Bacteria Identification from Chronic Apical Abscess and It's Sensitivity on Paste and Gel Calcium Hydroxide

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Abstract

Bacteria that can be found in root canal with chronic apical abscess consist of some Streptococcus and Staphylococcus species. They can be eliminated with calcium hydroxide by releasing the hydroxyl ion.

The research objective are identify species from Staphylococcus spp. and Streptococcus spp. in root canals which diagnosed chronic apical abscess and investigate their sensitivity against calcium hydroxide in paste and gel preparation.

The methods of this research is true experimental. The sample were taken from the root canal with chronic apical abscess. Streptococcus spp. were identified with RAPID STR and Staphylococcus spp. were identified with RAPID STAPH PLUS. All the samples were tested with calcium hydroxide in paste and gel for measure the diameter of inhibition zones. Six species of Staphylococcus spp. identified are member of coagulase negative Staphylococcus (CoNS), one species of Streptococcus spp. identified is a member of viridans streptococci. Calcium hydroxide in paste and gel preparation produces antibacterial effect with similar inhibition zone diameter on Streptococcus spp. and Staphylococcus spp that have been identified. Streptococcus spp. and Staphylococcus spp. that identified found from the root canal of chronic apical abscess are highly sensitive and have similar sensitiveness to calcium hydroxide in paste and gel preparation.

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Introduction

Dental abscess comprise of the complex mix of strict anaerob and facultative anaerob.[1] Chronic Apical Abscess is described as an inflammatory response to pulpal infection and necrosis characterized by a progressive onset, minimal or no pain, and occasional pus drainage via an associated sinus tract. Clinical symptoms are generally absent in a tooth with a recurrent apical abscess. Pulp vitality measurements will be negative, and the radiograph will have an apical radiolucency. Generally, the tooth is not sensitive to biting pain, although it can "feel different" to the patient as percussion is applied.[2] A chronic periapical abscess is a defense mechanism to the infection that attempts to prevent it from spreading to other areas of the body. Microorganisms that invade the root canal system are crucial in initiating and perpetuating periapical diseases, especially anaerobic bacteria that dominate the root canal microflora.[3]

Bacterial infection of the dental pulp ruins the pulp and then stimulates an inflammatory cell response and bone destruction in the periapical.[4,5] Infections of root canals are polimicrobial infections dominated by anaerobes.[6] Numerous medicaments have been widely used to treat chonic periapical inflammation in the root canal. Changes in the variety of bacteria isolated from a root canal with chronic periapical abscess and antibiotic sensitivity have been observed over time. The most often identified bacteria from chronic periapical abscesses are *Streptococcus viridans* and *Staphylococcus aureus*.[7]

Bacterial infection of the root canal is a critical factor in the development of the periapical

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disease—This is why endodontic treatment goals require eradicating microorganisms to preventing re-infection.[8] Due to the complexity of the anatomy and the variety of root canals, just instrumentation cannot provide adequate endodontic treatment. Consequently, endodontic treatment is chemomechanical – this means it is performed chemically through disinfection and mechanically through instrumentation with endodontic burs and needles.[9] Disinfection is accomplished by adding a medicament into the root canal. Intracanal medicaments are required to eradicate bacteria present in root canals and create an environment conducive to periapical tissue repair. The mechanical stage is performed instrumentally to form an ideal root canal that enables effective irrigation and obturation and provides adequate access to place the medicament.[10,11]

Since its discovery in 1920, calcium hydroxide [$Ca(OH)_2$] has become one of the most commonly used medicament components in dentistry.[12] Calcium hydroxide was selected because it has a pH of about 12.5, is antibacterial, and has been established as a source of many root canal infections.[8,11] Calcium hydroxide is used in dentistry to regulate bacteria, remove organic debris, heal periapical inflammation, prevent root resorption caused by inflammation, promote the development of hard tissue, and act as a temporary filler.[13]

Calcium hydroxide's antibacterial activity against staphylococcus spp. is mostly due to the staphylococcus bacteria's coagulation reaction, which is regulated by the staphylocoagulase enzyme.[14] Calcium hydroxide's antibacterial activity on Streptococcus spp. plays an important role in bacterial cell membrane permeability, protein denaturation, and enzyme inactivation, leading in the bacteria's death.[15] Calcium hydroxide's antibacterial action on these bacteria is affected by its consistency.

