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Original Article

Comparative antimicrobial efficacy of selected root canal irrigants on commonly isolated microorganisms in endodontic infection

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ABSTRACT

Objective: This study aims to evaluate and compare the antimicrobial efficacy of three selected root canal irrigants (BioPure MTAD, metronidazole, aztreonam) against microbes commonly isolated from polymicrobial microbiota of root canal infection. **Materials and Methods:** This study was designed with four experimental groups (Group I - *Bacteroides fragilis*, Group II - *Propionibacterium acnes*, Group III - *Enterococcus faecalis*, Group IV - *Candida albicans*) based on the microbes selected for the study. Group I and Group II bacteria were used to compare and evaluate antimicrobial effect of BioPure MTAD, metronidazole, aztreonam, and normal saline. Group III and Group IV bacteria were used to compare and evaluate antimicrobial efficacy of BioPure MTAD, aztreonam, and normal saline. Normal saline was used as a control irrigant in this study. Agar disc diffusion method was applied to assess and compare the antimicrobial action of selected irrigants. **Results:** Metronidazole was found to be the most effective root canal irrigant against *B. fragilis* and *P. acnes* among the tested irrigants. Mean zone of inhibition against *E. faecalis* has been shown to be maximum by BioPure MTAD, followed by aztreonam. Antifungal effect against *C. albicans* was only shown by BioPure MTAD. **Conclusions:** Overall, BioPure MTAD is the most effective root canal irrigant as it has shown an antibacterial effect against all the tested microorganisms. However, metronidazole showed maximum antibacterial effect against obligate anaerobes. Aztreonam also showed an antibacterial effect in the present study, raising its possibility to be used as a root canal irrigant in the future.

Key words: Agar disc diffusion method, BioPure MTAD, *Candida albicans*, *Enterococcus faecalis*

INTRODUCTION

Reduction or elimination of microflora during root canal treatment is achieved by the combined effect of both chemomechanical preparation and intracanal medications.[1,2] The removal of microorganisms and other irritants from the confines of the root canal is conducted by means of the mechanical action of instruments and the flow and backflow of the irrigating solutions.[3,4]

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BioPure MTAD (Dentsply/Tulsa, Tulsa, OK, USA), which is a mixture of a tetracycline isomer, citric acid, and a detergent,[5] has been recommended as a final rinse irrigant because of its antimicrobial properties and its ability to remove the smear layer.[6,7] It is less cytotoxic than most of the endodontic medicaments.[8,9]

Aztreonam, which is a synthetic monocyclic beta-lactam antibiotic, has a molecular structure different from older beta-lactam antibiotics as it has a less complex nucleus.[10] It has shown excellent activity against Gram-negative bacilli.[11]

Metronidazole, a nitroimidazole compound, is a broad-spectrum antibiotic and exhibits activity against anaerobic bacteria and protozoa. It demonstrates effective antibacterial activity against anaerobic cocci as well as Gram-negative and Gram-positive bacilli. In the treatment of periodontal disease, it has been used both topically and systemically.[12]

The objective of this research was to assess and compare the antibacterial and antifungal effectiveness of BioPure MTAD, aztreonam, and metronidazole against obligate anaerobic bacteria, i.e., *Bacteroides fragilis* and *Propionibacterium acnes*, facultative anaerobic bacteria, i.e., *Enterococcus faecalis*, and yeast, i.e., *Candida albicans*.

MATERIALS AND METHODS

The microorganisms selected for this study, i.e., *B. fragilis* ATCC 25285 (Group 1), *P. acnes* ATCC 6921 (Group 2), *E. faecalis* ATCC 29212 (Group 3), and *C. albicans* ATCC 10231 (Group 4), were obtained from the American type culture collection and stored at a temperature of −10°C to −20°C in a freezer.

Strains of the selected microorganisms were reactivated in the respective media at a given temperature and time.

Groups 1 and 2 - Activated in brain–heart infusion broth supplemented with hemin and menadione at 37°C for 48 h.

Group 3 - Activated in brain–heart infusion broth for 24 h at 37°C.

Group 4 - Activated in Sabouraud dextrose agar broth at 37°C for 24 h.

After the revival of microorganisms, four to five well-isolated colonies of each strain were picked up with a sterile loop, later dissolving them in respective test tubes containing 0.85% of 5 ml sterile saline to create a turbidity of 0.5 McFarland scale (1.5×10^8 CFU/ml) for the preparation of inoculum. The resulting suspension was then spread on agar plates with the help of a sterile cotton swab in a form so that a lawn growth was observed.

