

**BACTERIAL DIVERSITY OF DENTAL ABSCESS IN PATIENTS WITH
ODONTOGENIC INFECTION AND THEIR ANTIBIOTIC
SUSCEPTIBILITY**

Poonam. B. Chauhan* and Pratibha. B. Desai¹

Dept. of microbiology, K.B.S Commerce & Nataraj Professional Sciences college, Vapi –
396195 (Gujarat), India

Dept. of microbiology, Shree Ramkrishna Institution of Applied Sciences, Athwalines, Surat -
395001 (Gujarat), India

ABSTRACT

Twenty seven patients with odontogenic infection with a mean age of 41.2 years with 59.26% male and 40.74% female were found to be polymicrobial. The sample consisted of both aerobic (36%) and anaerobic (64%) bacteria. The study mainly focused on periapical and periodontal abscess as it was the found to be the prime cause of odontogenic infection. The periapical abscess was found to be 41% and periodontal abscess 59%. Among anaerobes *Peptostreptococcus micros* were found to be prominent followed by *Porphyromonas gingivalis*, *Bacteroides* spp., and *P. aeruginosa* among aerobic bacteria. Results also showed that maximum numbers of isolates were recovered from molar teeth with *Bacteriodes* spp., being the dominant one among anaerobes and *Pseudomonas aeruginosa* among aerobes, whereas in case of incisor teeth *Porphyromonas gingivalis* and *Streptococcus* spp., were found to be the eminent one among the anaerobes and *Staphylococcus aureus* among the aerobic bacteria. Antimicrobial susceptibility test were done against the isolates obtained from the abscesses using Kirby-Bauer disc diffusion technique. All the aerobic isolates were found to be susceptible to doxycycline hydrochloride and all obligate anaerobes were susceptible to tetracyclines.

Keywords: Odontogenic, periapical abscess, periodontal abscess, antibiotic susceptibility, Kirby-Bauer disc diffusion technique

Introduction

An abscess consists of a collection of pus into a cavity formed by tissue liquefaction. All the forms of abscess in the oral cavity like the dental abscess, dentoalveolar abscess, odontogenic abscess results in pus formation around the tooth and these terms are used as synonyms to describe the abscesses. The cause may be an endodontic infection (acute apical abscess) or a periodontal infection (periodontal abscess and pericoronitis). The acute apical abscess is the most common form of dental abscesses. Odontogenic infections can spread to any organ of the body resulting in lethal consequences (Clifton *et al.*, 2011) but mainly results in periapical abscess causing necrosis of the pulp leading to spread of the bacteria to the periapical tissue and periodontal abscess resulting in periodontal pocket and spreading of bacteria in the soft tissue (Stoll *et al.*, 1963; Held *et al.*, 1989).

Being polymicrobial, odontogenic infections can range from three to eight different species of bacteria and in rare cases can result in only one species. The infection is mainly dominated by the mixture of anaerobic and aerobic bacteria, followed by anaerobic and aerobic bacteria (Steven, 2012), where viridians group of streptococci and *Streptococcus anginosus* occur as facultative anaerobes and *Prevotella*, *Porphyromonas* and *Fusobacterium* spp. are found as strict anaerobes (Stefanopoulos *et al.*, 2004; Uluibau *et al.*, 2005; Rega *et al.*, 2006; Sanchez *et al.*, 2011). In spite of the improvement in the socioeconomic status and awareness of oral hygiene, many cases of odontogenic infections have been reported (Fereydoun *et al.*, 2013) For this reasons antibiotic treatment has to be effective in treating the infection (Peterson, 1997; Slots *et al.*, 1993; Thomas 2014) but unfortunately, even though the infections are treated successfully without identifying or knowing the etiological agents and without performing the susceptibility test, proper selection and dose of antibiotic is not done, probably resulting into resistant species (Citron *et al.*, 1991). Thus antibiotic susceptibility test is necessary to determine the appropriate therapy and dosage (level) of the chemotherapeutic agents.

