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Isolation and identification of anaerobic organisms in dentoalveolar abscess: A descriptive study

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Abstract

Aim: The present study was designed to assess the type of anaerobic microorganisms isolated from the Dentoalveolar abscess and their antibiotic sensitive pattern.

Materials and Methods: A hospital based descriptive cross-sectional study was conducted from January to December 2019; pus was aspirated from Dentoalveolar abscess in 60 patients, the specimen collected, transported anaerobically and was subjected to standard methods for isolation and identification of microorganisms, Antimicrobial susceptibility testing to different agents was carried out using the disc diffusion method, all demographic, clinical, laboratory data and results of cultures were collected for each case. Continuous variables are described as mean \pm SD and were compared using unpaired Student's t-test, for independent Categorical variables are described as (%) compared by chi square test or fisher exact test as appropriate. A p value $<$ 0.05 was considered significant.

Results: A total of 92 anaerobes were isolated, the prevalence of Gram-positive was high ($p < 0.001$) 33 of them were *Peptostreptococcus*, 29 *Peptococcus*, 16 *Fusobacterium*, 8 *Prevotella*, 6 were *Bacteroides*, Metronidazole was the most sensitive drug (100%) followed by Ciprofloxacin, Amoxicillin/clavulanic acid (90%), Clindamycin (85%) and Cefotaxime (75%). The Amoxicillin was 100% resistant.

Conclusions: Anaerobic bacteria are prevalent in Dentoalveolar abscess, they are subjected throughout life to a continuous challenge by antimicrobial agents used in clinical therapy, and high prevalence of bacterial resistance to ampicillin suggests the need for regular antibiotic susceptibility tests and rational use of antibiotics in the management of these infections.

Keywords: Abscess, amoxicillin, bacterial infections, metronidazole

Introduction

Dental or Dentoalveolar abscess is a denomination used to describe localized collection of pus in the alveolar bone at the root apex of the tooth. It usually occurs secondary to dental caries, trauma, deep fillings or failed root canal treatment. Microorganisms are capable of forming biofilms in root canals, hence making application of the "biofilm concept" plausible in such infections. Biofilms form not only within the root canal, but also on the root surface, in the apical region^[1]. After entering the periapical tissues via the apical foramen, these bacteria are capable of inducing acute inflammation leading to pus formation.

The pathogenesis of Dentoalveolar abscess is polymicrobial in nature, comprising of various facultative anaerobes, such as the viridans group streptococci and the *Streptococcus anginosus* group, and strict anaerobes, especially anaerobic cocci, *Prevotella* and *Fusobacterium* species^[2]. The presentation of bacterial infection of dental origin is constantly changing and is a measurable reflection of modern evolution of oral flora^[3, 4]. It has been suggested that, for the pathogenicity of strictly anaerobic gram-positive cocci the possession of a capsule (consisting of two, well characterised polysaccharides) a very important virulence factor of these bacteria^[5]. However, recent studies performed with advanced microbial techniques under strict anaerobic conditions have produced a different picture of flora causing these infections, approximately 5% of aerobic bacteria, 35% are due to anaerobic bacteria and remaining 60% are caused by both aerobic and anaerobic bacteria^[6]. Spreading odontogenic infections (SOIs) are the most common type of serious orofacial infection encountered by oral and maxillofacial surgeons that can spread rapidly through tissue planes, these include mediastinitis, pulmonary complications, sepsis, hypoxia, cardiac arrest and death^[7].

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The mixed flora that is present may also contain many organisms that are inherently resistant to the antibiotics being used, each approach is promising, but significant technical challenges remain for the full implementation of these into everyday clinical practice, because of these challenges, a range of novel approaches have been developed for detecting and treating 1. This study was aimed to isolate the anaerobic bacteria from Dentoalveolar abscess and to evaluate the antibiotic susceptibility of these bacteria.

Materials and methods

Setting and design

This was a Descriptive cross sectional Hospital based study conducted in Bowring and Lady Curzon Hospital, Bangalore for 1 year from January 2019 to December 2019, after obtaining institutional ethical clearance; we included 60 patients who visited the outpatient department of oral medicine and radiology for treatment and confirming about their participation without dropping out from the study. Purposive (Deliberate) critical case sampling was done to avoid bias. We included patients aged between 15 to 65 years with Dentoalveolar abscess with intraoral or extra oral swelling; (Figure 1) exclusion criteria included previous endodontic therapy of the affected tooth, teeth with periapical sinus/fistula, any generalized disorder or antibiotic therapy with in previous two months.

