A comparison of antimicrobial efficacy between Calcium Hydroxide and Mineral Trioxide Aggregate against resistant endodontic microorganisms

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Abstract:

Microorganisms and their by products are the main cause of pathological changes in dental pulp (root canals) and periapical region. Facultative bacteria and fungi have been identified in therapy resistant persistent endodontic infection. The objectives of this study was to evaluate the antimicrobial efficacy of Calcium hydroxide and Mineral Tri Oxide Aggregate (MTA) against therapy resistant endodontic microorganisms and compared between them. Six standard bacterial stains were used: Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, bacillus subtilis, Candida albicans and Enterococcus faecalis. The agar diffusion method on Muller-Hilton media was employed. The plates containing media were inoculated with the specified bacterial suspensions. Two standard holes were prepared on each microorganism inoculated plate with a copper puncher and one hole was completely filled with Ca $(OH)_2$ and other with MTA . The plates were then kept at environmental temperature for one hour to ensure prediffusion and then incubated at 37°C for 24 hours. After 24 hours, the diameters of inhibition zones were measured. Tests were replicated for thirty times for each sample and mean values were taken. Zone of inhibition as measured for Ca $(OH)_2$ and MTA were statistically analyzed with Student's t-Test .Both Ca$(OH)_2$ and MTA were found to produce zone of inhibition against Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), Bacillus subtilis (BTCC 17), and Candida albicans (BTCC 493). Ca $(OH)_2$ showed highest activity against S. aureus and lowest activity against P. aeruginosa which was similar to the activity range of MTA against the mentioned organisms. But both of them failed to produce any activity against E. coli and. E. faecalis. MTA was found to produce a lower efficacy than Ca $(OH)_2$ while comparing the zone of inhibition between them and statistically it was significant. Ca $(OH)_2$ and Mineral Tri Oxide Aggregate (MTA) showed antimicrobial efficacy against some therapy resistant microorganisms but they did not show antimicrobial efficacy against Escherichia coli and Enterococcus faecalis.

Keywords: *Calcium hydroxide, Mineral Tri Oxide Aggregate (MTA), antimicrobial efficacy.*

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Introduction:

Progress has been made in understanding the nature of root canal infection and periapical diseases. Success of endodontic treatment and retreatment depends on elimination of bacteria and their substrate from the root canal[1]. Asepsis and sterilization in the root canal system is usually accomplished by biomechanical preparation with intra-canal medicaments.It has been shown that instrumentation and antibacterial irrigation renders 50-70% of infected canals free of microorganisms while the remaining canals content vital bacteria. So the importance of antimicrobial medicaments in root canal therapy can not be over looked. Therefore, there has been a continuous search for new endodontic medicaments that present an ideal combination of good antimicrobial, mechanical, physiochemical and biological properties.

Calcium hydroxide has been used as an root canal medicament, but sometimes found to be resistant in failed endodontic cases, especially in presence of certain resistant microorganism like Enterococci faecalis, E.coli etc.[2] Because of relative inefficient activity of calcium hydroxide, concerning the treatment of persistent infection

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cases, new endodontic materials search has been even more incessant.

Mineral Trioxide Aggregate or MTA is a new promising material, have shown a significant improvement over other materials in endodontics. Perforation, pathological incomplete root formation, periapical surgery, resorption etc. are often associated with failed or resistant endodontic cases, in which situation MTA has been proven as most effective repairing restorative material.[3-7] But regarding these situations, the antimicrobial efficacy of MTA and calcium hydroxide especially against resistant microorganisms has not been clarified yet. Therefore the objective of this study is to evaluation of antimicrobial efficacy of calcium hydroxide and Mineral Trioxide Aggregate on resistant endodontic microorganisms. and compared between them.

