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Enumeration of anaerobic periodontal pathogens from tuberculosis and chronic periodontitis patients by paper points and curettes: CFU based evaluation

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AIM

The primary aim of the study was to enumerate the anaerobic bacterial load from tuberculosis patients. In this process, we also tried to evaluate an effective collection method for sub gingival bacteria.

INTRODUCTION

Oral hygiene of Tuberculosis patients is not an extensively studied aspect. The scarcity of literature proves it. The present paper speaks about the anaerobic periodontal pathogens' enumeration based on Colony Forming Units (CFU's). As per our knowledge, no such study has systematically evaluated anaerobic periodontal microbes from tuberculosis patients. The present study had also compared two widely used sampling techniques (paper point and curette).

The outcome of microbiological sampling depends on the technique used. Tanner and Goodson (1986) [4] discussed commonly used microbiological sampling devices. They opined the sampling tools as "dental approved" devices, which consisted of sampling using curettes, scalers, paper points, barbed broaches within cannulas, irrigation of periodontal pockets etc.

MATERIALS AND METHODS

Patients

Twenty subjects with tuberculosis from local government TB wards and twenty subjects with chronic periodontitis from CKS Theja Dental college (Age range: 18 - 55 years) were enrolled upon prior informed consent into the study. The study had been approved by the CKS Theja's Institutional Ethical Committee, Renigunta, Andhra Pradesh, India. All subjects had at least 1 periodontal with a probing depth more than 6 mm. The necessary subjects were advised SRP (scaling and root planing) once after our sample collection.

Sampling techniques

As we were confined only to know the anaerobic bacterial load in tuberculosis patients and chronic periodontitis patients via two different sampling methods and then to know the better technique between them as the outcome of microbiological sampling depends on the sampling technique used, we restricted ourselves from considering several other factors influencing the respective diseases. It was necessary to use both methods at the same time and at the same site from all the 20 subjects (per group) to maintain the accuracy and consistency.

Grouping according to sampling sequence: Cross-over design

A cross-over design was implemented to accurate the sampling results, wherein to balance the effect of the first sample on the second one, samples were taken in 2 opposite sequences in two patient groups and then depending on the sequence of sampling, patients were randomized into two groups (A and B) with 10 patients each.

In group A subjects, the first sampling was done using one # 40 absorbent paper point (Pearl Endopia, batch: 01A103, Pearl Dental Co., Ltd. Vietnam) by inserting into the pocket slowly with a sterile dental tweezers to the predetermined depth until tissue resistance. The paper point was left for 30 seconds, and then it was carefully removed without touching the adjacent unrelated tissues. The second sample was taken using sterile Gracey-curette 5-6 (Hu-Friedy, Chicago, USA) by inserting slightly as deep as possible into the pocket without applying any pressure on the tooth surface, in order to avoid a dislocation of subgingival plaque. As soon as the curette met tissue

resistance at the apical part of the pocket, subgingival sampling was performed with one single vertical stroke. In group B subjects, samples were taken in the opposite sequence, i.e. the first sample was taken with a sterile Gracey-curette 5-6 (Hu-Friedy, Chicago, USA), while the second one with one # 40 absorbent paper point (Pearl Endopia, batch: 01A103, Pearl Dental Co., Ltd. Vietnam).

Both the samples were then placed in the autoclaved transport media containing polypropylene screw cap vials for further analysis. After several test runs performed, 10-4 dilution was chosen as most suitable dilution for the colony forming units (CFU) determination. CFU/ml = Number of colonies per ml plated/ Total dilution factor (Benson, 2002) [1]. The serial dilutions were done using RTF (Himedia) medium and then were plated on Anaerobic Thyoglycolate Medium Base (ATMB) (Himedia) agar medium. The plates were anaerobically incubated in an anaerobic jar in an atmosphere of N2 80%, H2 10% and CO2 10% at 370C for 3-4 days (S. Doungudomdacha et. al, 2000) [2] and number colonies formed were calculated.

