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EVALUATION OF COMBINATION OF THREE ANTIMICROBIAL AGENTS AGAINST COMMON ENDODONTIC PATHOGENS.

Dental Science

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ABSTRACT

AIM: To determine antibacterial efficacy of three antibacterial agents and combination against endopathogens.
METHODOLOGY: Bacterial strains associated refractory endodontic conditions were used to determine MIC of Clindamycin (C), Metronidazole (M), Doxycycline (D) and combination CMD, against Candida albicans, Pseudomonas Aeruginosa, Escherichia Coli, Enterococcus Faecalis, Streptococcus Mutans, Bacillus subtilis subspe spizizenii, Actinomyces Actinomycetemcomitans. The data was analyzed using paired 't' test, one way ANOVA and Tucky's post Hoc HSD.
RESULTS: Clindamycin (C) inhibited C. albicans (90%) and S. mutans (90%) while P. Aeruginosa, E. Coli, E. Faecalis, B. subtilis and A. actinomycetemcomitans were resistant to it. Metronidazole (M) did not inhibit any of the bacteria. Doxycycline (D) inhibited C. albicans (90%), P. Aeruginosa (90%), and S. mutans (90%), while E. Coli, E. Faecalis, B. subtilis and A. actinomycetemcomitans were resistant. The combination CMD inhibited all the bacteria significantly.
CONCLUSION: Combination CMD can be used against resistant endopathogens for predictable results.

KEYWORDS

Antibacterial Agents, Combination, Endodontic Pathogens, Sensitivity.

INTRODUCTION:

Biomechanical preparation of root canals alone is not effective to render this system free of resident microbial population (1). Various intracanal medication and irrigants are used to disinfect root canals (2). Microorganisms situated in difficult areas of root canals cannot be eliminated easily. Calcium hydroxide, is not effective to eliminate microorganisms inside dentinal tubules (3). Thus, such microorganisms hinder the healing process. Eventually such teeth lead to a fate of endodontic failure (4).

Endodontic infections comprise of multiple microbial strains limiting single pragmatic antibiotic to disinfect root canals. Nonspecific antibiotics suppress most of the microbiological strains but allow residual micro-organisms to reestablish in root canals. Using such agents to eradicate endodontic microflora from the infected root canal systems through lesion sterilization and tissue repair have shown convincing results (5). Topical application of antimicrobial agents to the infected root canals can prove for eliminating fastidious microorganism. Among the antibiotics, penicillin such as amoxicillin are drug of choice. Other antimicrobial agents from lincosamide derivative (like clindamycin, erythromycin), nitroimidazole derivatives (like metronidazole) and tetracyclines (like minocycline, doxycycline) are recommended for patients allergic to penicillin. Doxycycline is effective to eliminate odontogenic anaerobic microorganisms (6). Metronidazole demonstrates limited efficacy against anaerobes causing periodontal and odontogenic infections (7). Thus, the present in-vitro study investigated antibacterial activity of clindamycin, doxycycline, metronidazole and combination CMD against bacteria encountered with endodontic infections.

MATERIALS AND METHOD:

The present in vitro study was carried after gaining clearance from Institutional Ethical Committee, letter no. DMIMS(DU)/IEC/2015-16/1744, Dated:31/12/2015.

MATERIALS USED –

Clindamycin HCL (C) and Doxycycline HCL (D) molecules were procured from Himedia Labs Pvt Ltd, Mumbai, India; while Metronidazole molecules (M) were procured from MP Biomedicals LLC France.

BACTERIAL STRAINS TESTED –

American Type Culture Collection bacterial strains were acquired from Himedia Labs Pvt Ltd, Mumbai, India -
Candida albicans (ATCC 10231)
Pseudomonas aeruginosa (ATCC 27853),
Escherichia coli (ATCC 25922),
Enterococcus faecalis (ATCC 35550),
Streptococcus mutans (ATCC 25175),
Bacillus subtilis subsp. spizizenii (ATCC 6633),
Aggregatibacter actinomycetemcomitans (ATCC 29523)

PREPARATION OF MICROBIAL INOCULA -

Bacterial isolates were suspended in BHI (Brain Heart Infusion) broth to prepare suspension (8).

