

Defining the normal periodontal microbial flora (selective) of oral cavity in health in the population of Hubli-Dharwad, Karnataka

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Abstract

Background: The oral cavity forms an indispensable part of the human microbiome, distributed within various niches. The mouth harbours at least six billion bacteria representing more than seven hundred species as well as other types of microorganisms. It is well known that there is no bacterial species found exclusively in healthy oral niches. There are various factors which affect the transition from oral commensals to pathogenic flora. With

this background the study was aimed to define and quantify the normal periodontal microbial flora of oral cavity in healthy individuals in Hubli-Dharwad population.

Materials and method: Hundred systemically healthy individuals were selected from out patient department of periodontics of SDM college of Dental sciences Dharwad. They were divided in to two groups. Group A –Fifty healthy individuals Group B -Chronic periodontitis

patients. Plaque sample was collected from dorsum of tongue, buccal mucosa, supragingival and subgingival areas. Plaque sample was immediately transferred to ependoff tubes and sent for FLUORESCENCE IN SITU HYBRIDIZATION (FISH). Aggregatibacter actinomycetemcomitans(AA), Porphyromonas gingivalis(PG), Prevotella intermedia(PI), Prevotella nigrescens(PN), Tennerella forsythensis(TN), Compylobacter rectus(CR), Treponoma denticola (TD), Fusobacterium nucleatum(FN), Actinomyces naeslundii (AN), Eikenella corrodens(EC), Capnocytophaga gingivalis(CG), Capnocytophaga ochrea(CO), Selenomonas noxia(SN), Streptococci(STRE) and Streptococcus sanguis(SG).

Results: We could observe various microorganisms in healthy individuals. The periopathogens were definitely lower in healthy individuals. We could also observe that the oral commensals were higher in healthy individuals. There was statistically significant difference in periopathogens in health and disease.

Keywords: Periopathogens, defining, microbial flora, Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis

Introduction

Periodontitis is a common chronic inflammatory disease characterized by destruction of supporting structures of the teeth. Etiology of periodontal disease is multifactorial in which microorganisms play a major role. The colonization of oral cavity starts about the time of birth. Within hours after birth, the sterile oral cavity becomes colonized by low numbers of mainly facultative and aerobic bacteria¹. Beginning the second day, anaerobic bacteria can be detected in the infant's edentulous mouth^{2,3}. Within two weeks after tooth eruption a more complex oral flora becomes established. It is estimated that more than 500 different species are capable of

colonizing adult mouth, and that any individual may typically harbour 150 or more different species^{4,5}. Most of the oral bacteria are commensal and beneficial. The current concept on etiology of periodontitis considers the presence of pathogenic species as etiologic factor. However the mere presence of putative periodontal pathogens in the gingival crevice is not sufficient to initiate or cause periodontal inflammation. An elevation in the relative proportion or number of these pathogens to reach critical mass seems more crucial to mount an effective tissue damaging process⁶. Even in health the periodontal pathogens may be present in the gingival crevice although in low number as member of normal resident flora⁷. The aims and objectives of this study was, to define and quantify the normal microbial flora of the human oral cavity in health in Hubli –Dharwad population.

Materials And Method

Hundred systemically healthy subjects aged between 18 to 50yrs from both gender 50 in each group were included. Patients visiting out patient department of periodontics SDM dental college Dharwad were included. They were divided in to two groups depending on periodontal health as chronic periodontitis⁸ and healthy subjects. Group A consisted of clinically normal oral and periodontal health. Group B consisted of chronic periodontitis with at least 20 teeth present, periodontal pocket depth \geq 5mm, clinical loss of attachment. Written informed consent was taken from all individuals. Permission was taken from institutional ethical committee. IRB no was 2016/S/perio/45. Inclusion criterias were systemically and periodontally healthy subjects, patients diagnosed as chronic periodontitis with at least 20 teeth present, periodontal pocket depth \geq 5mm and with clinical loss of attachment, age range between 18 to 50yrs. Both male and females were included. Exclusion criterias were pregnant