Materials and Methods:

The prospective comparative study was done in the Department of Conservative Dentistry and Endodontics, and the Department of Microbiology and Immunology, Bangabundhu Sheikh Mujib Medical University Hospital. *Staphylococcus aureus* (ATCC 25923*), Pseudomonas aeruginosa (*ATCC 27853), *Escherichia coli (*ATCC 25922), *Bacillus subtilis (*BTCC 17), *Candida albicans* (BTCC 493) and *Enterococcus faecalis* (clinically isolated) were collected (Fig 1) and preserved in crio vial with 20% glycerin broth and stored in liquid nitrogen at -196^{0} C temperature

Fig.-1: *Organisms containing plate*

Mueller Hinton agar media was poured in sterilized Petri Dishes and left till the media turned into gel form. Prepared potato dextrose media was collected and preserved in the same manner.

All the Prepared dishes were stored in refrigerator at 4^{0}C until use. Immediately before inoculation, all the media containing plates were dried in dryer to make moisture free.

From the collected microbial specimens, using a sterilized swab, a lawn of single microbiological strain other than Candida albicans were taken (Fig 2) and spreaded over a sterilized Mueller Hinton Plate.

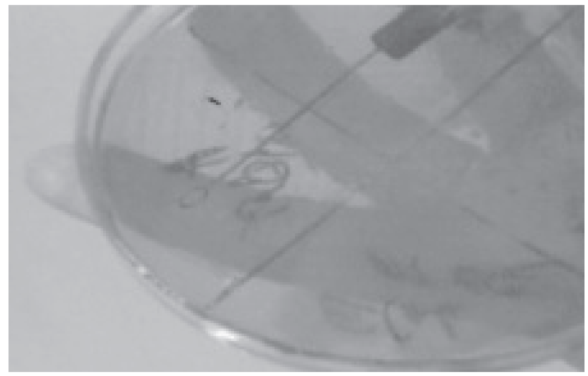

Fig.-2: *Lawn of organism is being taken for preparation of standard suspension*

Candida albicans was spreaded over a sterilized potato dextrose agar media. All the plates were incubated for 24 hours at 37ÚC in incubator. Different microbiological strains were sub-cultured in different plates. A standard microbiological suspension was prepared compare with 0.5 McFarland Scale. (0.5 McFarland Scale = 1.5×10^{8} CFU). The dried media in Petri dish was then inoculated with the prepared standard suspension of 0.5 McFarland Scale by sterile swab stick (Fig 3 & 4).

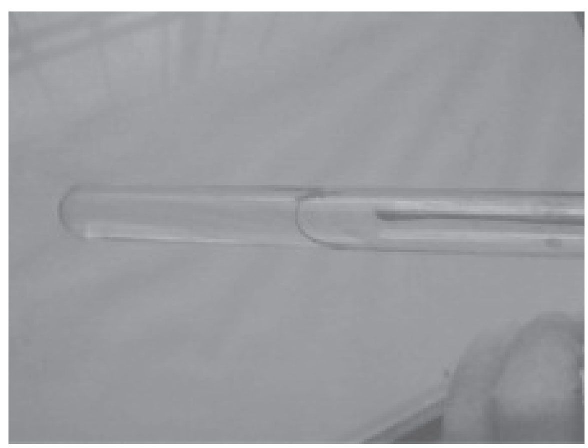

Fig.-3: *Organism being taken from standard suspension by sterilized swab*

Fig.-4: *Organism being inoculated on Muller Hilton media*

Now two standard holes of 3 mm diameter and 4 mm depth were prepared on each individual micro organism inoculated plate with a copper puncher (Fig 5).

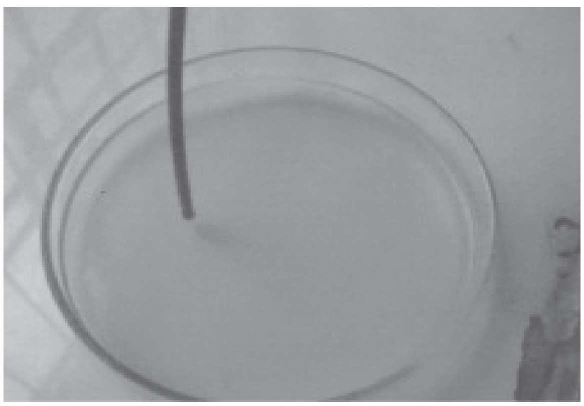

Fig.-5: *Punching of media with copper puncher*

Mineral trioxide aggregate paste (Pro root MTA, Dentsply, Tulsa, USA) or MTA paste was made in a creamy consistency. One prepared hole in inoculated media was then completely filled immediately by MTA following its preparation (Fig 6).