A brief case history was recorded and if clinical findings satisfied the inclusion criteria, the patient was informed about all the procedures to be performed during the study. Following that, if patient was ready to be a part of the study, the patient was asked to sign the consent form.

Procedure was explained to the patient. Patient was seated on a dental chair and draped with a patient drape. Patient was asked to rinse mouth with water. For each patient, the oral mucosa overlying the abscess was scrubbed with tincture of iodine; a sterile 18- gauge needle fitted to a 3-ml disposable syringe was passed through the alveolar mucosa into the abscess, from which the contents are withdrawn. The needle was sealed immediately by cork (Figure 2). The samples are transported to microbiology laboratory held at room temperature and processed within 30 mins.

Sample processing was done in the following way: Direct microscopy for Bacterial morphology: A thin smear of the pus sample was made on a clean glass slide and allowed to air dry. Smear was gently heat fixed, stained by Gram staining technique and examined under oil immersion objective of the light microscope, for the presence of pus cells, Gram positive and Gram negative organisms. The size, shape and arrangement of bacteria were noted.



Fig 2: Sample in the syringe with widen cork

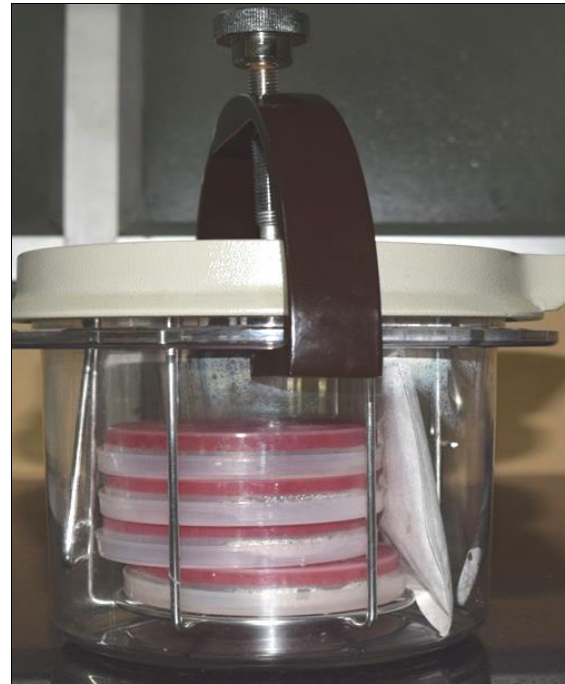


Fig 3: Anaerobic jar with plates and Gaspak



Fig 4: Growth of organisms



Fig 1: Dentoalveolar abscess in anterior palatal region

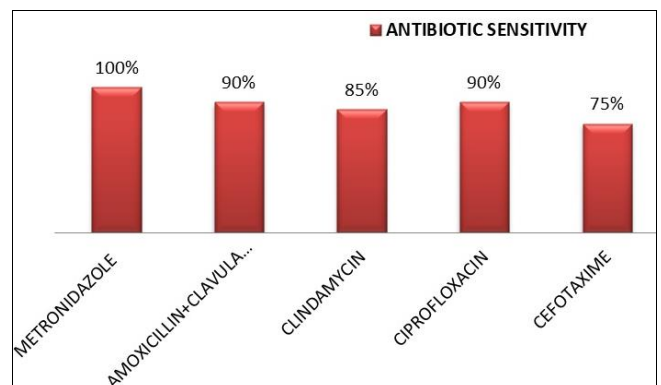


Fig 5: Antibiotic sensitivity pattern

Anaerobic culture

The sample was inoculated onto Brucella blood agar containing vitamin K and Hemin in which a Metronidazole disc was placed at the junction of primary and secondary streaking to identify the anaerobes presumptively. A MacConkey agar plate streaked with *Pseudomonas aeruginosa* ATCC strain which is an obligate aerobe was used as a negative control for anaerobiosis. The plates were immediately incubated anaerobically for 48 hours at 37°C in an anaerobic jar (Figure 3) (Hi media Anaerobic System Mark II LE 002 3.5L) with Gaspak (BD GasPak EZ Gas Generating Container Systems with indicator).