and lactating women, patients with systemic disease, patients who have undergone periodontal treatment, patients on antibiotics or who have taken in past six months, patients who are on mouth wash or used in past six months. The following parameters were recorded. Plaque index, (silness and loe) Gingival index (loe and sillness), probing pocket depth, clinical loss of attachment. Plaque samples were collected from four sites with area specific curette¹¹. The sites included Dorsum of tongue, Buccal mucosa, Supragingival, Subgingival areas. Plaque samples were collected with sterile curette and transferred to ependoff tubes and immediately transported to the laboratory. The presence and quantitative analysis of following microorganisms was done by FISH. The organisms included were *Aggregatibacter actinomycetemcomitans* (AA), *Porphyromonas gingivalis* (PG), *Prevotella intermedia* (PI), *Prevotella nigrescens*(PN), *Tannerella forsythensis*(TN), *Compylobacter rectus*(CR), *Treponema dneticola* (TD), *Fusobacterium nucleatum*(FN), *Actinomyces naeslundii* (AN), *Eikenella corrodens*(EC), *Capnocytophaga gingivalis*(CG), *Capnocytophag aochrea*(CO), *Selenomonas noxia*(SN), *Streptococci*(stre) and *Streptococcus sanguis*(SG).^{12,13,4,14,15,16,17} LAB METHOD - FLUORESCENCE IN SITU HYBRIDIZATION. (FISH) - Upon receipt of plaque sample in the laboratory, portion of plaque was transferred to avial containing ice cold paraformaldehyde (4%) fixed for 4hrs. Then it was washed 3 times with ice cold phosphate buffer saline (PBS) and resuspended in equal proportions of PBS and ethanol (96%) and stored at -20 degrees till use. The specificity of the probe was confirmed by testing against known strains of PG, PI, TF, TD and *Streptococcus mutans* etc. For preparation of slides 10µl of fixed sample was applied to gelatine coated slide. Then it was allowed to dry and was dehydrated by exposing the material to serial

concentrations of ethanol. The slides were then dried at 46⁰ for 10 min and FISH was performed. Fresh hybridization buffer was performed with 30% formamide concentration and oligonucleotide probe was diluted in this buffer to achieve a concentration of 20 picomoles in 100µl of buffer and applied to the plaque on the slide. It was kept in a moist petri dish and incubated at 48⁰c for 10 min, the slides were then washed with ice cold distilled water and then quickly air dried and mounted. They were wrapped in aluminium foil and stored at -20⁰ till studied under microscope. The demarked area of plaque was studied using fluorescent microscope with ×20, ×40 objectives. They were counted and graded by using a simple formula.¹⁸

no bacteria seen -0/negative

1-5 bacilli/smear-occasional/0.5

6-10/smear - 1+

10-100/smear - 2+

>100bacteria/smear - 3+

Clumps of bacteria seen - 4+

RESULTS

TABLE 1 BUCCAL MUCOSA : On Buccal mucosa anaerobic microorganisms were lower in number compared to healthy individuals. CG, TF were absent in healthy individuals. Streptococci were found more in healthy individuals. The difference between healthy and chronic periodontitis was statistically significant in relation to AN, CG, CO, STRE.

Table 2: Dorsum of Tongue : Most of the anaerobic microorganisms like PG, PN, TD, TF, PI, SN and EC were predominant on the dorsum of the tongue. AA were more in healthy individuals. There was a statistical significant difference between PG, TD, PI, PN, CO, STRE, SG and CR.

Table 3: Supragingival : AA, CO, SG, CR were more in healthy individuals compared to chronic periodontitis.

There was a statistical significant difference in health and chronic periodontitis in relation to TD, PN, SN, CO, STRE, SG.

Table 4 : Subgingival: AA, PG, FN, PI, PN, SN, TD, TF were lower in number in healthy individuals. There was a statistical significant difference between health and chronic periodontitis in relation to TD, TF, CO, STRE.

Note : List of abbreviations for microorganisms.

1. Aggregatibacter actinomycetemcomitans(AA)
2. Porphyromonas gingivalis (PG)
3. Prevotella inetermedia (PI)
4. Prevotella nigrescens (PN)
5. Tennerella forsythensis (TN)
6. Compylobacter rectus (CR)
7. Treponoma deticola (TD)
8. Fusobacterium nucleatum (FN)
9. Actinomyces naeslundi (AN)
10. Eikenella corrodens (EC)
11. Capnocytophaga gingivalis (CG)
12. Capnocytophaga ochrea (CO)
13. Selenomonas noxia (SN)
14. Streptococci (STRE) and
15. Streptococcus sanguis (SG)