Correlation between sampling techniques

The agreement of quantitative results of both sampling techniques was tested using Pearson correlation coefficient. Table 1 indicates correlation between both sampling procedures among tuberculosis subjects and Table 2 describes the same in chronic periodontitis subjects. While, Table 3 describes Spearman Rank Correlation between sampling methods in tuberculosis patients and Table 4 describes Spearman Rank Correlation between sampling methods in chronic periodontitis patients.

Figures 1, 2, 3 and 4 are the scatter plots of A v/s B sampling techniques for paper points in TB, curettes in Tuberculosis (TB), paper points in chronic periodontitis (CP) and curettes in chronic periodontitis (CP) respectively. Figures 5 and 6 represent scatter plots of 'A' sampling between Paper points and curettes in TB and Scatter plot of 'B' sampling between Paper points and curettes in TB respectively. Finally, figures 7 and 8 represent 'A' sampling between Paper points and curettes in Chronic Periodontitis and 'B' sampling between Paper points and curettes in Chronic Periodontitis.

Table 1: Pearson Correlation Coefficient between the sampling procedures – Tuberculosis

	Tuberculosis – CFU – 10^{-4} dilution			
	A sampling Paper Points	**B sampling** Paper Points	**A sampling** Curette	**B sampling** Curette
No. of Subjects	20	20	20	20
Mean	7.25	6.8	9.75	9.9
S.D	4.56387416	4.95877744	6.0687	6.72701
Pearson Correlation Coefficient 'R'	0.9535*		0.9701*	

* strong positive correlation

Table 2: Pearson Correlation Coefficient between the sampling procedures – Chronic Periodontitis

	Chronic Periodontitis – CFU – 10^{-4} dilution			
	A sampling Paper Points	**B sampling** Paper Points	**A sampling** Curette	**B sampling** Curette
No. of Subjects	20	20	20	20
Mean	8.95	8.95	11.5	11.5
S.D	10.460326	10.460326	12.8493	12.84933
Pearson Correlation Coefficient 'R'	1*		1*	

* strong positive correlation

Table 3: Spearman Rank Correlation in tuberculosis between paper points and curette techniques

	Tuberculosis – CFU – 10^{-4} dilution			
	A sampling		**B sampling**	
	Paper Points	Curette	Paper Points	Curette
No. of Subjects	20	20	20	20
Mean	7.25	9.75	6.8	9.9
S.D	4.56387416	6.0687	4.95877744	6.72701
Spearman Rank Correlation 'rho' R	0.876*		0.842*	

* strong positive correlation

Table 4: Spearman Rank Correlation in periodontitis between paper points and curette techniques

	Chronic Periodontitis – CFU – 10^{-4} dilution			
	A sampling		**B sampling**	
	Paper Points	Curette	Paper Points	Curette
No. of Subjects	20	20	20	20
Mean	8.95	11.5	8.95	11.5
S.D	10.460326	12.8493	10.460326	12.84933
Spearman Rank Correlation 'rho' R	0.974*		0.974*	

