Int J Pharm Bio Sci 2015 Jan; 6(1): (B) 245 - 251

Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ISOLATION OF AEROBIC AND ANAEROBIC BACTERIA IN TRANSIENT BACTEREMIA

CHITRA.N*[1] AND V.MANGAYARKARASI [2]

1 Assistant Professor, Department of Microbiology, SRM Dental college, Ramapuram campus, SRM University, Chennai, India.
2 Professor, Department of Microbiology, SRM Medical College, SRM University, Kattankulathur, Chennai, India.

ABSTRACT

The normal oral cavity has a large and diverse resident microflora. These microorganisms are generally considered non-pathogenic and as normal inhabitants of the oral cavity. Bacteremia in dentistry frequently occurs due to entry of these bacteria into the bloodstream following procedures such as periodontal probing, root canal treatment, orthodontic treatment, and oral hygiene procedures. Invasive procedures such as extractions, periodontal surgery and third molar surgery pose a higher risk of causing bacteremia. In a healthy person, bacteremia in the bloodstream is transient in nature and is countered by normal defense mechanisms. However, bacteremia may cause infective endocarditis (IE) in patients with cardiac anomalies or in immunocompetent or immunocompromised patients. The aims of this study were to isolate and identify the various types of aerobic and anaerobic bacteria causing transient bacteremia after third molar surgery and to find their prevalence.

KEYWORDS: **Aerobic, anaerobic, bacteremia, transient, isolation.**

*Corresponding author

CHITRA.N
Assistant Professor, Department of Microbiology, SRM Dental college, Ramapuram campus, SRM University, Chennai, India.

INTRODUCTION

A large and varied species of bacteria reside as normal flora in the oral cavity. Commensalism is shown by the bacteria which form the normal flora of the oral cavity. Commensalism, is a mechanism that benefits the microbes alone without harming the host. Such commensal bacteria are generally harmless but can become pathogenic under opportunistic conditions or if translocated to a different niche[1]. Biofilm formation is a very unique feature of the oral commensal bacteria. In the oral cavity, the oral biofilms if unchecked, after a period of time, can permanently establish themselves and reside on non-shedding tooth surfaces which is close to the dentogingival junction. Bacteremia can occur, when there is disruption of this integrity between the biofilm and the sub gingival epithelium.[2]

Transient nature of Bacteremia

According to the Centers for Disease Control and Prevention (CDC), Blood Stream Infections can be defined as the presence of viable bacteria in the blood (i.e., bacteremia) which is documented by a positive blood culture result[3]. Normal oral microbes can enter the bloodstream in relatively high numbers, during invasive dental procedures and circulate throughout the body, these bacteremias are however transient, which means that they do not last long and are generally clinically benign and self-resolving in persons who do not have an underlying illness or immune deficiency or a turbulent cardiac blood flow[3]. These microbes are usually eliminated by the reticuloendothelial system within a few minutes, and usually do not cause any clinical symptom, however there may be a slight increase in temperature. It is important, however, that in spite of an initial steep fall in the number of bacteria, a few bacteria survive in the circulation.[4]. The role of these persistent bacteria and how they survive host defenses need to be evaluated further, as they may well be the ones that evade the initial host immune response and have the opportunity to seed target organs and after a certain period of time, will start to multiply and can cause systemic infections .The most common complication due to transient bacteremia of dental origin is bacterial endocarditis[2].

Factors Responsible for Bacteremia of Dental Origin

Bacteria can enter the bloodstream from the oral cavity by a number of mechanisms and by various routes. The bacterial biofilm in the gingival tissue is harmoniously balanced so a break or damage of this oral niche can cause spread of the oral flora into the bloodstream .Routine every day activities such as chewing, brushing, and flossing, can breakdown the barrier between the gingival tissues and oral biofilm[5]. Most often in dentistry this disruption is due to tissue trauma caused by procedures such as scaling, probing and tooth extractions which can result in a breakage in capillaries or small blood vessels located near the plaque biofilms, which allows the entry of bacteria into the systemic circulation[6].

Major aerobic and anaerobic bacterial species recovered from bacteremias of dental origin and their virulence factors

Several predominant aerobic bacteria, which are normal residents of the oral cavity can cause Infective Endocarditis. Anaerobic bacteria can cause periodontal disease, however they very rarely are reported to cause IE[7]. Some of the oral bacteria especially the normal aerobic flora have special virulence factors which aid them in their entry and survival in the bloodstream**.** Viridans streptococci secreted factors could increase the production of interleukin-8, which is particularly important in lung pathology, such as acute respiratory distress syndrome (ARDS) *Streptococcus sps:* Ability to switch peripheral blood monocytes to dendritic cells which exhibit large numbers of adhesion molecules, contributing to adhesion to the injured endothelium and to fibrinogen in blood clots[8].

