

Bacteriology of Periapical Abscess and Antibiotic Susceptibility Pattern of the Facultative Anaerobes Isolated at Gandaki Medical College, Pokhara, Nepal

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Abstract

Periapical abscess is a commonly occurring infectious state to the health sector. Dental abscess and its consequences possess a substantial burden on communities, health care system, and individuals promising early diagnosis and relevant treatment. Spreading of a dental abscess might lead to a significant morbidity and mortality. A prospective hospital based study at Gandaki Medical College Teaching Hospital and Research Center Pvt. Ltd. was carried out to study the facultative anaerobes of periapical abscess and their antibiotic susceptibility pattern. Isolation of etiological agents was performed and causative agents were identified. Antimicrobial susceptibility testing was carried for isolates according to the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) by disk diffusion method. A total of 87 samples were studied and out of which *Streptococcus* sps. 56 (67.37%) and *Staphylococcus* sps. 31 (35.63%) were selected. Vancomycin was found to be the most effective antibiotic with 91.95% susceptibility, followed by Gentamicin (90.80%), Amoxicillin (87.35%), and Penicillin (68.96%). Metronidazole was found to be least sensitive with 36.78% susceptibility. The involvement of facultative anaerobes in causation of periapical abscess and the empirical therapy used at present are still susceptible to first-line β -lactam agents. Still a proper knowledge of these antimicrobial patterns in treatment is an actively involving process in order to address the antibiotic prescribing procedure.

Keywords: Dental Abscess; Facultative Anaerobes; Antibiotics, β -lactam

Introduction

Until 1990s dental abscess was not considered a medical issue and the clinical diagnosis was outlined topic. Dental abscess is formed from a collection of pus developed inside the teeth, gum, or in the bone holding the teeth. Generally, there are 3 different types of dental abscess viz Gingival, periodontal, and periapical. Periapical abscess and focal inflammation of the tooth root occurs by penetration of bacteria into the pulp area, mainly of preceding dental caries and plaque formation on the site, which may allow the entry of bacteria into the soft tissues of tooth [1]. Localization of pus in the alveolar region at the root apex of tooth is a clinical definition used to explain dentoalveolar abscess. An abscess at the end of a tooth is called periapical abscess. Primarily, dental infection is caused by trauma, dental caries, deep fillings or failed root canal treatment and then comes dental abscess infection. The abscess is typically associated with a bacterial infection, often accumulated in the soft pulp of the teeth. Bacterial agents colonized at the root canals occurring with the different dental infection. These bacteriological agents can easily form biofilms in the root canals and form abscess [2]. In case of periapical abscess, bacteria enter the tooth via tiny holes formed by tooth decay or caries. As a consequence, the hole will eventually penetrate the soft inner pulp of the tooth and become infected forming pulpitis. The progression of pulpitis open the way for bacteria to the alveolar bone leading to periapical abscess.

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Various bacteriological agents causing dental abscesses consist of the mixture of strict anaerobes and facultative anaerobes. About 20% of the dental abscess are solely caused by strict anaerobes and varies (6 - 60%) upon the recovery condition [3,4] and mixed aerobic dental infections are uncommon (6%) [4]. Polymicrobial characteristics of such dental infection and availability of cultivable and uncultivable microorganisms might create problems in diagnosis at microbiology laboratories.

Common dental infection causing genera consist of anaerobic *Streptococci*, *Fusobacterium* and followed by the black-pigmented anaerobes such as *Prevotella* and *Porphyromonas* species [5] and facultative anaerobes of viridans group *Streptococci* and the anginosus group *Streptococci*. *Staphylococcus aureus* has been frequently reported from acute dental abscess, ranging from 0.7% to 15% [3,4]. Antibiotics are never considered as an alternative for surgical drainage. The maintenance of an airways and abscess drainage is sine qua non condition. The period of infection and associated bacteremia risk can be reduced by taking antimicrobial therapy right after of diagnosis and before surgery drainage.