* strong positive correlation

Fig.1 A vs B Paper points sampling – TB

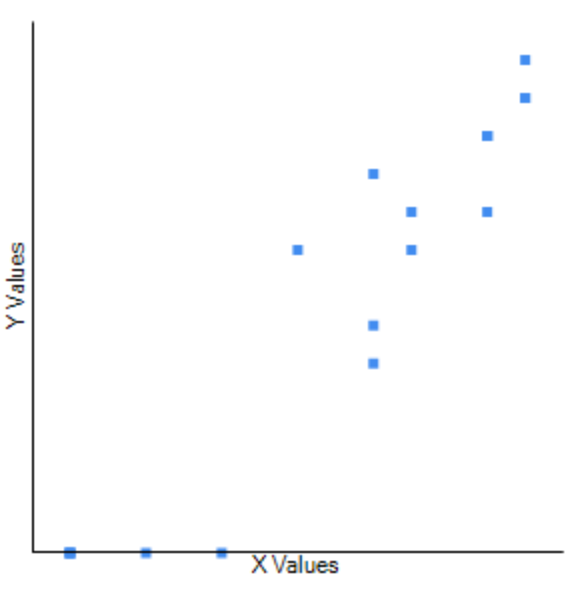


Fig.2 A vs B Curette sampling - TB

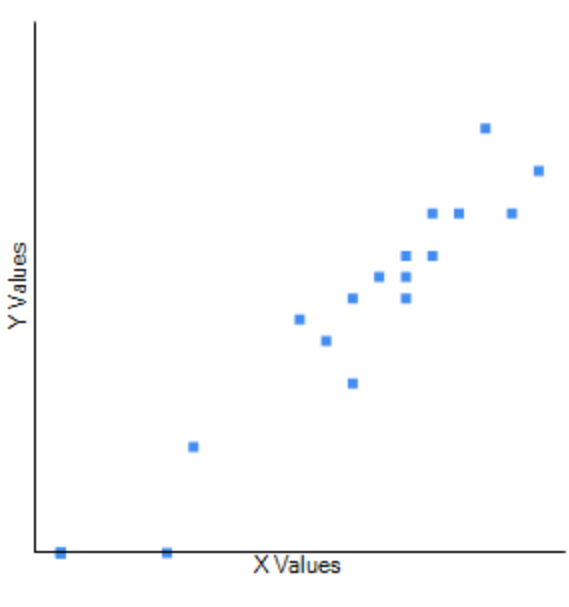


Fig.3 A vs B Paper points sampling – CP

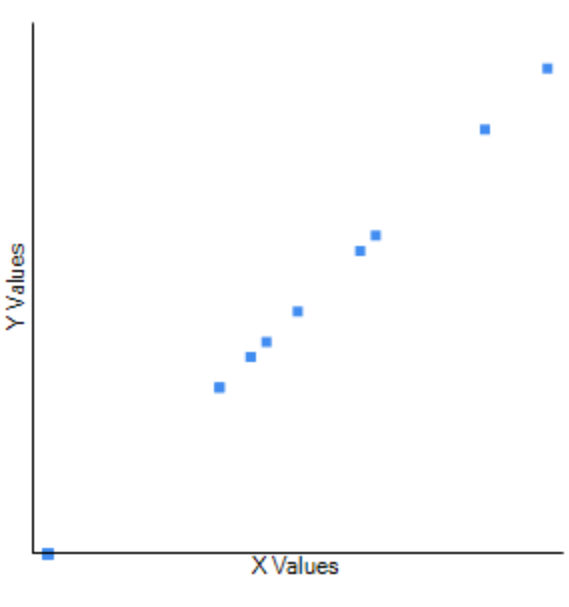