Materials and Methods

The samples were collected from patient suffering with dental abscess from different dental clinics in and around Vapi area. Samples for microbiological examination were obtained using

sterile paper point in sterile transport medium and processed in 4 hours of collection, the medium is vortexed for 30 seconds before further proceeding, the vortex sample were then inoculated in duplicates on various medias like Trypticase soy agar, Fusobacterium agar, Actinomyces agar, Polyethyl agar and Bacteroides bile agar plates and incubated aerobically as well as anaerobically at 37⁰C for 4-5 days. The resultant mixed colonies were further isolated on 5% Anaerobic sheep blood agar and 5% Trypticase blood agar plate in duplicates (Mohapatra *et al*, 2012). The isolates were studied morphologically and processed biochemically as well as enzymatically for identification and preceded for antibiotic susceptibility test using Kirby-Bauer disc diffusion method (Bauer *et. al*, 1996). Zone of inhibition were measured and recorded as being sensitive or resistant. The antibiotic disc (HiMedia) used were Ampicillin/sulbactam (A/S^{10/10}), Norfloxacin (NX¹⁰), Doxycycline Hydrochloride (DO³⁰), Gentamicin (GEN¹⁰), Co-Trimoxazole (COT²⁵), Ampicillin (AMP¹⁰), Roxythromycin (RO³⁰), Colistin (CL¹⁰), Cefotaxime (CTX³⁰), Penicillin-G (P¹⁰), Amoxyclav (AMC³⁰), Azithromycin (AZM¹⁵), Ciprofloxacin (CIP⁵), Amikacin (AK³⁰), Metronidazole (MT⁵), Clarithromycin (CLR¹⁵), Nalidixic Acid (NA³⁰), Tetracycline (TE³⁰), Erythromycin (E¹⁵), Chloramphenicol (C³⁰), Kanamycin (K¹⁰⁰⁰), Streptomycin (S¹⁰), Nethillin (NET³⁰), Clindamycin (CD²), Ceftriaxone (CTR³⁰), Vancomycin (VA³⁰), Ceftazidime (CAZ³⁰), Trimethoprim (TR⁵), Piperacillin (PI¹⁰⁰), Piperacillin/Tazobactam (PIT^{100/10}), Cefuroxime (CXM³⁰).

Statistical analysis was performed using single proportion test.

Results

Dental examinations were carried out in patients with odontogenic infections. In this case it was found that female were affected more (59%) in comparison to male (41%) with maximum numbers of isolates in the age group between 31- 60 years (Figure 1) where periapical abscess (n=11) and periodontal abscess (n=16) were analysed for the bacterial diversity and antibiogram. In the group of patients with periapical abscess, anaerobic bacteria were isolated in only 8 cases and both aerobic and anaerobic bacteria were found in 19 cases. From these 27 patients a total of 44 anaerobic and 23 aerobic bacterial strains were isolated (Table 1). The mean number of

isolates was 2.5 per patient in each case. Of the 44 anaerobic strains isolated, 4 (*Streptococcus* spp.) appeared to be microaerophilic as they were able to grow in 10% carbon dioxide.

Anaerobic gram-negative bacteria were more frequently isolated from patients with periodontal abscess than from patients suffering from periapical abscess ($P < 0.05$). In the group of patients with periapical abscess, it was found that all of the 27 isolates were from the mixed culture in that 17 were anaerobic bacteria (Table 1). *Peptostreptococcus* spp. and *Porphyromonas gingivalis* were more frequently associated with periodontal abscesses than with periapical abscesses ($P = 0.47$), the study recovered 5 genus of anaerobic bacteria (in the absence of aerobic bacteria) of 11 samples in case of periapical abscesses while the number was 7 of 16 samples in case of periodontal abscesses. In this study aerobic gram positive bacteria were more frequently isolated from periapical abscess whereas aerobic gram negative bacteria were more frequent from periodontal abscess. Result also showed that gram positive and gram negative anaerobic bacteria were more frequent in case of periodontal abscess. Moreover, there was more isolation of gram negative bacteria (aerobic and anaerobic) than gram positive bacteria (aerobic and anaerobic).