This prospective study was carried out to find the microbiology of periapical abscess of facultative anaerobes isolated and antibiotic pattern of the those isolates from the patients visiting Gandaki Medical College Teaching Hospital and Research Center, College of Dental Science, Pokhara, Nepal.

Materials and Methods

Materials

Nutrient agar, Mueller Hinton agar, Mannitol salt agar base, Metronidazole, Cefoxitin, Penicillin-G, Amoxicillin, Gentamicin, and Vancomycin were purchased from Himedia (Mumbai, India). Disposable cotton buds were purchased from Savas enterprises (Delhi, India). All the reagents used were of highest analytical grade.

Radiographic examination of periapical abscess

Only the patients having periapical abscess were included in our study. The diagnosis of periapical abscess was done on physical examination by inspection of the oral cavity and the site where the patient reports pain and swelling. However, the periapical lesion may not be easily identified because of tissue destruction created by inflammation and infection. Beside physical examination, we used radiographical identification to exclude other localization. X-rays initially help to identify the exact site of lesion.

Sample collection

A total of 87 samples were collected from the patients visiting Dental Science Department, Gandaki Medical College Teaching Hospital and Research Center Pvt. Ltd, Pokhara, Nepal. Correct and appropriate methods of sampling were employed for the collection of causative pathogens involved in the development of periapical abscess. Selection of sample type and appropriate method of sampling are crucial to diagnostic values. Purulent material through intact mucosa was squeezed after disinfection by antiseptic mouthwash (chlorhexidine) and collected with a disposable cotton swab. Application of antiseptic and appropriate method of sample collection were followed to reduce contamination from the normal oral flora.

Isolation and screening of microorganisms

Conventional identification method of culturing was employed for significant diagnostic yield under appropriate laboratory conditions on artificial culture media (Nutrient agar). Processing on artificial culture was done by incubating the microorganism at 37°C for 48 - 72h. Microorganisms grown was gram stained and further identified by biochemical characterization.

Microbiology of facultative anaerobes causing periapical abscess

Gram-staining was performed for the presumptive identification of the pathogens. Gram staining was carried according to standard technique using acetone-alcohol (1:1) as decolorizer. Appropriate biochemical tests were employed for the confirmatory identification of the pathogens. Microorganisms with grams positive reaction having cluster-like appearance and in chains were selected for further investigation. Microorganisms were selectively cultured on mannitol salt agar and blood agar. The cultured plates were incubated at 37°C. Colony morphology and biochemical characteristics were studied. The pure isolated colonies from culture plates were picked up and inoculated onto various biochemical medium for different biochemical tests.

Purity test

The inoculums used for the different biochemical tests were pure culture. The purity test was used to ensure that the inoculums used for the biochemical tests was pure culture. During the experiment, the same inoculum was sub-cultured on non-selective medium, before and after performing biochemical test, in order to confirm the purity of the inoculums.

Antibiotic susceptibility pattern of facultative anaerobes causing periapical abscess

Antibiotic susceptibility pattern was analyzed with Metronidazole, 5 µg (Himedia, India), Cefoxitin, 30 µg (Himedia, India), Penicillin-G, 10 µg (Himedia, India), Amoxicillin, 10 µg (Himedia, India), Gentamicin, 10 µg (Himedia, India), and Vancomycin, 30 µg (Himedia, India). Disc diffusion method was employed for determining the susceptibility pattern of the antibiotics used. Mueller Hinton agar plate was lawn cultured with appropriate pathogens. Antibiotic discs were applied and incubated at 37°C for 24 - 48h. Result interpretation was done on the basis of bacterial lysis around the antibiotic discs. Larger the zone of inhibition, more sensitive the antibiotic is.