After 48hrs plates were examined for growth and colony morphology of different colonies were recorded. (Figure 4) Transmitted light was used to look for hemolysis. Gram stained smears of the colonies were examined under oil immersion objective of the microscope. Catalase and Spot indole test were performed. Each colony were then subcultured onto brucella blood agar plates and based upon gram staining Nitrate disk, Bile esculin disk and Special potency disk were used for gram negative, Sodium polyanethol sulphonate (SPS) disk was used for gram positive and the plates were incubated in the anaerobic jar with Gaspak at 37°C for 48 hrs. Aerotolerance test was done to differentiate between obligate and facultative anaerobes. Zone of inhibition were measured after incubation and results were interpreted. Biochemical tests (catalase, Nitrate disk reduction test, coagulase, Bile test, Spot indole test, Sodium polyanethol sulphonate test, and Special potency antibiotic disc susceptibility) were performed.

Antimicrobial susceptibility testing was performed anaerobic isolates, by disc diffusion method. Colonies of bacteria are spread over Mueller-Hinton agar medium. Discs impregnated with antibiotics are placed by the help of sterilized forceps. This plate was again incubated for 12–24 h at 37°C. Zone of inhibition is measured by the help of the WHO quality control chart to access the sensitivity of the antibiotics (Amoxicillin, Amoxicillin and clavulanic acid, Clindamycin, Cefotaxime, Metronidazole, Ciprofloxacin, antibiotics were selected for testing).

Statistical analysis

Data are expressed as means \pm SD or percentages. To compare continuous variables, we used the unpaired Student's *t* test, Mann Whitney *U* test and Wilcoxon test. For categorical variables, we applied the Chi-square or Fisher's exact test. Comparison of proportions was performed using the Fisher's exact test. "P value" is considered to be significant if <0.05 . The Statistical software namely SAS 9.2, SPSS 15.0, Stata 10.1, MedCalc 9.0.1, Systat 12.0 and R environment ver.2.11.1 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc.

Results

The study population comprised of 60 patients, there were 33 males and 27 females (Table-1) Among 60 patients

constituting the study group, 54 cultures had positive results, and 6 cultures had no growth (Table -2), Total isolates obtained were 92, among that 6 cultures revealed no growth, 16 cultures revealed one isolate, 38 cultures revealed 2 isolates, among the 92 isolates obtained, 62 of them were Gram +ve, 30 were Gram -ve (Table - 2)

Among the 92 isolates obtained, the prevalence of Gram-positive was high ($p < 0.001$), 33 of them were *Peptostreptococcus*, 29 of them were *Peptococcus*, 16 of them were *Fusobacterium*, 8 of them were *Prevotella*, 6 of them were *Bacteroides*. (Table- 3)

Incidence of Positive cultures among the male and Female (Table-4) was not significant ($p=0.8482$) similarly the Incidence of Negative cultures (Table-5) was also not significant ($p=0.5657$)

Peptostreptococcus spp (35.86%) was isolated in 33 instances in which Metronidazole was the most sensitive drug (100%) followed by Amoxicillin/clavulanic acid (87.2%), Clindamycin (84.6%), Cefotaxime and Ciprofloxacin (71.8%) each. The most resistant drugs were amoxicillin (100%). *Peptococcus* (31.5%) was isolated in 29 instances in which Metronidazole was the most sensitive drug (100%) followed by Amoxicillin/clavulanic acid (90.5%), Clindamycin and Ciprofloxacin (88.9%) each, Cefotaxime (79.4%). The most resistant drugs were amoxicillin (100%).

Among the entire anaerobically cultured bacteria, Metronidazole was the most sensitive drug (100%) followed by Ciprofloxacin, Amoxicillin/clavulanic acid (90%), Clindamycin (85%) each and Cefotaxime (75%). The least effective drug was amoxicillin (100%) (Figure 5)

Table 1: Demographic Data

Parameter	Group
Number of patients	60
Age (average)	36
Sex (M:F)	33:27 (55%:45%)
Average isolates per patient	1.533

Table 2: Culture Results

Finding of culture	Number of specimens	Percentage (%)
Total positive cultures	54	90%
Total negative cultures	6	10%
1 isolate per culture	16	26%
2 isolates per culture	38	60%
Gram +ve	62	67%
Gram -ve	30	33%