The media used for the four groups were as follows:

Group 1 - Wilkins-Chalgren agar plate (HiMedia, Mumbai).

Group 2 - Brain–heart infusion medium (HiMedia, Mumbai) with 1% glucose.

Group 3 - Mueller-Hinton agar plate (HiMedia, Mumbai).

Group 4 - Sabouraud dextrose agar plate (HiMedia, Mumbai).

Antibiotic disc preparation

Antibiotic solution of BioPure MTAD was prepared by mixing powder and diluent.

Sterilized Whatman paper no. 1 was used to prepare 6-mm diameter discs, which were later soaked in 40 µl of normal saline and BioPure MTAD, respectively.

The company-made aztreonam and metronidazole antibiotic discs (BD Diagnostic, India) were also used for the test.

These antibiotic discs were then aseptically transplanted to the agar plate formerly incubated with microorganisms.

For Group 1 and Group 2, agar plates BioPure MTAD, aztreonam, metronidazole, and normal saline containing discs, and for Group 3 and Group 4, agar plates, BioPure MTAD, aztreonam, and normal saline prepared discs were transferred aseptically.

Group 1 and Group 2 agar plates were incubated in an anaerobic environment created using the Anoxomat system (Mart Microbiology BV, Netherlands) for 48 h.

Group 3 and Group 4 plates were incubated for 48 h at 37°C under the aerobic conditions, in an atmosphere of 10% CO_2, in a CO_2 incubator (New Brunswick, USA). All assays were repeated five times. Measurement of zones of inhibition was conducted by a Vernier caliper.

RESULTS

The results obtained were statistically evaluated using one-way ANOVA test and the intergroup comparison was done using Bonferroni multiple comparison test. Value of $P < 0.001$ was considered statistically significant.

- *Bacteroides fragilis* (Group 1) [Tables 1a and b]
- *Propionibacterium acnes* (Group 2) [Tables 2a and b]
- *Enterococcus faecalis* (Group 3) [Tables 3a and b]
- *Candida albicans* (Group 4) [Tables 4a and b].

DISCUSSION

Disc diffusion technique, a well-established method for antimicrobial research, has been applied in this study.[13,14] This method has an advantage that chemical properties of tested medicaments do not alter during the procedure and also it is less technique sensitive.[15]

Anoxomat system was used for anaerobic culture in the current study as earlier research[16] had shown that with the use of this system commendable growth is achieved, in terms of density and colony size. Evidently, the Anoxomat method is considerably reliable and appears to support the growth of strict anaerobes efficiently. Zone of inhibition around specimens was measured in the present study using a Vernier caliper at three different points as done in previous studies.[17,18] In the present study, all assays were repeated five times to establish reproducibility.[19]

Tabulated results demonstrated that metronidazole produced the maximum zone of inhibition against *B. fragilis* [Graph 1]. Vijaykumar *et al*.[20] and Whelan *et al*.[21] demonstrated that metronidazole was effective against *B. fragilis*, which is in agreement with the results obtained in the current study.

Antimicrobial efficacy against *P. acnes* in the existing study was shown to be maximum by metronidazole [Graph 2]. Effect of metronidazole on *P. acnes* has been reported with mixed findings in the literature. Gaetti-Jardim Júnior *et al*.[22] determined the positive antibacterial effect of metronidazole against *P. acnes*, and the results obtained were in accordance with the present study. Similarly, Effat Khodeinae *et al*.[23] demonstrated that 2% metronidazole showed adequate efficacy against *P. acnes* while evaluating the efficacy of 2% metronidazole gel against acne vulgaris. In contrast to the above results, few studies reveal no effect of metronidazole against *P. acnes*.[24]

Table 1a: Effect of root canal irrigants and their values of zone of inhibition against *Bacteroides fragilis*

Irrigants	Zone of inhibition (mm), mean±SD	Effect
BioPure MTAD	26.75±1.02	Sensitive
Metronidazole	30.00±1.63	Sensitive
Aztreonam	28.25±1.02	Sensitive
Normal saline	0±0.0	Resistant

ANOVA F=681.96, P<0.001 (significant). SD: Standard deviation

Table 1b: Intergroup comparison of mean zone of inhibition size for the irrigants against *Bacteroides fragilis*

	t	*P*	Significance
1 versus 2	4.22	<0.001	Significant
1 versus 3	1.94	0.0	Nonsignificant
1 versus 4	34.71	<0.001	Significant
2 versus 3	2.27	0.0	Nonsignificant
2 versus 4	38.93	<0.001	Significant
3 versus 4	36.66	<0.001	Significant