Quality control of the test

Quality control plays one of the important roles for correct result interpretation. Our experiment was carried at strict quality control. While using readymade dehydrated media, the manufacturer’s instruction for preparation, sterilization and storage were followed to prevent the alteration of the nutritional, selective, inhibitory and biochemical properties of the media. For strain and reagents, whenever a new batch of the same were prepared, a control smear was stained to ensure correct staining reaction. During experiment, quality control was applied at various steps as recommended by CLSI guidelines.

Results

During the study period patients who had clinical features suggestive of periapical abscesses with or without risk factors were studied. Patients visiting our hospital setting with intense, throbbing or sharp-shooting pain at the site of the abscess formation and complaining of tenderness when pressure was applied to the affected tooth and chewing on the side of the abscess and intraoral swelling observed on physical examination were selected for our study [6]. In some of the severe conditions, facial asymmetry was observed because of intense swelling. Radiographic findings (Figure 1) helped us to exclude other localization beside periapical abscesses from other forms. X-rays findings also help in identifying the exact site of lesion of periapical abscesses.

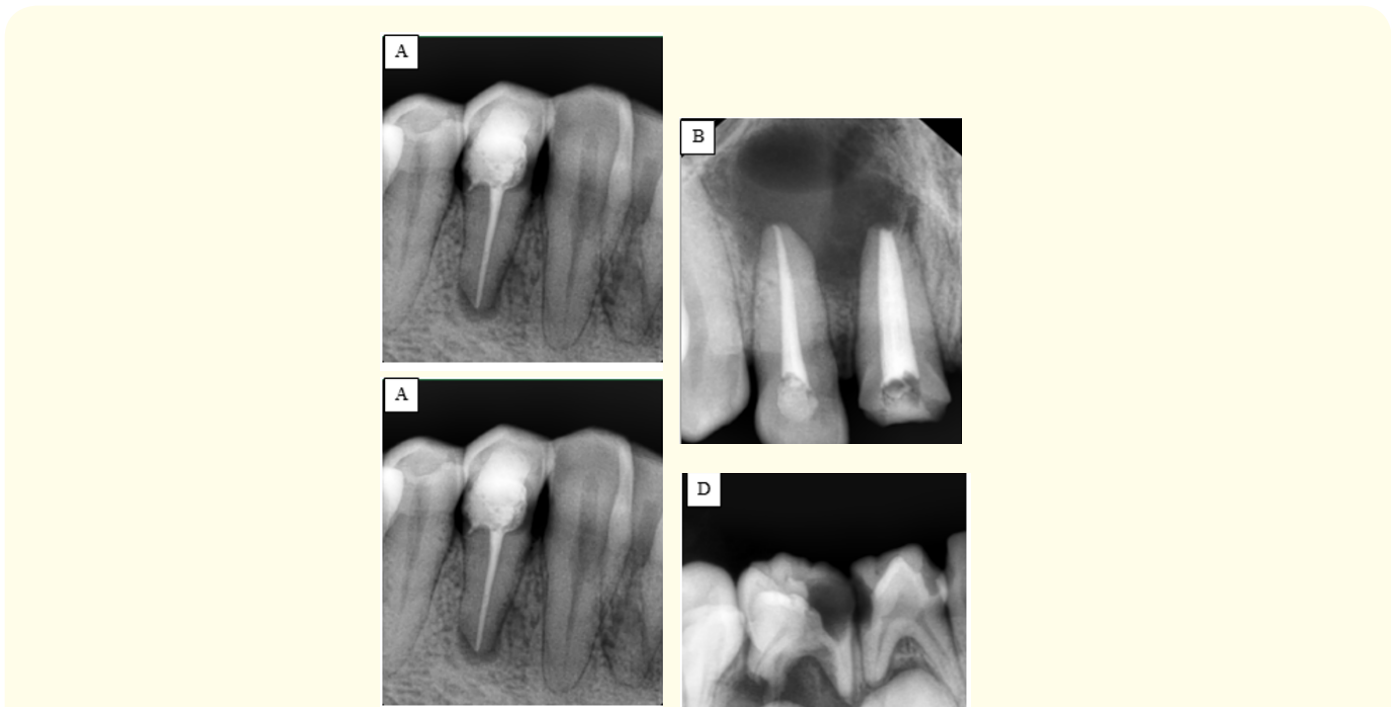


Figure 1: Radiographic examination. A: Periapical radiolucency in an endodontically treated lower right canine, B: Large periapical radiolucency indicative of chronic periapical abscess in relation to upper right central and lateral incisors, C: Retained root stumps of lower left first molar with periapical radiolucent area, D: Carious lower right deciduous second molar with periapical abscess and resorbed roots.