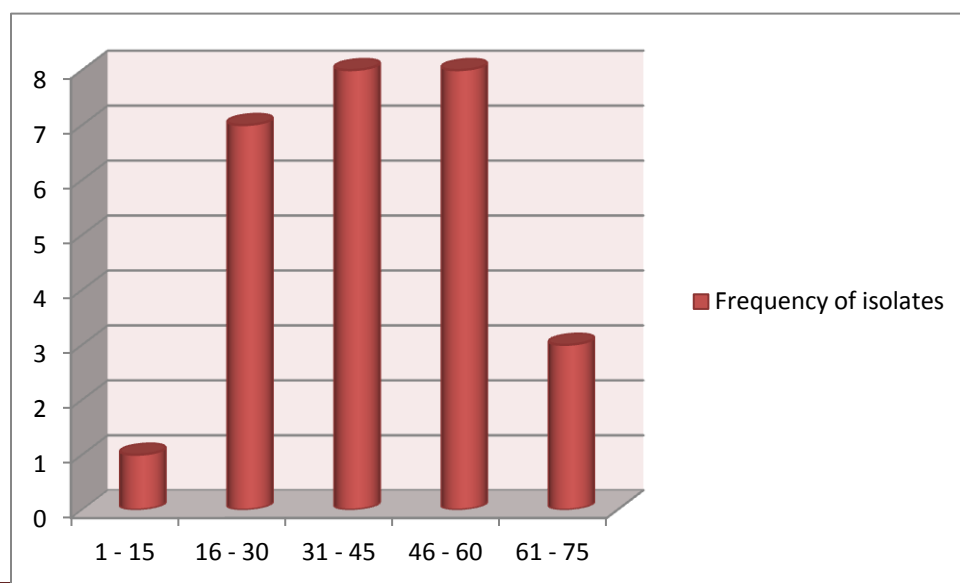


Figure 1: Age-wise distribution of isolates from patients suffering from odontogenic infections

The study revealed that different types of bacteria were isolated from different types of teeth (Table 2). Data showed that maximum numbers of isolates were recovered from molar teeth where *P. aeruginosa* was the most frequent followed by *Bacteroides* spp., *Peptostreptococcus* spp. and *P. gingivalis*. All the 5 isolates from premolar teeth were found to be in equal proportion, *S. aureus* was the prominent one followed by *Streptococcus* spp. and *P. gingivalis* among the 14 isolates from incisor teeth.

The 19 cases of molar teeth yielded 48 isolates of which 15 isolates were from periapical abscess and 33 from periodontal abscess, *P. aeruginosa* was the prominent bacteria in both the cases. The affected premolar from 2 cases harboured 5 bacterial isolates from only periodontal abscess. The 14 incisor samples resulted in 11 isolates from periapical abscess with *S. aureus* being the maximum and 3 from periodontal abscess with *P. gingivalis*, *P. intermedia/nigrescens* and *P. aeruginosa* (1 strain each).

The susceptibility test against the dental abscess microbes were carried out using commercially available antimicrobial discs (Hi-media) and were assayed by Kirby – Bauer disc diffusion method. Studies showed high rate of resistance against macrolide, lincosamide and to some extent to penicillin group of antibiotics. Tetracycline exerted a strong antimicrobial activity against both aerobic and anaerobic bacteria (Table 3 & 4).

Table 1: Number of bacterial strains isolated from periapical and periodontal abscess in patients with odontogenic infections

Bacterial species	periapical abscess (n=11)	periodontal abscess (n=16)
I. Aerobic bacteria	10	13
1. Gram-positive	6	4
<i>Staphylococcus aureus</i>	5	1
<i>Staphylococcus</i> spp.	0	2

<i>Enterococcus faecalis</i>	1	1
2. Gram-negative	4	9
<i>Klebsiella</i> spp	1	3
<i>P. aeruginosa</i>	3	6
II. Anaerobic bacteria	17	26
1. Gram-positive	3	4
<i>Peptostreptococcus</i> spp	3	4
2. Gram-negative	14	23
<i>Porphyromonas gingivalis</i>	1	4
<i>Porphyromonas</i> spp.	2	3
<i>Prevotella intermedia/nigrescens</i>	0	3
<i>Prevotella</i> spp.	0	3
<i>Fusobacterium nucleatum</i>	3	2
<i>F. necrophorum</i>	1	2
<i>Bacteroides fragilis</i>	2	1
<i>Bacteroides</i> spp.	3	2
<i>Streptococcus</i> spp	2	2
<i>Veillonella</i> spp	0	1
Bacteria:	27	40
Gram-positive	9	8
Gram-negative	18	32

Table 2: Microorganisms isolated from different types of teeth

Types of teeth (no.)	Incisor (6)	Premolar (2)	Molar (19)
<i>Peptostreptococcus</i> spp.	1	-	5
<i>Bacteroides</i> spp.	1	1	6
<i>Fusobacterium nucleatum</i>	1	1	3
<i>Fusobacterium</i> spp.	-	-	3
<i>Porphyromonas gingivalis</i>	2	-	4
<i>Porphyromonas</i> spp.	1	1	3
<i>Veillonella</i> spp.	-	-	1
<i>Streptococcus</i> spp.	2	1	1
<i>Enterococcus faecalis</i>	1	-	1
<i>Prevotella nigrescens/intermedia</i>	1	-	2
<i>Prevotella</i> spp.	-	-	3
<i>Klebsiella pneumonia</i>	-	-	4
<i>Pseudomonas aeruginosa</i>	1	-	8