Calcium hydroxide is often used as an intracanal medicament in dentistry as a paste and gel preparation. Calcium hydroxide paste preparation are more soluble and absorbent in root canals than gel formulations because the paste preparation has a higher concentration of propylene glycol than the gel preparation.[16] Propylene glycol has antibacterial properties and enhances calcium hydroxide penetration into dentinal tubules.[17,18]

The aim of this study was to isolate and classify microorganisms from root canals of clinically asymptomatic, intact, non-vital teeth with periapical pathosis to determine their sensitivity towards calcium hydroxide paste and gel.

Materials and methods

Patient Selection

The present study was approved by the Universitas Padjadjaran Medical Faculty Research Ethics Committee at Bandung, Indonesia and informed consent was obtained from all subjects.

The study used incisors, canines, premolars, and both first and second molars. A tooth with previous endodontic therapy, a periodontal pocket greater than 2 mm deep, periapical sinus/fistula, abnormal anatomy, calcified canals, a wide-open cavity with clinical exposure, tooth isolation is impossible, limited access to the apical region, the presence of marginal periodontitis, and some systemic disease or antibiotic therapy in previous three months were excluded from the study.

Each patient in the sample had at least one nonvital tooth that was intact and reported one or more of the following symptoms: percussion pain, palpation pain, fistula, and localized or diffuse swelling. These symptoms matched the criteria for chronic periapical abscesses described by Torabinejad.[19] Nonvital tooth determined by vitality tests with no responses to thermal stimuli. Periapical abscesses are identified by radiographic features ranging from lamina dura interruption to severe loss of periapical and interradicular tissues.

Collection of Sample

Microbiological culture sampling was carried out under strict aseptic conditions. A systematic root canal therapy protocol was developed. Restorations and carious lesions were entirely eliminated prior to root canal sampling. A rubber dam was used to isolate the tooth. To protect the contents of the radicular pulp tissue, the access cavity was prepared with a sterile round bur (Gates-Glidden, Dentsply-Maillefer, Ballaigues, Switzerland) and manually irrigated with sterile saline without the use of water syringe.

The coronal necrotic pulp tissue was carefully removed, and the coronal third of the root canal was subsequently enlarged to avoid

contamination of the paper point by coronal pulp residues. After obtaining access to the pulp, a sterile reamer/file/broach was inserted into the root canal up to the apical foramina and the root canal material was collected for aerobic and anaerobic culture using sterile paper point number 15 (Dentsply-Maillefer, Ballaigues, Switzerland) and maintained for 60 seconds. Placing a paper point in a medium transport vehicle and transporting it to the laboratorium as soon as possible.

Microbiological Procedure

Paper points containing materials of the root canals were immediately placed in microtube containing 450 µL of bulyon. The tubes were shaken in a mixer for 5 seconds. Serial 5-fold dilutions were made up and 50 mL of each serial dilution was plated onto media in Mueller-Hinton and blood agar. The plates were incubated at 37°C up to 48 hours to allow microorganism growth. Colonies were counted and characteristic colony in each culture base on shape, margin, diameter, and color. Bacteria were classified by Gram stain using a light microscope with 1000X magnification and and *RapID System* from *Remel*.

Identification of Bacteria

Streptococcus species identification used the *RapID STR System*, while Staphylococcus used the *RapID STAPH PLUS System*. The Staphylococcus spp bacteria found in this study were *Staphylococcus epidermidis, Staphylococcus warneri, Staphlyococcus saprofiticus, Staphlococcus haemoliticus, Staphlyococcus cohnii*, *and Staphlyococcus hominis*, while the Streptococcus spp bacteria found in this study were *Streptococcus salivarius*.

Calcium hydroxide sensitivity test

Calcigel® 2g *(Prevest Denpro Limited, India),* a calcium hydroxide gel composed of 45 percent calcium hydroxide, barium sulfate, methylcellulose, and purified water; and *UltraCal XS®* 1.2 mL (*Ultradent Product, Inc., USA*), a calcium hydroxide paste composed of 35% calcium hydroxide, barium sulfate, propylene glycol, methylcellulose, and purified water, was used in this analysis. Calcium Hydroxide powder for control using *EMSURE® ACS (Merck KGaA, Darmstadt, Germany)* 20 mg.

The method used is agar diffusion method with Müller-Hinton (AMH). The bacterial suspension with 0.1 mL Mc Farland turbidity was spread using sterile cotton swabs on AMH. A well was made at AMH with a diameter of 6.5 mm. The well was filled with test solutions divided into three groups: group 1: *UltraCal XS®* with 100% concentration; group 2: *Calcigel®* concentration 100%; and group 3: Calcium hydroxide powder for analysis EMSURE® ACS for positive control. AMH has incubated facultatively anaerobically into an exicator for 24 hours at 37ºC.