Discussion

The purpose of the study was to define the normal periodontal microbial flora in Hubli –Dharwad population. As per our knowledge is concerned this is the first study of this kind in this population. It was also decided to quantify the bacteria as it has been proved that number of bacteria increase in periodontal disease¹⁶. Thus the difference in number of periopathogens in health and disease can be a diagnostic feature. With this background it was decided to quantify or compare the periopathogens in periodontal health and disease. The microorganisms selected were Aggregatibacter actinomycetemcomitans (AA), Porphyromonasgingivalis (PG),

Prevotellainetermedia (PI), Prevotellanigrescens (PN), Tennerellaforsythensis (TN), Compylobacter rectus (CR), Treponomadeticola (TD), Fusobacteriumnucleatum (FN), Actinomyces naeslundi (AN), Eikenella corrodens (EC), Capnocytophaga gingivalis (CG), Capnocytophaga ochrea (CO), Selenomona snoxia (SN), Streptococci (stre) and Streptococcus sanguis (SG)^{12,13,4,14,15,16,17}as these microorganisms are increased in chronic periodontitis.

In our study CO and SG were greater in number in healthy subjects. These microorganisms are known to be protective and beneficial to the host and are typically found in high numbers at periodontal sites that do not demonstrate attachment loss (inactive sites) but in low numbers at sites where active periodontal destruction occurs^{16,19}. In our study AA was lower in number in buccal mucosa and subgingival areas of healthy individuals and it may be due to increased production of H₂O₂ by SG which is known to be lethal to cells of AA¹⁹. In our study PG, FN, TD, PI, PN were increased in number in chronic periodontitis compared healthy individuals^{12,13,4,14,15,16,17}

Actinomyces naeslundi was lower in number in subgingival plaque in CP compared to healthy individuals, and higher in number in supragingival, buccal mucosa and dorsum of tongue in CP compared to healthy individuals.

These results are similar to study done by William F Liljemark.²⁰ EC was lower in subgingival areas of CP compared to healthy subjects and other sites. CG was lower in number on dorsum of tongue compared to healthy and other sites in CP. The reason could be colonization may be either promoted or impeded by other bacteria competing in various ecosystem²¹.

Conclusion

We could see various microorganisms in healthy individuals. The periopathogens were definitely lower in healthy individuals. We could also observe that the oral

commensals were higher in healthy individuals. There was statistically significant difference in periopathogens in health and disease.

List of abbreviations for microorganisms

Aggregatibacter actinomycetemcomitans (AA), Porphyromonas gingivalis (PG), Prevotella intermedia (PI), Prevotella nigrescens (PN), Tennerella forsythensis (TN), Compylobacter rectus (CR), Treponoma denticola (TD), Fusobacterium nucleatum (FN), Actinomyces naeslundii (AN), Eikenella corrodens (EC), Capnocytophaga gingivalis (CG), Capnocytophaga ochrea (CO), Selenomonas noxia (SN), Streptococci (STRE) and Streptococcus sanguis (SG).

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Legend Tables

Table 1: Distribution of Micro-organism between healthy and chronic periodontitis on Buccal mucosa.

Micro-organism	Concentration	Chronic Periodontitis		Healthy group		p value
		N	%	N	%	
AA	No bacteria	42	84	43	86	0.764
	1-5 bacilli/smear	5	10	7	14	
	6-10 bacilli/smear	2	4	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	1	2	0	0	
PG	No bacteria	46	92	47	94	0.876
	1-5 bacilli/smear	3	6	2	4	
	6-10 bacilli/smear	1	2	0	0	
	10-100 bacilli/smear	0	0	1	2	
	>100 bacilli/smear	0	0	0	0	
FN	No bacteria	47	94	50	100	0.810
	1-5 bacilli/smear	3	6	0	0	
	6-10 bacilli/smear	0	0	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
TD	No bacteria	49	98	49	98	-
	1-5 bacilli/smear	1	2	1	2	
	6-10 bacilli/smear	0	0	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
TF	No bacteria	50	100	50	100	-
	1-5 bacilli/smear	0	0	0	0	
	6-10 bacilli/smear	0	0	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
PI	No bacteria	42	84	45	90	0.933
	1-5 bacilli/smear	6	12	4	8	