<i>Staphylococcus aureus</i>	3	-	3
<i>Staphylococcus</i> spp.	-	1	1
Total no. of Isolates	14	5	48

Table 3: Activity of different antimicrobial agents against odontopathogen (anaerobes)

Antibiotics	Activity of different antimicrobial agents against odontopathogen (anaerobes)						
	<i>Peptostr eptococc us</i> spp.	<i>Porphyro monas</i> spp.	<i>Prevotell a</i> spp.	<i>Bacteroi des</i> spp.	<i>Fusobact erium</i> spp.	<i>Streptoc occus</i> spp.	<i>Veillonell a</i> spp
Penicillin G	-	-	-	-	-	-	-
Ampicillin	-	-	-	-	-	-	-
Amoxyclav	+/-	+/-	+	+/-	+/-	-	+
Piperacillin	+/-	+	+	+/-	+	+/-	+
Piperacillin/Tazobactam	+/-	+/-	+	+/-	+	+/-	+
Ampicillin/Sulbactam	+/-	-	+/-	-	-	-	+
Cefuroxime	-	-	-	-	-	-	-
Ceftazidime	-	-	+/-	-	-	-	-
Ceftriaxone	+/-	+/-	+/-	+/-	+	+/-	+
Cefotaxime	+/-	+/-	+	+/-	-	+/-	+
Erythromycin	-	-	-	-	-	-	-
Clarithromycin	-	-	-	-	-	-	-
Roxythromycin	-	-	-	-	-	-	-
Clindamycin	-	-	-	-	-	-	-
Amikacin	-	+/-	+/-	-	+/-	+/-	-
Gentamicin	+/-	+/-	+	+/-	+	+/-	+
Streptomycin	-	-	+/-	-	-	-	-
Kanamycin	+/-	+/-	+/-	-	+	+/-	+
Netillin	+/-	+/-	+	+/-	+	+/-	+
Nalidixic Acid	-	+/-	+/-	+/-	+/-	+/-	-
Ciprofloxacin	-	+/-	+/-	+/-	+/-	+/-	-
Norfloxacin	+/-	+/-	+/-	+/-	+	+/-	-
Trimethoprim	-	-	-	+/-	-	-	-
Co-trimoxazole	-	+/-	-	-	-	-	-
Tetracycline	+	+	+/-	+/-	+	+/-	+
Doxycycline Hydrochloride	+/-	+	+	+/-	+	+	+
Chloramphenicol	-	+/-	+/-	+/-	+/-	-	+
Metronidazole	-	-	-	-	-	-	-

- Less than 30% sensitive strains; +/- 30-80% sensitive strains; + more than 80% sensitive strains.

Table 4: Activity of different antimicrobial agents against odontopathogen (aerobes)

Antibiotics	Activity of different antimicrobial agents against odontopathogen (aerobes)			
	<i>Staphylococcus</i> spp.	<i>Pseudomonas aeruginosa</i>	<i>Enterococcus faecalis</i>	<i>Klebsiella</i> spp.
Penicillin G	-	-	-	-
Ampicillin	-	-	+/-	+/-
Amoxyclav	+/-	+/-	+/-	+/-
Piperacillin	+/-	+/-	+	+
Piperacillin/Tazobactam	+/-	+	+	+
Ampicillin/Sulbactam	-	+/-	+	+/-
Cefuroxime	-	-	-	-
Ceftazidime	-	-	+/-	-
Ceftriaxone	+/-	-	+	-
Cefotaxime	+/-	+/-	+	+/-
Erythromycin	-	-	-	-
Clarithromycin	-	-	-	-
Roxythromycin	-	-	-	-
Clindamycin	-	-	-	-
Amikacin	-	-	+/-	-
Gentamicin	+	+	+	+/-
Streptomycin	-	-	-	+/-
Kanamycin	+/-	+	+	+/-
Netillin	+/-	+	+	+/-
Nalidixic Acid	+/-	-	+/-	-
Ciprofloxacin	-	-	-	-
Norfloxacin	+/-	+/-	+	+/-
Trimethoprim	-	-	+/-	-
Co-trimoxazole	-	+/-	+/-	+/-
Tetracycline	+/-	+	+	+
Doxycycline Hydrochloride	+	+	+	+
Chloramphenicol	+/-	+/-	+	+
Metronidazole	-	-	-	-

- Less than 30% sensitive strains; +/- 30-80% sensitive strains; + more than 80% sensitive strains.