PREPARATION ANTIBACTERIAL STOCK SOLUTIONS –

The stock solutions of all antimicrobial agents (C, M, D) were prepared by adding them to saline at concentration of 100 µg/ml (8).

DETERMINATION OF MINIMUM INHIBITORY CONCEN TRATION (MIC) -

From every stock solution, 1 ml of antibacterial solution was added to test tubes and diluted further two-fold. 5 µl of bacterial aliquot was added to these test tubes. The test tubes incubated at 37^0C for 24 hours. The MIC was determined by visual inspection and spectrophotometry (OD600:0.6-0.7). The broth without bacterial growth and least drug concentration was noted as MIC for that drug. The procedure was repeated in triplicates (8).

STATISTICAL ANALYSIS:

The data was analyzed with SPSS version 22.0 statistical package for MS Window (SPSS Inc. Chicago, IL, USA) using descriptive statistics. Intra group mean and standard deviation was analyzed using paired 't' test. Inter group mean and standard deviations were analyzed using One-way ANOVA while statistical significance with Tucky's Post Hoc HSD test ($P<0.05$).

RESULTS:

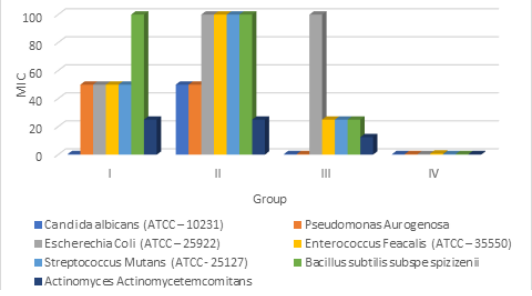
One-way ANOVA signifies the overall comparison. Table 1 and graph 1 shows MIC of various bacteria against different drugs. The highest resistance to clindamycin was shown by B. subtilis (100±5 mg), followed by E. faecalis (55±8.66 mg) whereas E. coli (51.67±2.89 mg) and P. Aeruginosa (50±5 mg) were moderately resistant. A. Actinomycetemcomitans (25±5 mg) displayed weak resistance while,

S. Mutans (0.47±0.12 mg) and C. Albicans (0.43±0.06 mg) exhibited least resistance. The highest resistance to metronidazole was exhibited by E. Coli (107.33±7.51 mg), E. Faecalis (100±6.04 mg) and B. Subtilis (96.67±5.77 mg). While moderate resistance was shown by C. Albicans (55±5 mg) and P. Aeruginosa (55±13.23 mg). The least resistance was shown by A. Actinomycetemcomitans (22.67±2.08 mg) and S. Mutans (21±3.61 mg). the highest resistance to doxycycline was shown by E. Coli (108±10.41 mg). Moderate resistance to doxycycline was shown by B. Subtilis (27.67±3.79 mg), E. faecalis (24.33±6.03 mg) and A. Actinomycetemcomitans (12.50±2.50 mg). The least resistance was exhibited by S. Mutans (0.77±0.25 mg) followed by C. Albicans (0.53±0.23 mg) and P. Aeruginosa (0.43±0.06 mg).

Table 1 - Comparison of MIC of various endodontic bacteria against different drugs

Sr. No.	Bacteria	Gr I (Clinda)	Gr II (Metro)	Gr III (Doxy)	Gr IV (CMD)	P value
1	C. albicans	0.4	50	0.4	0.4	<0.001**
2	P. Aeruginosa	50	50	0.4	0.4	<0.001**
3	E. Coli	50	100	100	0.4	<0.001**
4	E. Feacalis	50	100	25	0.8	<0.001**
5	S. Mutans	50	100	25	0.4	<0.001**
6	B. subtilis subspe spizizenii	100	100	25	0.4	<0.001**
7	A. Actinomyce-temcomitans	25	25	12.5	0.4	<0.001**

**Highly significant (p<0.001) one sample t test.



Graph 1 - MIC of antibacterial agents against various endodontic bacteria.