	6-10 bacilli/smear	2	4	1	2	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
PN	No bacteria	40	80	41	82	0.824
	1-5 bacilli/smear	8	16	8	16	
	6-10 bacilli/smear	2	4	0	0	
	10-100 bacilli/smear	0	0	1	2	
	>100 bacilli/smear	0	0	0	0	
SN	No bacteria	50	100	44	88	0.496
	1-5 bacilli/smear	0	0	6	12	
	6-10 bacilli/smear	0	0	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
AN	No bacteria	32	64	47	94	0.001*
	1-5 bacilli/smear	11	22	2	4	
	6-10 bacilli/smear	7	14	0	0	
	10-100 bacilli/smear	0	0	1	2	
	>100 bacilli/smear	0	0	0	0	
EC	No bacteria	41	82	46	92	0.356
	1-5 bacilli/smear	8	16	2	4	
	6-10 bacilli/smear	1	2	1	2	
	10-100 bacilli/smear	0	0	1	2	
	>100 bacilli/smear	0	0	0	0	
CG	No bacteria	30	60	50	100	0.005*
	1-5 bacilli/smear	15	30	0	0	
	6-10 bacilli/smear	5	10	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
CO	No bacteria	30	60	16	32	0.032*
	1-5 bacilli/smear	15	30	16	32	
	6-10 bacilli/smear	5	10	14	28	
	10-100 bacilli/smear	0	0	2	4	
	>100 bacilli/smear	0	0	2	4	
STRE	No bacteria	18	36	0	0	<0.001*
	1-5 bacilli/smear	20	40	1	2	

	6-10 bacilli/smear	11	22	11	22	
	10-100 bacilli/smear	0	0	18	36	
	>100 bacilli/smear	1	2	20	40	
SG	No bacteria	25	50	19	38	0.444
	1-5 bacilli/smear	13	26	21	42	
	6-10 bacilli/smear	10	20	7	14	
	10-100 bacilli/smear	2	4	2	4	
	>100 bacilli/smear	0	0	1	2	
CR	No bacteria	15	30	10	20	0.773
	1-5 bacilli/smear	23	46	25	50	
	6-10 bacilli/smear	10	20	11	22	
	10-100 bacilli/smear	1	2	2	4	
	>100 bacilli/smear	1	2	2	4	

Note: * significant at 5% level of significance (p<0.05)

Table 2: Distribution of Micro-organism between healthy and chronic periodontitis on Dorsum of tongue

List of abbreviations for microorganisms :

Micro-organism	Concentration	Chronic Periodontitis		Healthy group		p value
		N	%	N	%	
AA	No bacteria	33	66	24	48	0.364
	1-5 bacilli/smear	12	24	20	40	
	6-10 bacilli/smear	4	8	6	12	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
PG	No bacteria	24	48	38	76	0.043*
	1-5 bacilli/smear	16	32	10	20	
	6-10 bacilli/smear	10	20	2	4	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
FN	No bacteria	31	62	40	80	0.245
	1-5 bacilli/smear	13	26	9	18	
	6-10 bacilli/smear	6	12	1	2	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
TD	No bacteria	24	48	40	80	0.027*
	1-5 bacilli/smear	25	50	10	20	

	6-10 bacilli/smear	1	2	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
TF	No bacteria	24	48	37	74	0.133
	1-5 bacilli/smear	25	50	13	26	
	6-10 bacilli/smear	1	2	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
PI	No bacteria	18	36	34	68	0.037*
	1-5 bacilli/smear	30	60	16	32	
	6-10 bacilli/smear	1	2	0	0	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
PN	No bacteria	16	32	35	70	0.006*
	1-5 bacilli/smear	29	58	13	26	
	6-10 bacilli/smear	4	8	2	4	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
SN	No bacteria	21	42	26	52	0.824
	1-5 bacilli/smear	28	56	23	46	
	6-10 bacilli/smear	1	2	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
AN	No bacteria	32	64	38	76	0.772
	1-5 bacilli/smear	13	26	10	20	
	6-10 bacilli/smear	4	8	2	4	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
EC	No bacteria	23	46	26	52	0.904
	1-5 bacilli/smear	24	48	23	46	
	6-10 bacilli/smear	2	4	1	2	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
CG	No bacteria	40	80	37	74	0.854
	1-5 bacilli/smear	9	18	13	26	