Streptococcus intermedius
Hyaluronidase and chondroitin sulfate depolymerase break the ground substance[1].

Streptococcus anginosus
Binds to the endothelium, the basement membrane, and collagen[9].

Streptococcus sanguis
Platelet aggregation-associated protein; enhances platelet accumulation[8]. *Porphyromonas gingivalis* Cysteine proteinase (gingipain); degrades extracellular matrix proteins, such as laminin, fibronectin, and type IV collagen[1]. Fusobacterium nucleatum Inhibits binding of chemotactic peptides to neutrophils and in prevention of phagocytic activity[10]. *A.actinomycetemcomitans* EmaA (extracellular matrix protein adhesin A); forms antenna-like protrusions associated with the surface and mediates adhesion to collagen in connective tissues, facilitating spread[1]. Several recent studies show that some of the oral bacteria which invade the blood stream also have resistant genes which help them to resist the action of antibiotics in the bloodstream.

MATERIALS AND METHODS

Ethical Approval
This study has been approved by the Ethical committee of SRM University. The purpose of the study was explained and a written consent was obtained from all patients included in the study before collection of sample from the patients. This study was carried out in the Department of Microbiology, SRM Dental College and Hospital – Ramapuram , SRM University, Chennai ,India. The study comprised of patients who came to the Dept. of Oral and Maxillofacial Surgery, SRM Dental College and Hospital. All the patients were scheduled to undergo third molar surgery. 100 generally healthy patients, who came to SRM Dental College, Ramapuram , Chennai, were taken for the study. Of these, 50 were patients who had been given Amoxicillin prior to impaction (Group I) and 50 were patients who had not taken any antibiotics before impaction (Group II)[11]. Blood samples were collected at baseline, and after the surgical procedure.

Blood Collection and Processing
Before sampling the patients skin was cleaned with antiseptic – povidone iodine and 70% iso propyl alcohol and allowed to dry before collection of blood sample – to avoid risk of contamination. Each blood sample (10 ml) was collected using an IV cannula(BD Venflon – Becton Dickinson India(P) Ltd) placed in the antecubital vein [12,13]. Before local anesthetic injection was given, the baseline blood sample was collected; the second sample was collected after the surgical procedure. Blood samples were inoculated into the Aerobic culture media bottles (Hi media Mumbai, India - BHI broth with SPS) and Anaerobic culture media bottles (Hi media Mumbai, India - BHI and RCM broth).

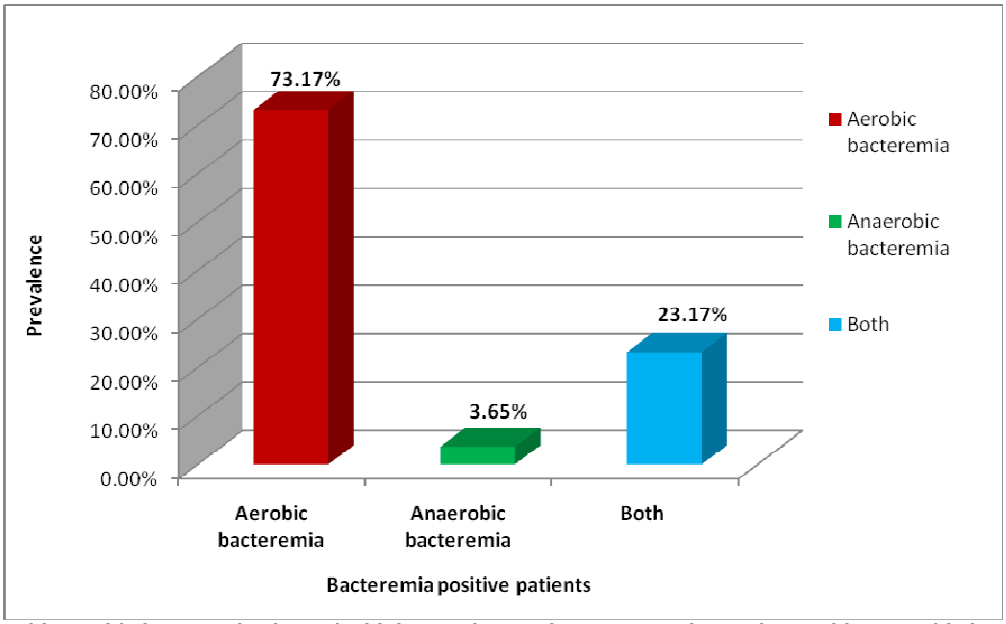
Microbiological Analysis
The blood culture bottles were transferred to the Department of Microbiology, SRM University within 10 minutes of collection. The blood culture bottles were incubated separately for aerobic and anaerobic cultivation. Blood samples which were collected were incubated for 48 hours at 37˚C .Culture bottles were examined for turbidity and subcultures were done. If no growth was observed the bottles were further incubated for a period of 7 days. If there is still no growth detected in the bottles, a blind subculture was done before discarding the sample as negative. For aerobic culture, positive blood cultures were subcultured on blood agar and chocolate agar plates and incubated aerobically in a CO_2 incubator at 37˚C with 5%-10% CO_2 for 24-72 hours and observed for growth of bacterial colonies. For anaerobic cultures, positive blood cultures were subcultured onto blood agar and brucella agar plates[7] supplemented with haemin and vitamin K and incubated in anaerobic jar with gaspak(Hi-media Ltd).On plates with growth, the colonies were isolated and subcultures were made. The isolated bacteria were identified using established and conventional methods, including colony morphology, grams staining, aerotolerance testing, determination of sensitivity to special profile antibiotic discs (Hi-