Discussion:

In the present study, patient ranged in the age group between 31 to 60 years with preponderance of female patients as compared to that of male patients that conflict from the report of many findings (Bahl *et al.*, 2014; Whitesides *et al.*, 2000; Rega *et al.*, 2006). The oral cavity is characterized by harbouring indigenous microbiota. The ability of microorganisms to colonize the different oral surfaces depends mainly on their binding potential.

Some differences were noted in types and numbers of the bacteria in both the cases i.e. periapical and periodontal abscesses. Mixed infections consisting of strict anaerobic and facultative anaerobic bacteria were observed in periapical as well as in periodontal abscesses as documented in many studies (Kuriyama *et al.*, 2000; Bahl *et al.*, 2014). Aerobic bacteria were more frequently isolated from the patients with periodontal abscesses, and especially *Staphylococcus* spp., was a more common finding. It is well documented that *Staphylococcus* spp. causes abscesses in other parts of the body as compared with oral cavity due to the virulence factors it possesses (Justyna *et al.*, 2011). It is also known that the anaerobic infection is enhanced by aerobic bacteria by lowering the oxidation-reduction potential (Onderdonk *et al.*, 1976).

The present study showed a greater diversity of anaerobic and facultative species and is the characteristic of the abscess flora as reported Brook *et al* (1981). The frequency of *Porphyromonas gingivalis* was highest among other anaerobic gram negative rod which is in accordance as reviewed by Morten Enersen *et al.*, 2013 followed by *Porphyromonas* spp. and *Prevotella intermedia/nigrescens* amongst the patients with periodontal abscess. As documented the pathogenicity is due to the presence of capsule, lipopolysaccharide, fimbriae, outer membrane vesicles, hemolysin, extracellular and cell-bound enzymes (Hofstad, 1984; Leandro *et al.*, 2013; Jin *et al.*, 2013). It was observed that aerobic microorganisms were isolated from about one third of all infections, but then always together with anaerobic bacteria which are in agreement with the findings of von Konow *et al.*, 1981 and 1983.

In a report limited to the bacteriology of periapical abscesses, our result of 2.5 species per specimen agrees with the report of Oguntebi *et al.*, (1982) and closely with the result of Nagendra *et al.*, (2012) of 2.2 isolates per patients, where Brook *et al.*, (1981) and Betsy *et al.*, (1983) recovered an average of 4.9 and 4.5 species per specimen respectively. It is, however,

interesting to note that Gram-positive anaerobic bacteria, especially *Peptostreptococcus* spp., were isolated with high rates in both periapical abscess and periodontal abscess whereas Socransky *et al.*, 1998 reported *Peptostreptococcus* spp. as the second major group in dental abscess.

The present study showed that the isolates are susceptible to large number of antibiotics indicating that these antimicrobial drugs can be used to reduce the incidence and severity of infection and disease. Among the tetracycline group of antibiotics, doxycycline hydrochloride was found to be more effective in comparison to tetracycline (Kennath *et al.*, 2014). It was also noted that these group was effective even against β -lactams resistant bacteria. Also it was noted that second generation quinolones was more effective than first generation quinolone, whereas in the study Arul *et al.*, 2014 found third generation cephalosporin to be more effective than second generation. Penicillin was ineffective against most of the pathogens in odontogenic infections which is in partial agreement to the findings of Kuriyama *et al.*, 2000, where Yuvaraj V, 2015 in his study found penicillin to be eighty percent effective against odontopathogens.

The conclusions of this study are that microbiological specimens from periodontal abscess contain more gram-negative anaerobic rods than do specimens from periapical abscess, and that *Peptostreptococcus* spp. and *P. gingivalis* is the most frequently isolated bacteria in periodontal abscess. Since the result from the present study reveals that majority of the isolates showed less sensitivity against the antibiotics used indicating that patient should be motivated and made aware of the consequences thereby preventing the transformation of microorganisms into deadly strains.

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