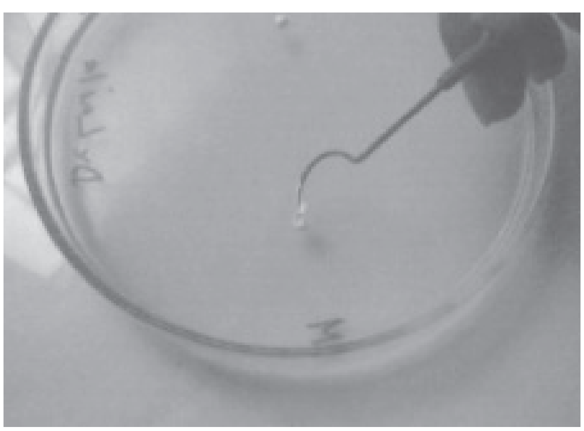

Fig.-6: *Placement of prepared MTA into the punched cavity on organism inoculated media*

Calcium hydroxide paste was directly poured into another hole from its tube with the help of a needle supplied by manufacturer (Fig 7).

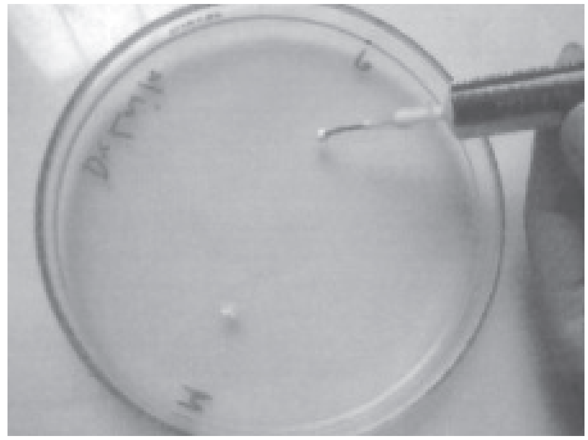

Fig.-7: *Placement of $Ca(OH)_2$ into the punched cavity on organism inoculated media*

All the micro organism inoculated plates were maintained at room temperature for 1 hour to allow pre diffusion of the materials, and then incubated at 37^0C for 24 hours. After 24 hours, plates were taken out of the incubator and observed for formation of zones of microbial inhibition (Fig.8).

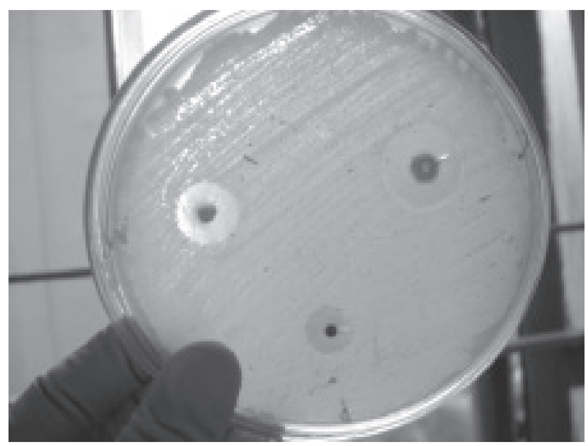

Fig.-8: *Zone of Inhibition on Muller Hilton media*

The zones were then measured with a millimeter ruler with accuracy of 0.5mm. Data were posted on respective data sheets. Tests were replicated for thirty times on each sample.

After completion of the procedure, data were collected and posted on data sheets. The statistical analysis was done for the test of significance.