media Ltd), biochemical and fermentation tests[14]. Smears were prepared on glass slides and subjected to grams staining. The morphology of the bacteria like shape, arrangement etc was observed to identify the genus of the bacteria and biochemical tests and identification kits (Hi-media latex kits, Mumbai, India), and (Microxpress identification kits – Tulip diagnostics (P) Ltd, India) were used to identify the species.

RESULTS

No bacteria were isolated from samples collected at baseline. Bacteria were isolated from 82 of the 100 subjects included in the study, in blood samples which were collected after the surgical procedure. The number of patients positive for bacteremia in Group I was 46(92%) and in Group II 36 (72%). The total prevalence of bacteremia when both groups were included was (82%).On an average 3-1 bacterial species per bacteremia patient was detected in blood samples collected after completion of surgical procedure. A total of 161 bacterial isolates was isolated from the blood cultures. 73.17% of the subjects were positive for only aerobic bacteria, 23.17% of the subjects had both aerobic and anaerobic bacteria and only 3.65% had detectable anaerobic bacteria. The most frequently isolated aerobic bacteria were *Streptococcus viridians*(76.8%). Other predominant bacteria isolated were *Moraxella catarrhalis*(43.9%) and *Staphyloccus sps*(34.1%). A high number of aerobic bacteria were isolated when compared to the anaerobic bacteria isolated. The most frequently isolated anaerobic bacteria were *Peptostreptococcus* sps(6.09%) followed by *Actinomycetes sps*(4.87%) and *Bacteroides fragilis*(4.87%).

Figure 1
Bacteremia positive patients after third molar surgery (n=82)