1 - BioPure MTAD, 2 - Metronidazole, 3 - Aztreonam, 4 - Normal saline

Table 2a: Effect of root canal irrigants and their values of zone of inhibition against *Propionibacterium acnes*

Irrigants	Zone of inhibition (mm), mean±SD	Effect
BioPure MTAD	26.00±0.82	Sensitive
Metronidazole	29.25±1.82	Sensitive
Aztreonam	0±0.0	Resistant
Normal saline	0±0.0	Resistant

ANOVA F=307.37, P<0.001 (significant). SD: Standard deviation

Table 2b: Intergroup comparison of mean zone of inhibition size of irrigants against *Propionibacterium acnes*

	t	*P*	Significance
1 versus 2	7.96	<0.001	Significant
1 versus 3	63.68	<0.001	Significant
1 versus 4	63.68	<0.001	Significant
2 versus 3	71.64	<0.001	Significant
2 versus 4	71.64	<0.001	Significant
3 versus 4	0.0	0.0	Nonsignificant

1 - BioPure MTAD, 2 - Metronidazole, 3 - Aztreonam, 4 - Normal saline

Table 3a: Effect of root canal irrigants and their values of zone of inhibition against *Enterococcus Faecalis*

Irrigants	Zone of inhibition (mm), mean±SD	Effect
BioPure MTAD	29.25±1.63	Sensitive
Aztreonam	8.5±1.23	Sensitive
Normal saline	0.0±0.0	Resistant

ANOVA F=516.12, P<0.001 (significant). SD: Standard deviation

Metronidazole is a potent bactericidal, which is efficacious against anaerobes that are composed of

Table 3b: Intergroup comparison of mean zone of inhibition size for irrigants against *Enterococcus faecalis*

	t	*P*	Significance
1 versus 2	24.9	<0.001	Significant
1 versus 3	35.10	<0.001	Significant
2 versus 3	10.20	<0.001	Significant

1 - BioPure MTAD, 2 - Aztreonam, 3 - Normal saline

Table 4a: Effect of root canal irrigants and their values of zone of inhibition against *Candida albicans*

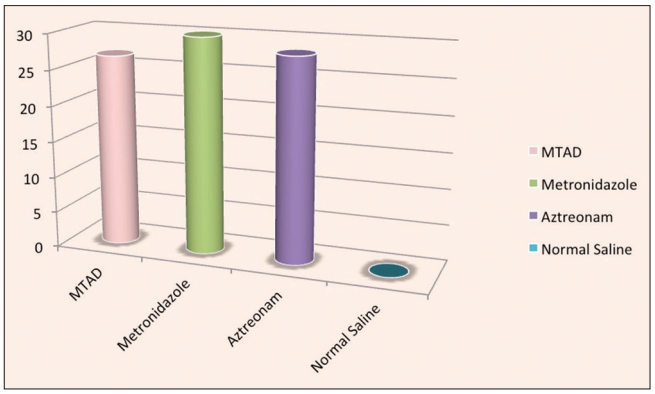
Irrigants	Zone of inhibition (mm), mean±SD	Effect
BioPure MTAD	8.50±0.82	Sensitive
Aztreonam	0.0±0.0	Resistant
Normal saline	0.0±0.0	Resistant

ANOVA *F*=430.50, *P*<0.001 (significant). SD: Standard deviation

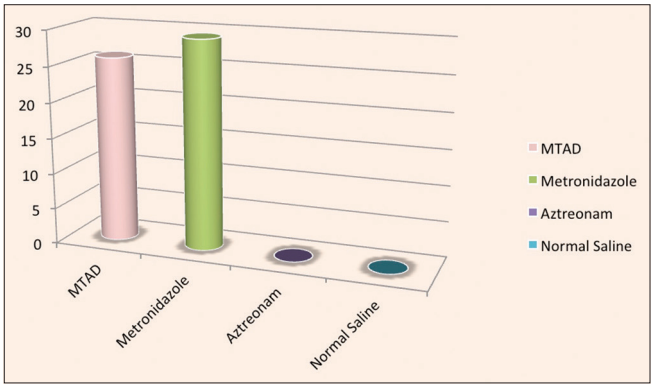
Table 4b: Intergroup comparison of mean zone of inhibition size of irrigants against *Candida albicans*

	t	*P*	Significance
1 versus 2	25.50	<0.001	Significant
1 versus 3	25.50	<0.001	Significant
2 versus 3	00.00	0.00	Nonsignificant