Table 3: Incidence of Various Anaerobic Bacterial Isolates

Isolated	Number of isolates	Percentage (%)
Gram Positive		
Peptostreptococcus	33	35.86
Peptococcus	29	31.52
Gram Negative		
Fusobacterium	16	17.4
Prevotella	8	8.7
Bacteroides	6	6.52

Table 4: Demographic Comparison of Positive Cultures rates

Total (60)	Positive cultures	Incidence rate	95% Confidence Interval	Incidence rate difference	Incidence rate ratio	P-value
Male(33)	29	0.8788	0.5885 to 1.2621	-0.04714	0.9491	0.848
Female(27)	25	0.9259	0.5992 to 1.3668			

Table 5: Demographic Comparison of Negative Cultures rates

Total (60)	Negative cultures	Incidence rate	95% Confidence Interval	Incidence rate difference	Incidence rate ratio	P-value
Male(33)	4	0.1212	0.033 to 0.3104	0.04714	1.6364	0.5657
Female(27)	2	0.07407	0.0089 to 0.2675			

Discussion

Procedural Aspects- In microbiological studies involving Dentoalveolar abscess, the investigators were initially faced with a number of methodical problems such as

- Specimen contamination by non-involved oral flora.
- Loss of certain microorganisms during their transport.
- Various handling procedures and,
- An inability to culture certain fastidious microorganisms due to inappropriate atmospheric conditions and culture media used.

Since Dentoalveolar abscess are induced by microorganisms that are part of the normal oral microflora, it is therefore extremely important to disinfect the mucosal surface overlying the abscess, thus, a variety of sampling techniques have been used in past studies, previous studies have used iodine solution for surface disinfection^[8, 9]. Few used 70% ethyl alcohol solution^[10, 11].

In our study for each patient, the oral mucosa overlying the abscess was scrubbed with tincture of iodine; a sterile 18-gauge needle fitted to a 3-ml disposable syringe was passed through the alveolar mucosa into the abscess, from which the contents are withdrawn, the needle was sealed immediately by cork.

Specimen transport

Most studies do not describe the method used in transporting the specimen from the patient to the laboratory for culture; an ideal transport medium keeps the microbes alive and preserves their proportions in the sample.

In our study after the aspiration the needle was sealed immediately using wooden cork, the specimens were transported in the same syringe to microbiology laboratory and processed within 30 mins, this method was employed in the previous studies^[12, 13].

Culture techniques

The majority of early investigation on dental periapical abscesses actually failed to utilize adequate anaerobic culture techniques^[14].

Shah A *et al.* used culture media containing chocolate agar or Mac Conkey agar medium. Plates were incubated at 37°C for 12–24 h in an aerobic atmosphere.^[15] In a study by Kityamuwesi R *et al.* specimen was immediately inoculated in a transport medium, Soybean casein digest broth - BD BACTEC Plus + Anaerobic/F Medium.^[11] Habib A *et al.* inoculated swab immediately into a tube of thioglycollate broth, the specimens were incubated for 24 hours at 37°C. Then sub cultured onto 2 solid agar plates, one blood agar plate for aerobic incubation for 24 hours and one brain heart infusion agar for anaerobic incubation for 48-72 hours^[16]. The use of an anaerobic chamber is currently considered the best technique available for recovery of stringent anaerobes since specimens and cultures can be protected from oxygen at every stage of the procedure.

Specimen processing and Identification- all the anaerobic and aerobic plates were examined. The colonies of bacteria were identified by their macroscopic and microscopic appearance. Gram smear and aerotolerance testing was done. If the colony