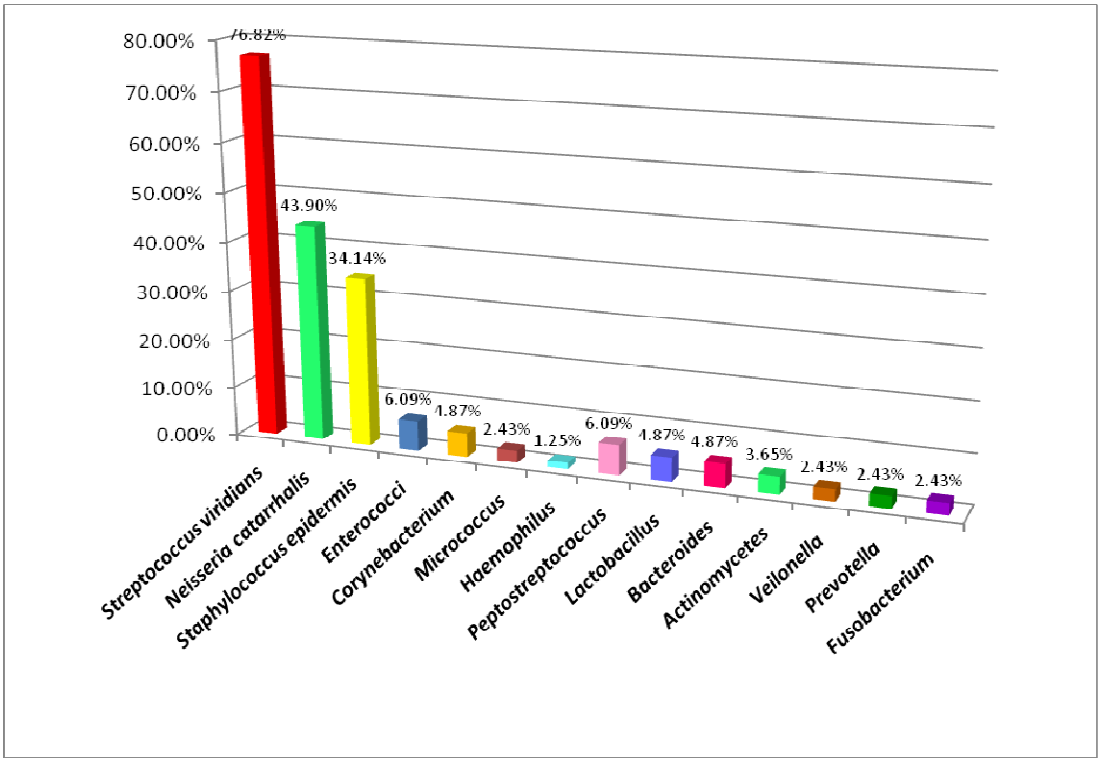


Patients with aerobic bacteremia showed a high prevalence when compared to patients with anaerobic bacteremia.

Table 1
Bacterial isolates from bacteremia positive patients (n=161)

BACTERIA	No.of Isolates
AEROBIC BACTERIA	
Streptococcus mitis	32
Streptococcus mutans	18
Streptococcus salivarius	6
Streptococcus anginosus	3
Streptococcus sanguis	4
Moraxella catarrhalis	36
Staphylococcus epidermidis	26
Staphylococcus saprophyticus	2
Corynebacterium sps	4
Enterococci	5
Micrococcus	2
Haemophilus influenza	1
ANAEROBIC BACTERIA	
Peptostreptococcus anaerobius	4
Peptostreptococcus assacharolyticus	1
Lactobacillus acidophilus	4
Bacteroides fragilis	4
Actinomycetes israelii	2
Actinomycetesodontolyticus	1
Veilonella sps	2
Prevotella melanogenica	2
Fusobacterium nucleatum	2

Figure 2
Total bacterial isolates obtained after third molar surgery



Streptococcus viridians group of bacteria (76.8%) showed highest prevalence followed by Moraxella catarrhalis (43.9%) and Staphyloccus sps(34.1%).

DISCUSSION

Bacteremia is known to result from a number of conservative dental procedures. In the present study, the prevalence of bacteremia was high (82%) when compared to other similar studies. Different rates of bacteremia have been reported after different procedures in dentistry. In a similar study done in Spain by Tomas et al, 2008 the prevalence of bacteremia following third molar surgery was found be (69.2%).[12] Takai et al found the incidence of bacteremia after various oral and maxilla facial surgical procedures to vary from(23% to 58%) [13]. Previous studies by Heimdahl et al and Rajasuo et al, reported that the majority of bacteria isolated from blood cultures after dental procedures are anaerobic[4,7]. However, in our study the majority of the bacteria isolated were aerobic bacteria which are normal oral commensals, probably due to the inclusion of only subjects with moderately healthy oral cavity. In a study based on vascular access related infections by Anandhilakshmi et al, it was found that most of the bacteria isolated from blood were skin micro- organisms, and aerobic bacteria such as *Staphylococcus epidermidis* and *Enterococci* showed the highest prevalence in their study[16], unlike the present study where all the bacteria isolated from blood were normal oral micro –organisms, however in our study also aerobic bacteria showed higher prevalence. This study is in accordance with studies conducted by Tomas et al and D.Babu et al where the aerobic bacteria *Streptococcus viridians* group and *Staphylococcus epidermidis* showed a high prevalence rate after invasive and surgical procedures [12,15]. The Viridans Group of Streptococci was the most frequently isolated bacteria in the present study (76.87%), similar results were obtained by Tomas et al, where Viridans Group of Streptococci was the most frequently isolated bacteria which showed a prevalence of (87.9%).[12]

CONCLUSION

The results of this study indicate that in persons with moderately healthy oral cavities, who undergo invasive dental procedures such as third molar surgery the prevalence of aerobic bacteremia is higher than anaerobic bacteremia. Pre-medication with amoxicillin does not seem to completely prevent transient bacteremia from occurring during dental surgical procedures. The importance of further studies in this field with alternative drugs such as ceftriaxone, ciprofloxacin, azithromycin and moxifloxacin is necessary. Since the oral aerobic commensals are known to be a major cause of infective endocarditis, further detailed research has to be carried out, on the virulence factors and virulence genes of such bacteria, as these virulence factors are thought to facilitate their entry, multiplication and survival in the bloodstream.