	6-10 bacilli/smear	1	2	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
CO	No bacteria	28	56	12	24	0.027*
	1-5 bacilli/smear	17	34	30	60	
	6-10 bacilli/smear	3	6	6	12	
	10-100 bacilli/smear	2	4	2	4	
	>100 bacilli/smear	0	0	0	0	
STRE	No bacteria	23	46	0	0	<0.001*
	1-5 bacilli/smear	15	30	6	12	
	6-10 bacilli/smear	8	16	10	20	
	10-100 bacilli/smear	2	4	17	34	
	>100 bacilli/smear	2	4	17	34	
SG	No bacteria	36	72	12	24	<0.001*
	1-5 bacilli/smear	5	10	20	40	
	6-10 bacilli/smear	5	10	11	22	
	10-100 bacilli/smear	3	6	6	12	
	>100 bacilli/smear	1	2	0	0	
CR	No bacteria	17	34	4	8	0.006*
	1-5 bacilli/smear	17	34	30	60	
	6-10 bacilli/smear	15	30	12	24	
	10-100 bacilli/smear	0	0	4	8	
	>100 bacilli/smear	1	2	0	0	

Note: * significant at 5% level of significance (p<0.05)

Table 3: Distribution of Micro-organism between healthy and chronic periodontitis at Supragingival

Micro-organism	Concentration	Chronic Periodontitis		Healthy group		p value
		N	%	N	%	
AA	No bacteria	36	72	33	66	0.246
	1-5 bacilli/smear	6	12	14	28	
	6-10 bacilli/smear	7	14	3	6	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
PG	No bacteria	28	56	41	82	0.059
	1-5 bacilli/smear	14	28	8	16	
	6-10 bacilli/smear	7	14	1	2	

	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
FN	No bacteria	29	58	36	72	0.640
	1-5 bacilli/smear	16	32	12	24	
	6-10 bacilli/smear	3	6	2	4	
	10-100 bacilli/smear	2	4	0	0	
	>100 bacilli/smear	0	0	0	0	
TD	No bacteria	19	38	34	68	0.046*
	1-5 bacilli/smear	25	50	16	32	
	6-10 bacilli/smear	6	12	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
TF	No bacteria	23	46	35	70	0.132
	1-5 bacilli/smear	26	52	14	28	
	6-10 bacilli/smear	0	0	1	2	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
PI	No bacteria	16	32	29	58	0.101
	1-5 bacilli/smear	28	56	21	42	
	6-10 bacilli/smear	6	12	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
PN	No bacteria	18	36	36	72	0.008*
	1-5 bacilli/smear	24	48	13	26	
	6-10 bacilli/smear	6	12	1	2	
	10-100 bacilli/smear	2	4	0	0	
	>100 bacilli/smear	0	0	0	0	
SN	No bacteria	20	40	36	72	0.036*
	1-5 bacilli/smear	23	46	14	28	
	6-10 bacilli/smear	4	8	0	0	
	10-100 bacilli/smear	3	6	0	0	
	>100 bacilli/smear	0	0	0	0	
AN	No bacteria	32	64	41	82	0.318
	1-5 bacilli/smear	12	24	8	16	
	6-10 bacilli/smear	4	8	1	2	

	10-100 bacilli/smear	2	4	0	0	
	>100 bacilli/smear	0	0	0	0	
EC	No bacteria	22	44	23	46	0.960
	1-5 bacilli/smear	20	40	21	42	
	6-10 bacilli/smear	7	14	6	12	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
CG	No bacteria	33	66	35	70	0.932
	1-5 bacilli/smear	17	34	14	28	
	6-10 bacilli/smear	0	0	1	2	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
CO	No bacteria	25	50	11	22	0.036*
	1-5 bacilli/smear	16	32	29	58	
	6-10 bacilli/smear	9	18	8	16	
	10-100 bacilli/smear	0	0	1	2	
	>100 bacilli/smear	0	0	1	2	
STRE	No bacteria	18	36	0	0	<0.001*
	1-5 bacilli/smear	25	50	1	2	
	6-10 bacilli/smear	5	10	14	28	
	10-100 bacilli/smear	0	0	25	50	
	>100 bacilli/smear	2	4	10	20	
SG	No bacteria	29	58	16	32	0.029*
	1-5 bacilli/smear	13	26	14	28	
	6-10 bacilli/smear	6	12	15	30	
	10-100 bacilli/smear	1	2	5	10	
	>100 bacilli/smear	1	2	0	0	
CR	No bacteria	18	36	9	18	0.092
	1-5 bacilli/smear	17	34	28	56	
	6-10 bacilli/smear	11	22	11	22	
	10-100 bacilli/smear	4	8	1	2	
	>100 bacilli/smear	0	0	1	2	