Due to polymicrobial nature of such infection and problem of cultivable and uncultivable microbes, we selected microorganisms with Gram positive reaction having cluster-like appearance and in chains were only selected for further investigation (Figure 2).

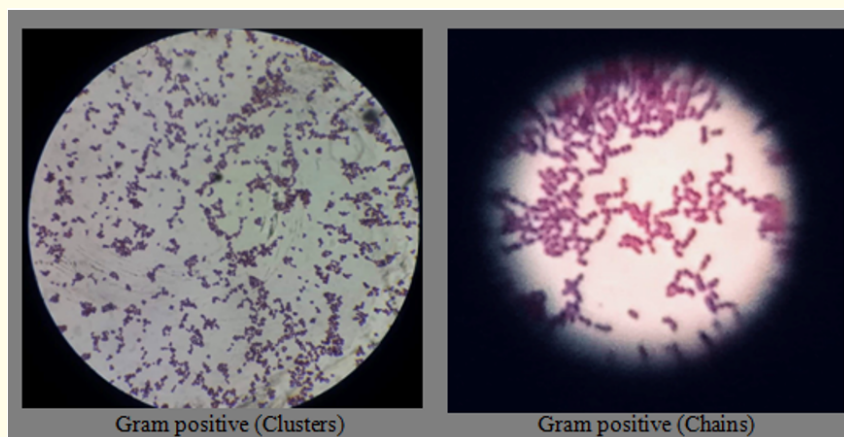


Figure 2: Gram stained microorganism in clusters and chains.

Out of 87 samples suspected of periapical abscesses, *Streptococcus* spp. (67.37%) were isolated twice the *Staphylococcus* spp. (35.63%). Facultative anaerobes that belong to the viridans group *Streptococci* and the anginosus group *Streptococci* are very common present in dental abscess as well as *Staphylococcus aureus* has been frequently reported from acute dental abscess, ranging from 0.7% to 15% [3,4]. The spectrum of microorganisms causing periapical abscesses is shown in table 1.

Etiological agents	Total No. (%)
<i>Streptococcus</i> spp.	56 (64.37)
<i>Staphylococcus</i> spp.	31 (35.63)
Total	87 (100)

Table 1: Spectrum of microorganisms causing periapical abscesses.

It is recommended by world health organization that every unit identify the etiological agents and antimicrobial susceptibilities to select an appropriate regime for treatment. Figure 3 shows the antibiotic pattern of *Staphylococcus* spp. Antimicrobials must never be considered as the replacement of surgical drainage or debridement. In most of the case, antimicrobial therapy started soon after diagnosis and before surgery can reduce the time of infection and minimize the chance of bacteremia.