REFERENCES

1. N.B.Parahitiyawa., Microbiology of odontogenic bacteremia: beyond endocarditis. Clinical Microbiology Reviews, Vol.22, no.1, 46-64,(2009)
2. Daly C.G.,Bacteremia due to periodontal probing: a clinical and microbiological investigation. Journal of Periodontology ,72, 210-214,(2001).
3. Harald Seifert, The clinical importance of microbiological findings in the diagnosis and management of bloodstream infections. Clinical Infectious Diseases 48:S238-45, (2009)
4. Heimdahl A, Hall G Hedberg M, Sandberg H,Soder Po, Tuner K and Nord Ce, Detection and quantitation by lysis-filtration of bacteremia after different oral surgical procedures. J Clinical Microbiol, 28, 2205-2209,(1999).
5. Peter B Lockhart, Michael T Brennan, Howell C Sasser and Philip C Fox, Bacteremia associated with tooth brushing

and dental extraction. Circulation, 117, 3118-3125,(2008).
6. Debelian Gj, Olsen I and Tronstad L.Systemic diseases caused by oral microorganisms. Endodontics and Dental traumatology, 10, 57-65, (1994)
7. A.Rajusuo, S.Nyfors, A.Kanervo, H.Jousiemies-somer, C.Lindqvist and R.Surronen.,Bacteremia after plate removal and tooth extraction. Int J Oral and Maxillofac.Surg, 33, 356-360,(2004).
8. Hahn, C. L., H. A. Schenkein, and J. G. Tew, Endocarditis-associated oral streptococci promote rapid differentiation of monocytes into mature dendritic cells. Infect. Immun. 73:5015–5021(2005)
9. Allen, B. L., B. Katz, and M. Hook, Streptococcus anginosus adheres to vascular endothelium basement membrane and purified extracellularmatrix proteins. Microb. Pathog, 32:191–204,(2002).
10. Van Dyke, T. E., E. Bartholomew,R. J. Genco, J. Slots, and M. J. Levine, Inhibition of neutrophil chemotaxis by soluble bacterial products. J.Periodontol. 53:502–508,(1982).
11. Chitra.N, Amoxicillin resistance in transient bacteremia after third molar surgeries. Journal of Pharmacy Research,8(3),277-280,(2014)
12. Tomas I, F Pereira, R Llucian, R Poveda, P Diz and Jv Bagan, Prevalence of bacteremia following third molar surgery. Oral Diseases,14, 89-94,(2008).
13. Sumie Takai, Tomoari Kuriyama, Maki Yanagisawa, Kiyomasa Nakagawa and Tadahiro Karasawa,Incidence and Bacteriology of bacteremia associated with various oral and maxillofacial surgical procedures. Oral Surg Oral Med Oral Pathol Oral Radiol Endod, 99, 292-8, (2005).
14. Murray PR, Baron EJ, Pfaller MA, Tenover FC,Yolken RH (eds.) Manual of Clinical Microbiology , ASM press: Washington DC, 2007, 9th edition.
15. D.Babu, N. Reddy, D.Swaroop and K.Babu, Evaluation of bacteremia following periodontal probing in gingivitis and periodontitis patients. The Internet Journal of Dental Scienc, Vol 9(2), (2011).
16. Anandhi Lakshmanan, Radha Madhavan, and Padmanabhan ,Vascular access related infections among haemodialysis patients in Tritiary care centre, Tamilnadu. Int J Pharm Bio Sci , 3(3),(B)7-15(2012).
17. Chitra.N, Moraxella catarrhalis bacteremia associated with third molar surgery. Int J Pharma Bio Sci, Jan;5(1):(B) 609-615,(2014).
18. Rafael Poveda Roda, Yolanda Jimenez, Enrique Carbonell, Carmen Gavalda and Maria Margaix Munoz, Bacteremia originating in the oral cavity. Med Oral Patol Oral Cir bucal,jun 1:13(6),E355-62, (2008).
19. Roberts GJ, Jaffray EC, Spratt D and Petrie A, Duration ,Prevalence and intensity of bacteremia after dental extractions in children,Heart, 92, 1274-1277,(2006).
20. WA Coulter, A Coffey, Idf Saunders and A M Emmerson, Bacteremia in children following Dental Extraction. J.Dent Res, 69(10),1691-1695,(1990).