Calcium hydroxide sensitivity testing was performed using the disk diffusion method on blood agar media. For anaerobic development, plates were incubated at 37 ºC for 48 hours in an anaerobic jar and then examined for an inhibition zone. Bacterial sensitivity may be determined by examining the inhibition zone or areas free of bacterial colonies on the medium agar surface and measure using a caliper with a 0.05 mm accuracy. Bacterial susceptibility may be categorized into four categories based on the diameter of the inhibition zone: extremely sensitive (diameter greater than 20 mm), sensitive (diameter 10-20 mm), fair (diameter 5-10 mm), and resistance (diameter less than 5 mm).

Results

According to it's characteristics, a bacterial colony is classified. These characteristics include shape, border, warning, and diameter (Table).

Shape	Margin	Diameter	Color
Circular	*Entire*	<1 mm	White
Rizoid	*Lobate*	1-2 mm	White
	Undulate	2-3 mm	Yellow
	Serrate	3-4 mm	Beige
	Filamentous	4-5 mm	Brown
		8 mm	Transparent

Table 1. Colony characteristics.

Each type of colony that appears on each sample is observed macroscopically and microscopically. Colonies suspected of *Staphylococcus* spp. and *Streptococcus* spp. has macroscopic characteristics as a circular-shaped. The *circular* colony was then observed under microscope with Gram staining. Only colonies that have a *coccus* shape with Grams (+) can proceed to the next stage of identification. Table show the colony observations by macroscopic and microscopic in each sample.

Sample	Code Colony	Macroscopic					Microscopic		
		Shape	Margin	Color	Diameter	Count	Shape	Gram	Formation
I	A	*Circular*	*Entire*	White	1-2 mm	5	*Coccus*	(+)	Staphylo
	B	*Circular*	*Entire*	Yellow	1-2 mm	3	*Coccus*	(+)	Staphylo
	C	*Circular*	*Serrate*	White	3-4 mm	10	*Coccus*	(+)	Strepto
II	D	*Circular*	*Entire*	White	2-3 mm	1	*Coccus*	(+)	Staphylo
	E	*Circular*	*Entire*	White	1-2 mm	1	*Coccus*	(+)	Staphylo
	F	*Circular*	*Entire*	White	1-2 mm	2	*Coccus*	(+)	Staphylo
	G	*Circular*	*Entire*	Yellow	<1 mm	2	*Coccus*	(+)	Staphylo
	H	*Circular*	*Entire*	Yellow	<1 mm	1	*Coccus*	(+)	Staphylo
III	I	*Circular*	*Entire*	White	2-3 mm	3	*Coccus*	(+)	Staphylo
	J						*Coccus*	(+)	Staphylo
	K	*Circular*	*Entire*	White	2-3 mm	1	*Coccus*	(+)	Staphylo
IV	L	*Circular*	*Entire*	Yellow	2-3 mm	9	*Coccus*	(+)	Staphylo
	M	*Circular*	*Entire*	White	2-3 mm	5	*Coccus*	(+)	Staphylo
	N						*Coccus*	(+)	Strepto
V	O	*Circular*	*Entire*	White	<1 mm		*Coccus*	(+)	Staphylo
VI	P	*Circular*	*Entire*	White	2-3 mm	15	*Coccus*	(+)	Staphylo
	Q	*Circular*	*Entire*	White	3-4 mm	1	*Coccus*	(+)	Staphylo

Table 2. Colony characteristics as macroscopic and microscopic.

Coccus Gram (+) with chain or cluster arrangement are then catalase tested. The positive catalase test result showed genus *Staphylococcus* while bacteria with a negative catalase test result showed genus *Streptococcus*. Species from *Streptococcus* genus bacteria are known using RapID STR while species from *Staphylococcus* g*enus* bacteria are known using RapID STAPH *PLUS*. The microcode result from the RapID System are entered into the *ERIC software* to determine the species, probability percentage, probability level, bioscore value and biofrequency level which can be seen in Table 1.