Fig.4 A vs B Curette sampling – CP

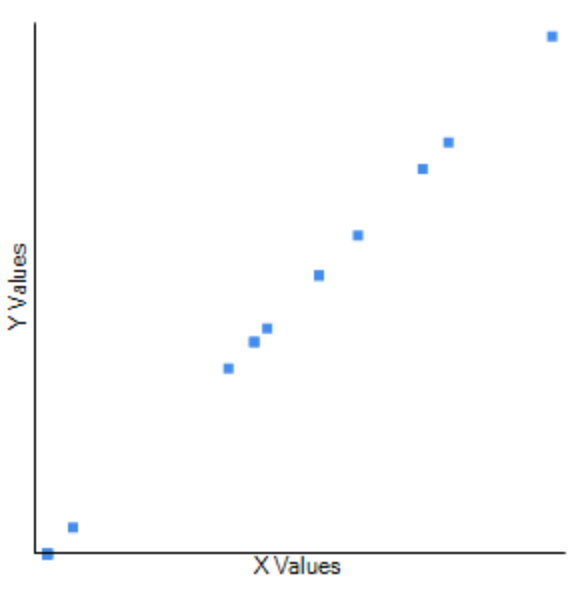


Fig.5 Scatter plot – 'A' sampling between Paper points and curettes in TB

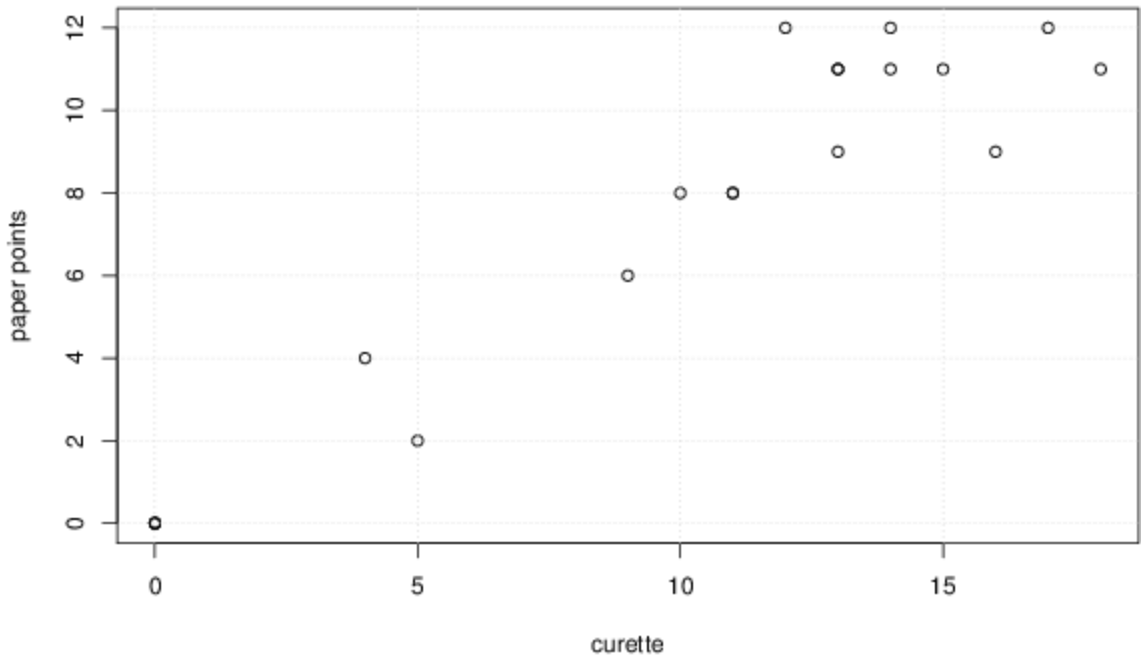


Fig.6 Scatter plot – 'B' sampling between Paper points and curettes in TB