Data were processed and analyzed using one way ANOVA with multiple comparisons facilitated by Student's t-Test test. A p value < 0.05 was considered significant.

Results:

Both $Ca(OH)_2$ and MTA were found to produce zone of inhibition against *Staphylococcus aureus* (ATCC 25923), *Pseudomonas aeruginosa* (ATCC 27853), *Bacillus subtilis* (BTCC 17), and *Candida albicans* (BTCC 493).(Fig 8) MTA showed highest activity against *S. aureus* and lowest activity against *P. aeruginosa* which was similar to the activity range of $Ca(OH)_2$ against the mentioned organisms. But both of them failed to produce any activity against *E. coli* and. *E. faecalis*. MTA was found to produce a lower efficacy than $Ca(OH)_2$ while comparing the zone of inhibition between them and statistically it was significant.

The findings of the study derived from data analysis are documented in tabular form (Table I- Table III).

Table- I

Efficacy of MTA and calcium hydroxide on different microorganisms

Microorganisms#	Zone of inhibition# produced around holes (mm)	
	MTA	Calcium hydroxide
S. aureus	14.65	24.48
P. aeruginosa	8.20	12.20
E. coli	0	0
E. faecalis	0	0
B. subtilis	10.70	15.83
Candida albicans	12.27	15.73

Means of thirty times assays.

Table-I shows that both MTA and Calcium hydroxide produced highest zone of inhibition against S. aureus But failed to produced any zone of inhibition against E coli and E faecalis.

Table- II

Compare of efficacy of MTA and calcium hydroxide on different microorganisms

Microorganisms#	Zone of inhibition produced around holes (mm)		p-value#
	MTA (n = 180)	Calcium hydroxide (n = 180)	
S. aureus	14.65 ± 0.53	24.48 ± 0.61	< 0.001
P. aeruginosa	8.20 ± 0.25	12.20 ± 0.25	< 0.001
E. coli	0	0	———
E. faecalis	0	0	———
B. subtilis	10.70 ± 0.70	15.83 ± 0.69	< 0.001
Candida albicans	12.27 ± 0.45	15.73 ± 0.69	< 0.001

Data were analysed using Student's t-Test and were presented as mean ± SD.

Table-II shows that In the culture media containing microorganisms like *S. aureus, P. aeruginosa, B. subtilis* and *Candida albicans*, the zone of inhibitions produced around the holes of MTA was observed to be significantly smaller than those produced around calcium hydroxide suggesting that the efficacy of calcium hydroxide is better than that of MTA on the four organisms ($p < 0.001$ in each case). However, neither calcium hydroxide nor MTA was found effective on *E. coli* and *E. faecalis* as no zone of inhibition was found to be produced in the media containing culture of these two organisms.

Table- III

Comparison of zone of inhibition between MTA and Calcium hydroxide

Zone of inhibition(mm)	Group		p-value#
	MTA (n = 180)	Calcium hydroxide (n = 180)	
	7.64 ± 5.76	11.38 ± 8.89	< 0.001

Data were analyzed using Student's t-Test Test and were presented as mean ± SD.

Table-III compares the zone inhibitions produced around the holes in culture plates of irrespective of different microorganisms. The zone of inhibition produced around holes containing MTA was observed to be significantly smaller (7.64 ± 5.76) compared to that produced around holes containing calcium hydroxide (11.38 ± 8.89 mm) irrespective of microorganisms ($p < 0.001$).

Discussion:

In the present study both Calcium Hydroxide and Mineral Trioxide Aggregate (MTA) showed antimicrobial activity against *S. aureus, P. aeruginosa, B. subtilis and C.albicans.* The findings are similar to the findings of Sipert et al.[8] who using similar methodology observed in vitro antimicrobial activity of MTA and calcium hydroxide based sealers (i.e. sealapex, fill canal, and Portland cements) against those organisms.