Sample	Code Colony	Rapid Test Identification Result	Filum	% Probability	Probability Level	*Bioscore*	Biofrequency
I	A	*K. sedentarius*	*Actinobacteria*	99,50%	*Implicit*	1/4	*Typical*
	B	*Micrococcus spp.*	*Actinobacteria*	96.78%	*Adequate*	1/126	*Acceptable*
	C	*G. morbillorum*	*Firmicutes*	99,90%	*Satisfactory*	1/86	*Acceptable*
II	D	*S. epidermidis*	*Firmicutes*	86,63%	*Inadequate*	1/6116	*Not applicable*
	E	*S. saprophyticus*	*Firmicutes*	84,64%	*Inadequate*	1/165	*Not applicable*
	F	*S. haemolyticus*	*Firmicutes*	99,90%	*Adequate*	1/4137	*Rare*
	G	*K. sedentarius*	*Actinobacteria*	99,61%	*Implicit*	1/4	*Typical*
	H	*Kocuria rosae*	*Actinobacteria*	99,90%	*Adequate*	1/196	*Acceptable*
III	I	*S. epidermidis*	*Firmicutes*	99,90%	*Adequate*	1/799	*Atypical*
	J	*S. epidermidis*	*Firmicutes*	99.15%	*Satisfactory*	1/51	*Acceptable*
	K	*S. warneri*	*Firmicutes*	99,90%	*Questionable*	1/14776	*Very rare*
IV	L	*S. saprophyticus*	*Firmicutes*	99,90%	*Adequate*	1/257	*Acceptable*
	M	*S. cohnii*	*Firmicutes*	98,87%	*Presumtive*	1/3040	*Rare*
	N	*S. salivarius*	*Firmicutes*	99,90%	*Questionable*	1/18504	*Very rare*
V	O	*S. haemolyticus*	*Firmicutes*	99,81%	*Adequate*	1/3141	*Rare*
VI	P	*S. cohnii*	*Firmicutes*	>99.9%	*Adequate*	1/131	*Acceptable*
	Q	*S. hominis*	*Firmicutes*	>97.51%	*Satisfactory*	1/46	*Acceptable*

Table 1. RapID System Identification Result.

The Staphylococcus spp bacteria found in this study were *S. epidermidis, S. warneri, S. saprofiticus, S. haemoliticus, Staphlyococcus cohnii*, *and S. hominis*, while the Streptococcus spp bacteria found in this study were *S. salivarius*.

The bacterial sensitivity test to calcium hydroxide of paste and gel was carried out by looking at the average diameter of the inhibitory zone, from three replications (Table 2).

Bacteria	Paste (*UltraCal XS®*)	Gel (*Calcigel®*)	Powder (EMSURE®)
S. warneri	9,97	9,70	9,37
S. saprofiticus	12,20	11,90	11,73
S. epidermidis	12,58	10,78	11,15
S. haemoliticus	9,87	9,85	9,83
S. cohnii	9,67	9,92	9,40
S.hominis	11,07	10,30	10,50
Streptococcus salivarius	10,92	11,08	11,23

Table 2. Inhibition Zone diameter Average for Calcium Hydroxide gel and paste (in mm).
*Extremely sensitive (diameter greater than 20 mm), sensitive (diameter 10-20 mm), fair (diameter 5-10 mm), and resistance (diameter less than 5 mm).

Statistical analysis with the one-way ANOVA test has been done. The statistical analysis concluded that there was no statistical difference between diameter of inhibitory zone from paste, gel, and powder of Calcium hydroxide, with p-value = 0.66 (p> 0.05).

Discussion

The bacteria found in this study were *S. epidermidis, S. warneri, S. saprofiticus, S. haemoliticus, S. cohnii, S. hominis*, which are coagulase-negative bacteria, and also *Streptococcus salivarius* (*Streptococcus* viridans group). This founding in line with other studies that found the presence of *Staphylococcus coagulase negative* (CoNS) is 5.33% in chronic periapical abscesses. *Staphylococcus coagulase negative* (CoNS) is a commensal bacterium on the skin and healthy mucosa that is not detrimental or likely to be beneficial. Coagulase-negative Staphylococci are also the most bacteria identified from dentoalveolar abscess in another studies.[1] This group of bacteria can act as the main opportunistic pathogen when they enter sterile body cavities.[20]

The Streptococcus found in this study is S. salivarius from the viridans group.[21] This result is similar to other studies that found the presence of *S. viridans* in chronic periapical abscesses is 9.33% with standard identification methods through catalase and coagulase tests.[7] *S. viridians* were also found 13.3% in dental abscesses in another study that identified bacteria by biochemical test methods. [22] α-

Hemolytic Streptococci, the Streptococcus viridans species are usually non pathogenic opportunists.[23]

Bacterial sensitivity test to calcium hydroxide conducted in this study was carried out by calculating the diameter of the inhibitory zone that appeared on the agar plate. Fernanda et al. tested antibacterial activity using the diffusion method, incubated 24 hours, then a bacterial inhibition zone was formed around well. Diameter of the bacterial inhibition zone is measured using a digital caliper (mm).[16,24]