Note: * significant at 5% level of significance (p<0.05)

Table 4: Distribution of Micro-organism between cases and controls at surface Sub gingival

Micro-organism	Concentration	Chronic Periodontitis		Healthy group		p value
		N	%	N	%	
AA	No bacteria	26	52	27	54	0.431
	1-5 bacilli/smear	9	18	15	30	
	6-10 bacilli/smear	12	24	8	16	
	10-100 bacilli/smear	3	6	0	0	
	>100 bacilli/smear	0	0	0	0	
PG	No bacteria	24	48	36	72	0.078
	1-5 bacilli/smear	14	28	14	28	
	6-10 bacilli/smear	9	18	0	0	
	10-100 bacilli/smear	3	6	0	0	
	>100 bacilli/smear	0	0	0	0	
FN	No bacteria	26	52	33	66	0.190
	1-5 bacilli/smear	14	28	17	34	
	6-10 bacilli/smear	10	20	0	0	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
TD	No bacteria	17	34	30	60	0.031*
	1-5 bacilli/smear	19	38	20	40	
	6-10 bacilli/smear	11	22	0	0	
	10-100 bacilli/smear	3	6	0	0	
	>100 bacilli/smear	0	0	0	0	
TF	No bacteria	12	24	31	62	0.005*
	1-5 bacilli/smear	26	52	15	30	
	6-10 bacilli/smear	10	20	4	8	
	10-100 bacilli/smear	2	4	0	0	
	>100 bacilli/smear	0	0	0	0	
PI	No bacteria	28	56	38	76	0.293
	1-5 bacilli/smear	16	32	11	22	
	6-10 bacilli/smear	5	10	0	0	
	10-100 bacilli/smear	1	2	1	2	
	>100 bacilli/smear	0	0	0	0	
PN	No bacteria	23	46	37	74	0.078
	1-5 bacilli/smear	19	38	13	26	

	6-10 bacilli/smear	7	14	0	0	
	10-100 bacilli/smear	1	2	0	0	
	>100 bacilli/smear	0	0	0	0	
SN	No bacteria	20	40	33	66	0.125
	1-5 bacilli/smear	25	50	17	34	
	6-10 bacilli/smear	3	6	0	0	
	10-100 bacilli/smear	2	4	0	0	
	>100 bacilli/smear	0	0	0	0	
AN	No bacteria	40	80	35	70	0.827
	1-5 bacilli/smear	10	20	14	28	
	6-10 bacilli/smear	0	0	1	2	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
EC	No bacteria	23	46	18	36	0.610
	1-5 bacilli/smear	16	32	24	48	
	6-10 bacilli/smear	8	16	6	12	
	10-100 bacilli/smear	3	6	2	4	
	>100 bacilli/smear	0	0	0	0	
CG	No bacteria	32	64	32	64	0.938
	1-5 bacilli/smear	16	32	14	28	
	6-10 bacilli/smear	2	4	4	8	
	10-100 bacilli/smear	0	0	0	0	
	>100 bacilli/smear	0	0	0	0	
CO	No bacteria	45	90	30	60	0.040*
	1-5 bacilli/smear	5	10	13	26	
	6-10 bacilli/smear	0	0	4	8	
	10-100 bacilli/smear	0	0	1	2	
	>100 bacilli/smear	0	0	2	4	
STRE	No bacteria	19	38	0	0	<0.001*
	1-5 bacilli/smear	23	46	1	2	
	6-10 bacilli/smear	6	12	9	18	
	10-100 bacilli/smear	2	4	14	28	
	>100 bacilli/smear	0	0	26	52	
SG	No bacteria	28	56	20	40	0.178
	1-5 bacilli/smear	13	26	11	22	

	6-10 bacilli/smear	8	16	12	24	
	10-100 bacilli/smear	0	0	7	14	
	>100 bacilli/smear	1	2	0	0	
CR	No bacteria	23	46	23	46	0.190
	1-5 bacilli/smear	23	46	14	28	
	6-10 bacilli/smear	3	6	7	14	
	10-100 bacilli/smear	0	0	4	8	
	>100 bacilli/smear	1	2	2	4	

Note: * significant at 5% level of significance ($p < 0.05$)