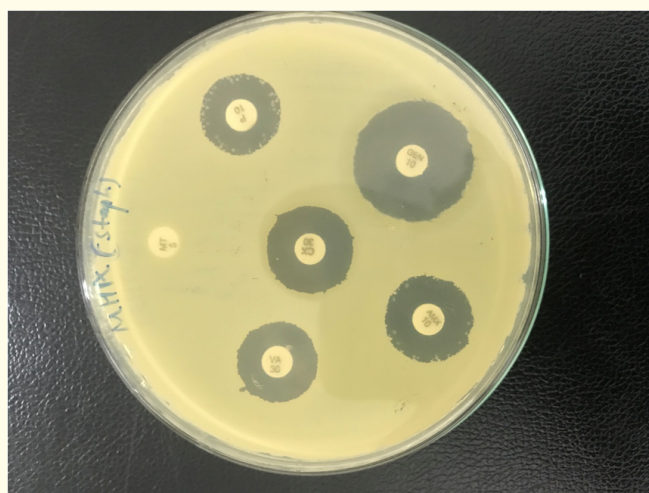


Figure 3: Antibiotic susceptibility pattern of *Staphylococcus* spp.
 P: Penicillin (10 µg); GEN: Gentamicin (10 µg), AMX: Amoxycillin (10 µg), CX: Cifoxitin (30 µg), VA: Vancomycin (30 µg), MT: Metronidazole (5 µg).

In our study (Table 2), Vancomycin was found to be the most effective antibiotic with 91.95% susceptibility, followed by Gentamicin (90.80%), Amoxicillin (87.35%), and Penicillin (68.96%). Metronidazole was found to be least sensitive with 36.78% susceptibility. Amoxicillin remains the antimicrobial of first choice and if resistance to amoxicillin then the use of either metronidazole [7]. or amoxicillin in combination with clavulanic acid [8] are considered as alternative to empirical therapy of first-line β -lactam agents. Till now, empirical therapy to periapical abscess suggests that most isolates are susceptible to first-line β -lactam agents.

Microorganisms	No.	Pen	Gen	Amx	Cif	Van	Met
<i>Streptococcus</i> sps.	56	75	94.64	91.07	-	89.28	30.35
<i>Staphylococcus</i> sps.	31	58.06	83.87	80.64	77.41	96.77	48.38

Table 2: Antibiotic susceptibility pattern of the bacterial isolates.

Pen: Penicillin; Gen: Gentamicin; Amx: Amoxicillin; Cif: Cefoxitin; Van: Vancomycin; Met: Metronidazole; “-”: Not tested.

Discussion

Nowadays, oral infections have been recognized as one of the priority-based health consequences. In the late stages of infections, they are very severe and economical loss to treat [9]. Periapical abscess refer to the virulence of bacteria, host resistant factor, and surrounding structures most commonly on the grounds of dental caries and tooth decay. Serious conditions arising from the spread of dental abscess might lead to significant morbidity and mortality. Periapical abscess is polymicrobial. Many of these microorganisms are commensal hosts of the oral flora, entering the pulp area and leading to the formation of abscesses during the tooth is breached or mechanical trauma [10]. As a consequence, various developmental and acquired clinical conditions have been linked with periapical abscess infections, such as abnormal development of enamel (dens invaginatus/dens evaginatus) and dentin malformation as observed in various dental infections (such as dentine dysplasia, dentinogenesis imperfect, familial hypophosphatemia, and osteogenesis imperfect). Sometimes, acquired cases might include buccal cysts that become infected [11]. The variable microbiological and clinical manifestation pattern of periapical abscesses requires the need for a perspective study of the causative agents and their drug susceptibility pattern to combat with the consequences.

Etiological agents involved in causation of dental abscesses consist of the complex mix of strict anaerobes and facultative anaerobes. Depending upon the cultural conditions and recovery, strict anaerobes and facultative varies by a ratio of 1.5 - 3:1 in mixed infections [12,13].

Antimicrobial resistance is becoming a burning global problem to control multi drug resistance. Antibiotics resistant are generally regarded as major public health conditions and have relevant implication on health and patient care. Resistance to drugs is associated with high morbidity and mortality, economic burden and sometime prolonged hospitalization. World health organization and the European Commission have identified the significance of studying the emergence and determinants of resistance pattern and the need for relevant strategies mechanism for its control.

Conclusion

Early diagnosis and proper treatment are pivotal for the management of a periapical abscess, since this might lead to loss of involved tooth. The involvement of facultative anaerobes in causation of periapical abscess and the empirical therapy used at present are still susceptible to first-line β -lactam agents.

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