OBJECTIVES:

1. To isolate and identify bacteria from dental caries, gingivitis and periodontitis.
2. To compare the isolates in study and control group.
3. To determine the antibiotic susceptibility patterns.

MATERIALS AND METHODS:

STUDY GROUP:

A prospective study done in 100 samples after taking approval from Institutional Ethical Committee.

25→ Dental caries
25→ Gingivitis
25→ Periodontitis
25→ Control group

STUDY PERIOD: From December 2016 to August 2018.

SELECTION CRITERIA FOR THE CONTROL GROUP:

- absence of any orodental infection
- absence of oral manifestations of any systemic disease.

SAMPLE COLLECTION:

The carious material is taken from the caries, for gingivitis-sterile absorbent paper points are placed in the base of the pocket, for periodontitis-crevicular fluid is collected.

SAMPLE PROCESSING:

- Three samples were collected from each case :
- direct smear,
- inoculation into BHI broth
- inoculation into RCM broth.

- After 24 hours of incubation in BHI broth, subculture was made on MaC Conkey and blood agar plates, and the organisms were identified by standard biochemical reactions.
- After 48 hours of incubation in RCM broth, subculture was made from the meat particles on 5% sheep blood agar supplemented with vitamin K and haemin; and incubated in anaerobic jar with Gaspak and Pseudomonas as biological control.
- Antibiotic susceptibity was done for both aerobic and anaerobic isolates.

- Foul odour upon opening jar gives a clue for the presence of anaerobes in the culture after incubation
- The anaerobic blood agar plate was streaked to ensure even lawn of heavy growth. Antibiotic discs Kanamycin (K) 1 mg, Vancomycin (V) 5µg, Colistin (Cl) 10µg were placed on the blood agar plate and incubated anaerobically. These discs aid in identification of anaerobes.
- Metronidazole (M) 5µg and Clindamycin(Cd) 2µg discs were also placed to know the susceptibility.
- The same colony subcultured on the anaerobic plate was used for aerotolerance testing on blood agar and Gram staining.

- **IDENTIFICATION OF ISOLATES FROM ANAEROBIC CULTURES:**

TABLE 1:

Organism	Gram stain	Culture on BA	Pigmentation	V	K	Cl
Peptostreptococcus sp.	GPC-pairs,chains	Convex, shiny colonies with circular edges	-	S	R	R
Bacteroides sp.	GN Pleomorphic	Smooth, white to gray colonies	-	R	R	R
Porphyromonas sp.	GN coccobacilli	Shiny, smooth colonies	Brown	S	R	R
Prevotella sp.	GN coccobacilli	Dry, round colonies	Black	R	R	S
Fusobacterium sp.	GNB straight or slightly curved with pointed ends	Large, smooth white to gray colonies	-	R	R	S

STREPTOCOCCUS MUTANS:

- **Culture on Blood Agar:** Small, gray, alpha hemolytic colonies are seen
- **Gram Stain** : Gram positive cocci in pairs and chains are seen.
- **Biochemical reactions :** Optochin – Resistant
  VP – Positive
  Arginine – Negative
  Urease – Negative
  Beta galactosidase – Positive

RESULTS:

SEX AND AGE WISE DISTRIBUTION:

TABLE 2 & 3 :

Gender	Caries	Gingivitis	Periodontitis
Male	8	10	11
Female	17	15	14

Age group(Years)	Caries	Gingivitis	Periodontitis
21-30	5	7	2

31-40	9	8	3
41-50	8	7	7
51-60	3	3	13

Case wise distribution of isolates in the study group(n=120)

Table 4:

Case	Anaerobic isolates	Aerobic isolates	Total
Caries	20	20	40
Gingivitis	22	20	42
Periodontitis	26	12	38
Total	68(56.6%)	52(43.3%)	120

Nature of infection:

Table 5:

Nature of infection	Number
Monomicrobial	28
Polymicrobial	42

- Out of the 42, two were isolated from 34 cases and three were isolated from 8 cases.
- The most common combination was Peptostreptococcus and Porphyromonas.

TOTAL ANAEROBIC ISOLATES FROM THE STUDY GROUP (n=68)

Table 6:

Isolate	Caries	Gingivitis	Periodontitis	Total
Peptostreptococcus sp.	4	8	5	17(25%)
Veillonella sp.	5	6	3	14(20.5%)
Lactobacillus sp.	10	2	1	13(15.8%)
Bacteroides sp.	0	2	1	3(3.6%)
Porphyromonas sp.	0	2	8	10(12.1%)
Prevotella sp.	0	0	2	2(2.43%)
Fusobacterium sp.	1	2	6	9(13.2%)

TOTAL AEROBIC ISOLATES FROM THE STUDY GROUP (n=52)

Table 7:

Isolate	Caries	Gingivitis	Periodontitis	Total
S.mutans	11	5	1	17(29.3%)
S.aureus	2	6	3	11(17.4%)
CONS	2	3	3	8(13.7%)
Klebsiella	3	5	4	12(20.6%)
Pseudomonas sp.	2	--	--	2(3.44%)
Escherichia coli.	--	1	1	2(3.44%)

COMPARISON OF ANAEROBIC ISOLATES IN THE STUDY(n=68) AND CONTROL GROUP (n=25) :

Table 8:

Isolate	Study group	Control group
Peptostreptococcus sp.	17	7
Veillonella sp.	14	3
Lactobacillus sp.	13	0
Bacteroides sp.	3	0
Porphyromonas sp.	10	0
Prevotella sp.	2	0
Fusobacterium sp.	9	1

In both the study group and control group, the most common isolate was Peptostreptococcus sp.