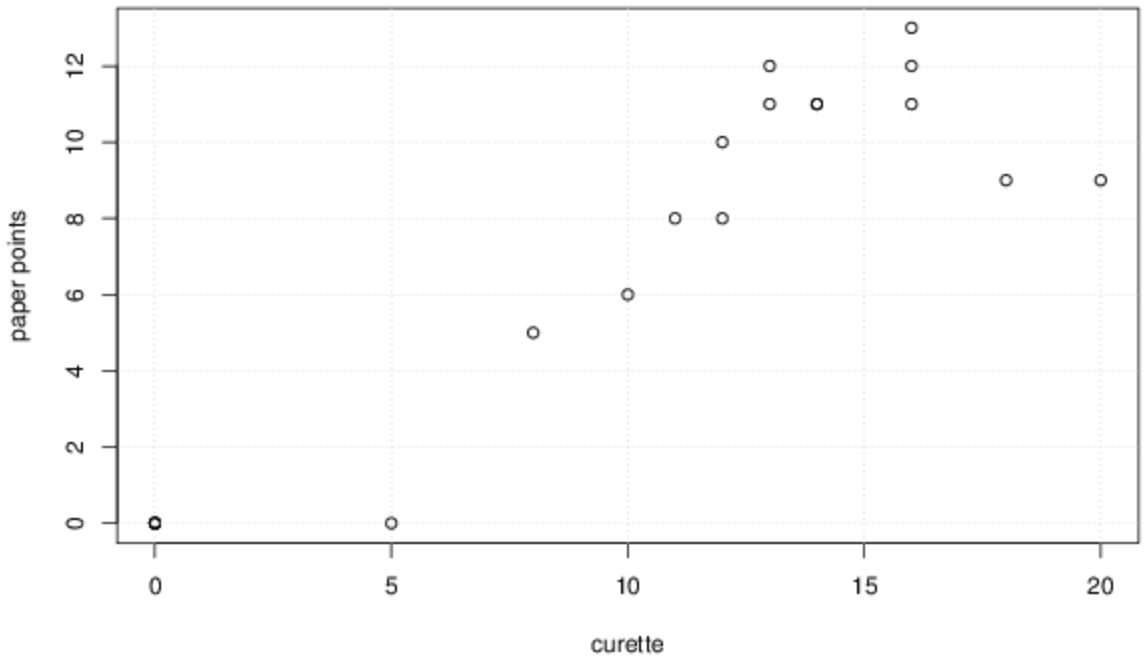


Fig.7 Scatter plot – 'A' sampling between Paper points and curettes in Chronic Periodontitis

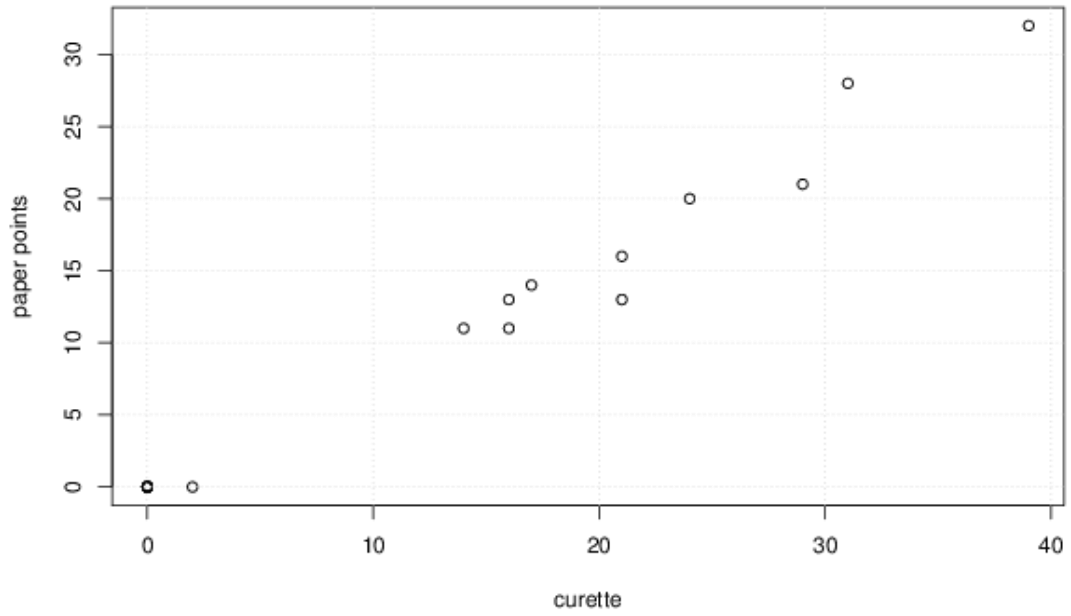
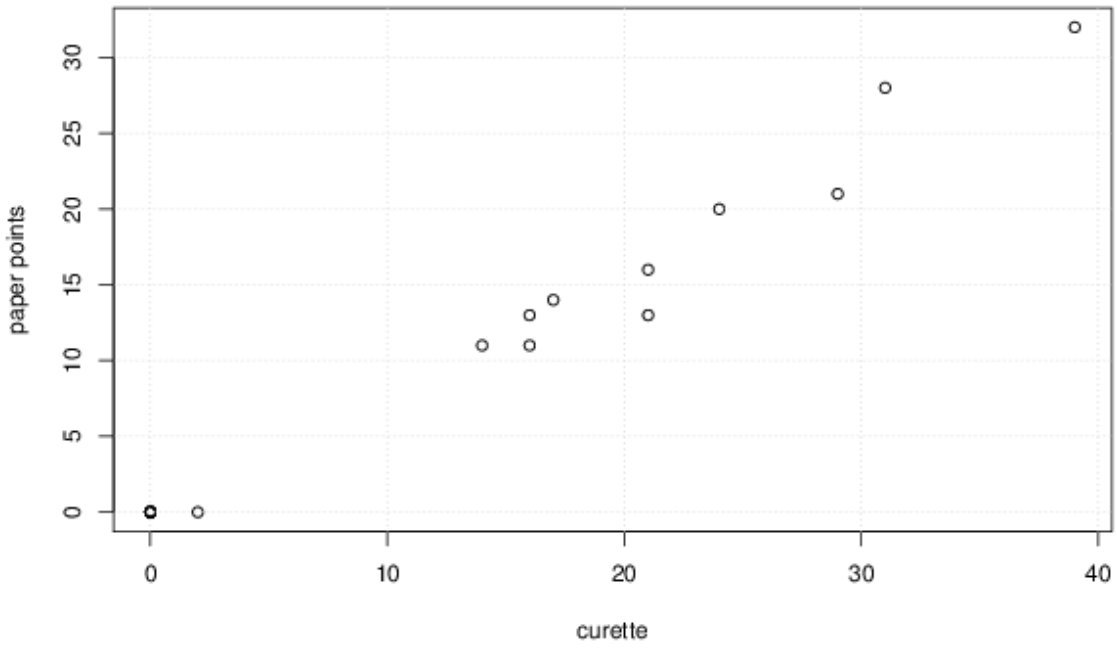