DISCUSSION:

Endodontic pathogens most commonly identified from root canal may include aerobic bacteria like Bacillus subtilis, candida and Pseudomonas, as well as anaerobic bacteria like Aggregatibacter, Enterococcus, Escherichia and Streptococcus (9). In endodontic infections, Gram-negative anaerobic bacteria are the predominantly responsible for characteristic clinicopathologic features of periradicular conditions. Candida albicans were isolated in cases with pulp necrosis, symptomatic as well as asymptomatic chronic apical periodontitis, and unsuccessful endodontic treatment. Presence of isolates of B. subtilis as transient strains were identified in cases of refractory endodontic and apical periodontitis cases (10), and are associated with biofilm formation in accessory canals, apical ramifications, space between obturating material and dentinal walls as well. E. faecalis enters in root canals during endodontic procedures, as local environment is altered due to inadequate biomechanical preparation or sterilization, and starts growing to higher number. It exhibits virulence due to presence of adhesins the helps to invade and adhere to dentinal tubules, presence of capsular polysaccharide and antibiotic resistance (11). Bacterial endotoxins play a major role in initiation and spread of inflammation as well as propagating periradicular lesions (12). A. actinomycetemcomitans is inhabitant component of oral microbial ecology and frequently isolated in periodontal infections. S. mutans is a major pathogen associated with dental caries and endodontic infections. Being deeply harboured in dentinal caries, they become primary source of endodontic infection. P. aeruginosa with its biofilm and other bacterial species exhibits its virulence and resistance to antimicrobial therapies (13). When residing in mixed microbial ecology, these bacteria exhibit complex association and diversified behaviours altogether. They enhance survival of each other, either due to presence of extracellular proteins, or mucopol ysaccharide favouring attachment and preventing penetration of antimicrobial agents in bacteria (14).

Elimination of all the endodontic pathogens, along with their byproducts, is the key factor is success of endodontic treatment. Systemic antibiotic is an empirical treatment to eradicate causative agents from root canals, that may not prove successful in chronic infections or pulpless teeth. As the quantity of drug reaching the root canals in such teeth is insignificant, it does not impede bacterial growth (10]). Systemic antibiotic therapy is also not advisable in case of refractory endodontic conditions or periradicular lesions. To counteract these flaws, the concept of topical drug application was launched, which proved to be successful eliminating endodontic lesions and healing diseases of periradicular tissues (12).

Microbial ecology associated with endodontic infections comprise of facultative as well as obligate aerobes and anaerobes (10). In the present study, among the antibacterial agents tested, it was observed that, metronidazole exhibited as least sensitivity. Facultative anaerobes develop resistance to metronidazole if its dose is altered. Hence, metronidazole is advised to use in combination with penicillin to treat endodontic infections. These results are in agreement previous studies (14). Doxycycline, a broad-spectrum antibiotic from tetracycline group is reported effective against a wide range of endodontic microorganisms. It has been used as intracanal topical antibiotic, as final root canal irrigant with other additives, smear layer and pulp solvent, dentine hardener and anti-inflammatory agent against root resorption (15). The results of this study in relation to doxycycline are in agreement with other studies. Clindamycin, at subinhibitory concentrations, helps decline toxin production and enhances opsonization and phagocytosis of microbial cells (16). In this study, clindamycin showed highest sensitivity against almost all the bacterial isolates used and results of this study are in agreement with previous studies (14).

CONCLUSION:

Incorporation of these antimicrobial agents as intracanal agents can be effective in eliminating endodontic lesions and decreasing therapeutic failures. However, concentration-dependent activity, would likely be better suited for this application than time-dependent.

Conflict of interest - Declared none
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