COMPARISON OF AEROBIC ISOLATES IN THE STUDY (n=52) AND CONTROL GROUP (n=11):

Table 9:

Isolate	Study group	Control group
S.mutans	17	6
S.aureus	11	0
CONS	8	4
Klebsiella sp.	12	3
Pseudomonas sp.	2	1
Eschericia coli	2	0

In both the study and control group, the most common isolate was S.mutans.

Percentage of susceptibility of the bacilli isolated:

Table 10:

Organism	Ipm	Pit	M	Ctr	Ak	Amc	Cot
Lactobacillus sp.	84.7	46.2	46.2	23.1	84.7	84.7	0
Bacteroides sp.	100	66.6	33.3	66.6	33.3	33.3	0
Porphyromonas sp.	60	50	50	40	30	40	20
Prevotella sp.	50	0	0	0	0	0	0
Fusobacterium sp.	88.8	77.7	55.5	55.5	33.3	44.4	22.2
Klebsiella sp.	91.6	66.6	-	83.3	75	58.3	16.6
Pseudomonas sp.	100	50	-	100	50	0	0
E.coli	100	100	-	50	0	0	0

Percentage of susceptibility of the cocci isolated:

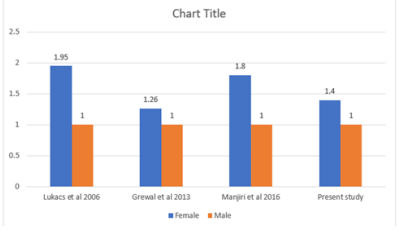
Table 11:

Organism	M	E	P	Cip	Cot	Cd	Amc
Peptostreptococcus sp.	86.2	0	0	47.5	41.1	35.2	64.7
Veillonella sp.	100	0	0	7.1	0	0	14.2
S. aureus	--	81.8	27.7	36.3	45.4	27.7	75
S.mutans	--	70.5	11.7	58.8	47	58.8	64.7
CONS	--	75	75	25	37.5	75	37.5

DISCUSSION:

Graph 1:

Female to male ratio in comparison with other studies:



- Females outnumbered males in all the three infections.

Polymicrobial nature of the orodental infections has been brought out in this study.

There were a total of 145 isolates from 100 samples. This correlated with the study of Saini et.al – 138 isolates from 100 samples.

- The ratio of aerobes to anaerobes decreased as the lesion progressed superficial to deep.

Table 12:

Study	Caries	Gingivitis	Periodontitis
Present study	1	0.90	0.46
Saini et.al	0.90	0.72	0.56

Comparison of isolates with the other studies:

Table 13:

Anaerobes:

Organism	Present study Caries	Present study Gingivitis	Present study Periodontitis	M.Sharma et.al 2011 Caries	M.Sharma et.al 2011 Gingivitis	M.Sharma et al 2011 Periodontitis	Saini et al.2003 Caries	Saini et al.2003 Gingivitis	Saini et al. 2003 Periodontitis
Peptostreptococcus sp.	4	8	5	0	0	0	6	9	6

Veillonella sp.	5	3	3	2	2	3	6	5	3
Lactobacillus sp.	10	2	1	6	2	1	13	4	2
Bacteroides sp.	0	2	1	2	0	7	0	0	0
Porphyromonas sp.	0	2	8	0	0	0	2	1	14
Prevotella sp.	0	0	2	0	0	0	2	3	4
Fusobacterium sp.	1	2	6	0	0	0	2	5	10

Table 14:
Aerobes:

Organism	Present study Caries	Present study Gingivitis	Present study Periodontitits	M.Sharma et.al 2011 Caries	M.Sharma et. al 2011 Gingivitis	M.Sharma et.al 2011 Periodontitis	Saini et.al 2003 Caries	Saini et.al 2003 Gingivitis	Saini et.al 2003 Periodontitis
S.mutans	11	5	1	8	4	2	12	3	2
S.aureus	2	6	3	2	0	0	3	6	3
CONS	2	3	3	0	0	0	1	3	4
Klebsiella spp.	3	5	4	1	2	0	3	6	4
Pseudomonas spp.	2	0	0	0	0	0	3	0	1
Escherichia coli	0	1	1	0	0	0	0	0	1

This may be due to variations in local factors like food habits, geographical distribution, usage of tooth paste etc.

CONCLUSION:

- Orodental infections are mostly polymicrobial in nature, predominantly caused by anaerobic bacteria
- The isolation of anaerobes increased as the lesion progressed from superficial to deep i.e., from caries to periodontitis.
- In the control group, there are more number of aerobic isolates. In the study group, there are more number of anaerobic isolates.
- The most common anaerobic and aerobic organisms isolated in both the study and control group are Peptostreptococcus sp. and Streptococcus mutans respectively.
- Some of the anaerobes have even shown resistance to Metronidazole. So, antibiogram for the orodental infections need to be done.

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