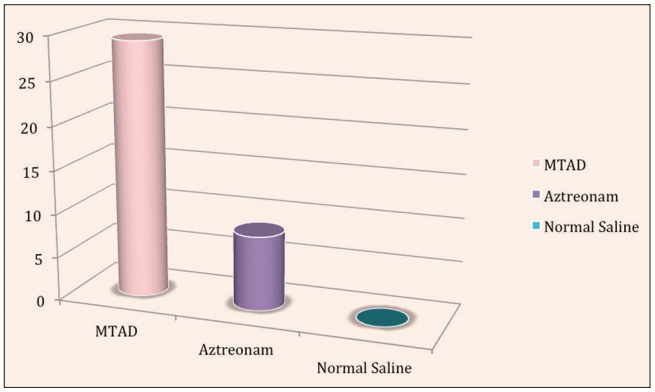
1 - BioPure MTAD, 2 - Aztreonam, 3 - Normal saline



Graph 1: Comparison of mean zone of inhibition of root canal irrigants against *Bacteroides fragilis*



Graph 2: Comparison of mean zone of inhibition of root canal irrigants against *Propionibacterium acnes*



Graph 3: Comparison of mean zone of inhibition of root canal irrigants against *Enterococcus faecalis*

electron transport components, i.e., ferredoxin, which are capable of donating electrons to metronidazole, developing highly reactive nitro radical anions that kill susceptible organisms by a radical-mediated mechanism.[18] This could be a possible reason for its high antimicrobial efficacy against *B. fragilis* and *P. acnes*.

In the present study, metronidazole was not used to test antimicrobial efficacy for *E. faecalis* and *C. albicans* because it is ineffective against facultative anaerobes. Literature has already proven its lacks of antimicrobial effect against *E. faecalis*.[19] Krishna *et al*.[25] in their study demonstrated that 10% metronidazole was not effective against *E. faecalis* among the tested intracanal medicaments. Yujra *et al*.[26] demonstrated that long-standing metronidazole therapy preferred the establishment of *C. albicans* in the oral cavity of rats.

In the current study, maximum mean zone of inhibition against *E. faecalis* was revealed by BioPure MTAD [Graph 3], which is in accordance with several other studies.[7] Newberry *et al*.[8] in their study identified that seven out of the eight tested strains of *E. faecalis* growth were affected when MTAD was used as a final irrigant. The presence of doxycycline defines the superior antimicrobial effect of MTAD against *E. faecalis*.[9] However, results of this research are in contradiction to reports which refute the antimicrobial effect of MTAD against *E. faecalis*.[10]

In the present study, antifungal effect against *C. albicans* was shown only by MTAD this result obtained was in agreement with the result given by Arslan *et al*.,[27] who demonstrated antimicrobial action of MTAD against *C. albicans* and *E. faecalis*. The antifungal effect of MTAD can be attributed to the better flow and penetration properties of doxycycline.[12] Contrary to

the above results, a study conducted by Ashari *et al*.[28] showed that MTAD is ineffective against *C. albicans* and its substantivity may be altered when used in conjunction with NaOCl.

MTAD in the current research has revealed substantial zone of inhibition against both *B. fragilis* and *P. acnes*. This may be possibly due to the occurrence of doxycycline as earlier research has confirmed the action of doxycycline on *B. fragilis* and *P. acnes*[11] in inhibiting the protein synthesis.

Aztreonam in the present study showed significant antimicrobial efficacy against *B. fragilis* and mild antibacterial activity against *E. faecalis* but was completely inactive against *P. acnes* and *C. albicans* microorganisms. Aztreonam, a bactericidal antibiotic, acts by interrupting with the formation of the bacterial cell wall.[2] It causes lysis and death of Gram-negative bacteria by binding to the penicillin-binding protein-3 (PBP-3),[3] which might be the reason for its activity against *B. fragilis*. Poor affinity has been shown for the PBP-3 of Gram-positive and anaerobic bacteria which could be the reason behind its mild activity against *E. faecalis*.

CONCLUSIONS

On the basis of results obtained in the current study, it may be stated that BioPure MTAD can be recommended as an effective alternative to the currently used root canal irrigants due to its superior antimicrobial properties against both aerobic and anaerobic microbial organisms as well as *C. albicans*. Metronidazole and aztreonam, on the other hand, are, however, not significantly efficacious against all strains of microbes commonly present in the root canal, thus limiting their use as an effective antimicrobial root canal irrigant.

Although the present study was done with strict adherence to all the scientific protocols, still further studies are required to evaluate the efficacy of the different irrigants, which may further widen their horizon in the field of endodontics.

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Conflicts of interest
There are no conflicts of interest.

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