The reason for antimicrobial activity of MTA has been explained by the study of Duarte et al[9] who demonstrated that the antimicrobial activity is seem to be related with elevated pH. Furthermore MTA contains calcium oxide, which when mixed with water; forms calcium hydroxide and induces an increase in pH by dissociation of calcium and hydroxide ions. An increase in pH level (pH 12.5), creates an unfavorable environment for microbial growth[10] because high pH is considered as bactericidal. Hydroxyl ions kill bacterial cells by damaging the cytoplasmic membrane, protein denaturation and damaging the DNA. Torabinejad et al[11] observed an initial pH of 10.2 for MTA, rising to 12.5 in 3 h. The antimicrobial activity of calcium hydroxide may also be related to ionization with subsequent release of hydroxyl ions and an increase in pH levels (pH 12.5). The antimicrobial activity of MTA-based materials against Candida albicans observed in the present study can also be explained by the sensitivity of this strain to high pH. Al-Nazhan and Al-Judai[12], demonstrated that at a stable concentration of 50 mg/ml, white MTA was able to eliminate C. Albicans in vitro for up to three days.

Present study findings solely contradict with the study findings of Filho et al.[10] who observed in vitro antimicrobial activity of Endodontic sealers, MTA based cements and Portland cement. They showed that all above mentioned organisms including E.coli and E. faecalis also inhibited by those materials. However, the difference between our study and the study of Filho et al[10] may be due to using double layered agar plates and different concentration of microorganisms.

In the present study E. coli and E. faecalis were found to be resistant against the anti microbial activity of MTA and calcium hydroxide. Using agar diffusion method, Sipert et al[8] while observing in vitro antimicrobial activity for sealapex, fill canal, Pro Root MTA, and Portland cements found no antimicrobial activity of MTA and Portland cement against E. coli. Ribeiro et al[13] in an anaerobic condition, observed in vitro antimicrobial activities for MTA, calcium hydroxide and Portland cement; but found no antimicrobial activity against E. coli and E. faecalis. Miyagak et al [14] also showed MTA and calcium hydroxide containing sealer have no antimicrobial effect against E.coi, E. faecalis etc with same methodology. Studies have shown that E. faecalis got killed only at a pH greater than 10-12 due to an inbuilt proton pump which enables it to survive in such alkaline environments.[15] The materials may also need direct contact with the bacteria for acting.

While comparing the antimicrobial activity of MTA and calcium hydroxide, although the mechanism of action of antimicrobial activity of MTA and calcium hydroxide is more or less same, in the present study, Mineral Trioxide Aggregate showed an antimicrobial activity lower than calcium hydroxide. This result is similar with previous study.[8,10,13,16] This variation in antimicrobial activity between MTA and Ca(OH)2 may be due to different diffusion and dissociation capabilities of two materials. Some substances have difficulty in dissociating and diffusing in agar (semi-solid medium), not expressing their real antimicrobial effect.[17] A material that diffuses more easily will probably provide larger zones of microbial growth inhibition.[18] However, great care was taken to keep the plates for 1 h at room temperature to allow the diffusion the agents through the agar and then incubated.

Although used by many authors, differences in agar medium, diffusion capacity of inhibitory agents, bacterial strains and cellular density, as well as anaerobic atmosphere may interfere with formation of inhibition zones around materials used in antimicrobial testing. [8,13,16] However, there is not a consensus regarding to a gold standard test for the appraisal of antimicrobial testing of cements and other solutions used in dental therapy.

Conclusion:

According to the study findings, it can be concluded that MTA, though it was found effective against *Staphylococcus Aureus, Pseudomonas aeruginosa, Bacillus subtilis, and Candida albicans;* but it showed a lower efficacy than $Ca(OH)_2$. On the other hand both $Ca(OH)_2$ and MTA found inactive against *E.coli* and *E. faecalis*. So considering all these findings it can be recommended that for achieving asepsis in endodontic infection, $Ca(OH)_2$ is preferable and should be used but continuing search should carry on to find out more effective material even against *E.coli* and *E. faecalis*.

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