Fig.8 Scatter plot – 'B' sampling between Paper points and curettes in Chronic Periodontitis



RESULTS

The colony forming units from tuberculosis patients revealed the presence anaerobic pathogens. Irrespective of the technique used, there were considerable amount of anaerobic load recorded among tuberculosis patients. It reveals a hidden risk of periodontitis among tuberculosis patients. The strict anaerobic conditions facilitated the growth of only anaerobes suppressing the aerobic bacteria. Similar results recorded in chronic periodontitis subjects didn't amuse as it is quite evident from their periodontal status itself. However, the load was more in CP than in TB. It was always a higher load obtained by curette sampling, irrespective of sampling techniques and methods in both clinical conditions. Yet, the results obtained via both techniques were statistically significant. It was an interesting observation in chronic periodontitis cases, wherein the colony forming units didn't vary via both techniques, though a strong reason couldn't be determined.

The statistical analysis of various sampling conditions and methods, revealed a significant correlation results among the procedures followed. As demonstrated in Fig. 1, and Table 1 a strong significance of 0.953 was obtained when the CFU of anaerobic bacteria eluted from the paper points and a strong significance value of 0.970 obtained from curette (Fig. 2) collection via comparing two sampling methods 'A' and 'B' in TB patients elucidates the efficiency of bacterial sampling. Significant Correlation Coefficient value of 1 was obtained in a similar test performed in chronic periodontitis patients as depicted in Table 2 and fig. 3, 4.

Spearman Rank Correlation results depicted in Table 3 denotes a strong correlation value of 0.876 in 'A' sampling technique and 0.842 in 'B' sampling technique comparing sampling methods of paper points and curettes among tuberculosis patients. Figures 5 and 6 represent the scatter plot of both values on comparison. Similar test results of Chronic periodontitis patients depicted in Table 4 with a Spearman Rank Correlation value of 0.974 via both techniques suggested a strong correlation between the sampling methods. The figures 7 and 8 represent the scatter plot of the 'A' sampling between Paper points and curettes in Chronic Periodontitis and 'B' sampling between Paper points and curettes in Chronic Periodontitis.

DISCUSSION

As most of our results were self-explanatory, we restrict our discussion to minimum. As per the aim of the study, we found a significant anaerobic bacterial load among tuberculosis patients. The cross-over design facilitated the various possibilities of collection and minimized the errors. The statistical evaluation clearly concludes that both the techniques of paper points and curettes can be used for subgingival sampling. Our results were in agreement with an earlier study by (Jervoe, 2007) [3]. However, due to the ease of sampling and comfort of patient while collection, we personally recommend the paper points for subgingival collection. Further long term analysis of subgingival bacteria using advanced techniques of PCR could evaluate and identify the responsible pathogens and help in treatment of the clinical condition.

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