Calcium hydroxide has antibacterial activity against *staphylococcus*. This corresponds to several researchers who stated that direct contact of calcium hydroxide to S. *aures* in the first 1 hour led to a decrease the bacteri by 1.3 x 10^5 and after 6 hours the number of *S. aureus* decreased by 6.8-7.2 x 10^4. The decrease of *S. aureus* is quite significant after incubation for 6-20 hours.[25] The results of this study are also in line with the discovery of an inhibitory zone diameter of 15.7±0.3^a x ± SE (mm), in testing the antibacterial effect of calcium hydroxide on *S. aureus.*[16]

Antibacterial effect of calcium hydroxide against *staphylococcus spp.* caused due to the coagulation reaction of *S. aureus* mediated by the enzyme *staphylocoagulase*. *Staphylocoagulase* is an extracellular protein produced by *S.aureus.* The thrombin-staphylocoagulase complex is formed when prothrombin and *staphylocoagulase are* mix. The *thrombin-staphylocoagulase* complex will become active and initiate the polymerization of fibrin. Fibrin monomers will form and aggregate into soluble fibrin, then converted into insoluble fibrin by activated *cross-linking* enzymes, factor XIII-a, and *fibrin stabilizing factor fibrinoligase*. The presence of calcium ions produced by calcium hydroxide will induce the occurrence of the reaction. [26]

The paste (*UltraCal XS®*) tested on *S. hominis* showed a significant difference in the diameter of the inhibitory zone ($p<0.05$), which was 11.07 mm compare by the gel (*Calcigel*) of 10.30 mm ®and powder (EMSURE) of 10.50 mm. Calcium hydroxide has a strong antibacterial against *S. hominis. S. hominis* produces biofilms as a virulence factor. Biofilms are mucous layers composed of polysaccharides, proteins, and microbial cells that form a matrix and produces bacterial protection from antibiotics or other substances, or the body's immune response. According to Sabran, hydroxyl ions produced by calcium hydroxide significantly inhibit the formation of bacterial biofilms. The nutrient in the biofilm environment is modified due to calcium hydroxide, thereby suppressing the growth rate of microorganisms.[27,28]

Calcium hydroxide has antibacterial activity against Gram-positive bacteria such as the *Staphylococcus epidermidis, S. warneri, S. saprofiticus, S. haemoliticus, S. cohnii, S. hominis*, and *Streptococcus salivarius*. The virulence factor for Gram-positive bacteria is Lipoteichoic acid for bacterial adhesion and the formation of biofilms as bacterial resistance to disinfectants, antibiotics, and the host's immune response. Calcium hydroxide can damage or modify the structure of lipoteichoic acid by deactivating some of the lipid components because calcium hydroxide creates an alkaline environment due to hydroxyl ions release. Calcium hydroxide can weaken lipoteichoic acid to stimulate macrophages. The teichoic acid exposed to calcium hydroxide cannot produce tumor necrosis factor-alpha (TNF-α).[29]

Calcium hydroxide's antibacterial activity against *S. viridans* is due to the release of hydroxyl ions. The hydroxyl ion reacts with lipids, proteins, and nucleic acids to form lipid peroxides, increasing the permeability of the bacterial cell membrane, denaturing proteins, and inactivating enzymes, resulting in cell death.[15]

Another studies, suggest to use calcium hydroxide powder mixed with alkaline water for better disinfection and prevent post operative flare up.[30] Calcium hydroxide paste and gel preparation had no significant effect on the inhibition zone's diameter cause they have the same ingredients (calcium hydroxide, barium sulfate, methylcellulose, and distilled water). Calcium hydroxide paste is more efficient than calcium hydroxide gel in dentistry since calcium hydroxide gels dissolve in root canals and are not absorbed in dentin hydroxyapatite.[16] Calcium hydroxide paste in syringe can deliver the paste more conveniently, efficient time and very suitable for root canal medicament.[31] Propylene glycol is used in calcium hydroxide paste to aid its penetration to the root canals because propylene glycol has a more remarkable ability to penetrate the dentinal tubules than purified water, can penetrate more easily through the

apical foramen, and have an antibacterial effect against anaerobic bacteria. [18,32,33]

Conclusions

The Staphylococcus spp bacteria found in this study were *Staphylococcus epidermidis, Staphylococcus warneri, Staphlyococcus saprofiticus, Staphylococcus haemoliticus, Staphlyococcus cohnii*, and *Staphlyococcus hominis*, while the Streptococcus spp bacteria found in this study were *Streptococcus salivarius*. All the Staphylococcus and Streptococcus that found in this research had strong sensitivity to Calcium hydroxyde paste and gel. Further research into the efficacy of these bacteria in conjunction with other medications is recommended.

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Declaration of Interest

The authors declare that there is no conflict of interest.

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