grows on aerobic culture, it is unlikely to be an obligate anaerobe (except *Clostridium septicum* and *Actinomycetes*). If no growth occurs on aerobic culture, the organism is presumptively identified by means of Gram's reaction, colony characters, and available biochemical tests. Anaerobic bacteria were further cultivated on Anaerobic agar with addition of 50 mg/ml kanamycin to selectively inhibit facultative anaerobes and aerobes while permitting growth of strict anaerobes^[11]. Biochemical tests (catalase, oxidase, coagulase, indole test) were performed to identify the species^[16]. In our study isolates were identified based on microscopic characteristics, aero tolerance test, and colonial characteristic and biochemical tests comparable with previous studies^[17]. Significant improvement in the routine diagnostic yield from acute dental abscesses has occurred with employment of meticulous specimen collection and processing on selective and nonselective agars under appropriate atmospheric conditions. However, despite the close attention to detail, it is apparent that many genera of bacteria have yet to be cultured. The studies using swabs of purulent material have demonstrated poor recovery of strict anaerobes and low mean numbers of isolates per sample (range 1.0-1.1). Major limitation of past cultural studies is that a large percentage of the oral microflora does not grow on conventional artificial culture media in the laboratory.^[18] Our study has revealed 90% positive cultures of anaerobic microorganisms (54/60 cases). A total of 92 isolates were recovered, accounting for 1.5 isolates per specimen. This finding is comparable with other workers^[16, 17].

Our study has revealed *Peptostreptococcus* (35.8%), *Peptococcus* (31.5%), *Fusobacterium* (17.4%), *Prevotella* (8.7%), *Bacteroides* (6.5%), regarding aerobic isolations, the finding are comparable to other workers but the proportion of *Peptostreptococcus* (35.8%) isolation is much more than that reported by other workers. In studies *Fusobacterium* is frequently reported in infections of the head and neck with reports indicating that *Fusobacterium* species can be detected in up to 52% of specimens^[19].

It is worth visualizing, that isolated microorganisms are not same in each case but differ from one individual to other, i.e., *Bacteroides*, *Fusobacterium*, *Peptostreptococcus*, *Peptococcus* and *Eubacterium* and these bacteria have been found out to be the main causative agent of endodontic infection^[20]. A study by Habib A *et al.*, showed 87 patients with (58.0%) had anaerobic bacterial infection. 71 patients (47.3%) had single bacterial isolate and 16 patients (10.7%) had multiple bacterial isolates. The most common isolated organism was *Prevotella* spp. (63 patients 42%).^[16] Among the entire anaerobically cultured bacteria, Metronidazole was the most sensitive drug (100%) followed by Ciprofloxacin, Amoxicillin/clavulanic acid (90%), Clindamycin (85%) each and Cefotaxime (75%). The least effective drug was amoxicillin (100%)

Kityamuwesi R *et al.* concluded that all Viridans Streptococci isolates were resistant to penicillin G, ampicillin and tetracycline, but retained susceptibility to vancomycin, all *Staphylococcus aureus* strains were resistant to cotrimoxazole and, but susceptible to vancomycin, and amoxicillin/clavulanate. All the gram negative isolates were susceptible to

amikacin and imipenem, but had poor susceptibility rates to ceftazidime, cotrimoxazole and ampicillin [11].

In a study by Rugarabamu SE *et al.*, majority of these organisms were susceptible to β -Lactam and β -lactamase inhibitor antibiotics such as Penicillin, Clindamycin, Metronidazole, Cefalosporin and Carbapenem [17]. Habib A *et al.*, isolated *Peptostreptococcus* spp (26.7%) in 40 instances in which Metronidazole was the most sensitive drug (100%) followed by Amoxicillin/clavulanic acid (87.2%), Clindamycin (84.6%), Cefotaxime and Ciprofloxacin (71.8%) each. The most resistant drugs were amoxicillin (100%). Prevotellaspp (42%) was isolated in 63 instances in which Metronidazole was the most sensitive drug (100%) followed by Amoxicillin/clavulanic acid (90.5%), Clindamycin and Ciprofloxacin (88.9%) each [16].

Conclusion

Anaerobic bacteria are prevalent in Dentoalveolar abscess, Furthermore, as commensal organisms anaerobes are subjected throughout life to a continuous challenge by antimicrobial agents used in clinical therapy, resulting in selection of resistant strains. Because the emergence of clinically significant antimicrobial resistance may complicate the outcome of head and neck infections, the susceptibility patterns of anaerobes have profoundly influenced therapeutic decisions in this context in recent years, with a major impact on the antimicrobial therapy of Dentoalveolar abscess.

Future scope

The bacteriological studies have shown variations in the microbiology of acute dental infections, further studies required on regular basis with advanced microbial techniques to isolate & test antibiotic susceptibility of microorganisms in order to provide guidelines for use of proper antibiotics in the management of these infections.

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