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Dr. Mishan Manohar Jaiswal
M.D.S, Conservative Dentistry
and Endodontics, Senior
Resident, Department of
Dentistry, Nalanda Medical
College & Hospital, Bihar, India

Dr. Neha Kumari
D.S, Pedodontics and Preventive
Dentistry, Dental Practitioner.
Consultant Pedodontist, Dental
Life Care, Param Parwati
market, 1st Floor, Vijay Nagar,
Bailey Road, Patna, Bihar, India

Dr. Sarita Singh
M.D.S, Prosthodontics and
Crown & Bridge, Senior
Resident, Department of
Dentistry, Nalanda Medical
College & Hospital, Patna,
Bihar, India

Corresponding Author:
Dr. Sarita Singh
M.D.S, Prosthodontics and
Crown & Bridge, Senior
Resident, Department of
Dentistry, Nalanda Medical
College & Hospital, Patna,
Bihar, India

Original article

Evaluation of microscopic flora and its antibiotic susceptibility from RCT failure cases: An *in vivo* study

Dr. Mishan Manohar Jaiswal, Dr. Neha Kumari and Dr. Sarita Singh

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Abstract

Background: The persistence of disease in periapical tissues is most commonly associated with difficulties or mishaps which occur during or after initial endodontic treatment.

Aims and Objective: To isolate and identify the organisms in root canal failed cases & its current antibiotic susceptibility pattern.

Materials and Methods: study was conducted on 51 patients attending the OPD of dental Department Nalanda Medical College and Hospital, Patna. The results were analyzed using VITEK-2 automated analyser (Bio Merieux UK).

Results: A total of 18 types of bacteria were isolated and among them 17.3% cases reported E- faecalis and another 15.2% cases reported E-cloacae complex. Pseudomonas aeruginosa accounted for 8.7% while 6.5% constituted of E-faecium, Escherichia coli etc.

Conclusion: Enterococcus species was found in majority of cases indicates that it is the predominant bacteria responsible for endodontic failure.

Keywords: Micro-organisms, RCT, vitek-2

Introduction

It is evident that an infected root canal system is a unique niche for the selective species of microorganisms [1]. The influence of bacterial persistence in the root canals on treatment outcome is an important issue in endodontics because bacteria have been shown to play a major role in persistence or emergence of apical periodontitis lesions after root canal treatment [2]. The continuity of periapical diseases is mostly associated with strain or mishaps which occur during or after endodontic treatment. Insufficient aseptic control, poor access cavity design, missed canals, inadequate instrumentation and leaking temporary or permanent restorations are the main factors that may be critically important in post treatment disease [3]. Despite thorough mechanical instrumentation and disinfection of the root canal system, microbes have been recovered at subsequent treatment sessions and at the end of the treatment. Such residual organisms and the leakage of bacterial products are likely to play a role in treatment failures [4, 5]. Recently growing significance has been placed on extraradicular infection as a cause of persisting apical periodontitis. The extraradicular occurrence of several anaerobic bacteria has been reported in post-treatment apical periodontitis lesions. A number of potentially endodontopathic bacteria have been found in asymptomatic periapical inflammatory lesions which were refractory to endodontic treatment. From active site of periodontal diseases anaerobic black-pigmented *Bacteroides* species and anaerobic gram-positive rods and cocci have been isolated. Facultative *Streptococcus* species have also been isolated, but appear to play a minor role [6]. In the case of prolonged endodontic therapy, the antibiotic prescription can play an important role in efficient treatment. Recently, technological advances in bacterial isolation have made the dental profession aware that anaerobic microorganisms predominate in infected root canals.

Aims and objectives

1. To isolate and identify the organisms in root canal failed cases.
2. To know the predominant organisms causing root canal failure.

3. To determine the current antibiotic susceptibility pattern of the isolates from failed endodontic treatment cases.

Methodology

The study was conducted on 51 patients attending the OPD of dental Department Nalanda Medical College and Hospital, Patna.

None of the patients had received any systemic antibiotic therapy in the preceding 3 months. All of the teeth had demonstrated radiographic signs of chronic apical periodontitis. All patients had completed root canal therapy more than 1 year previously. All the samples were taken with the sterile instrument.

A rubber dam was applied, and the operating field was disinfected using moller's protocol. The root filling was removed using Gates Glidden drills, protaper retreatment files, Hedstrom file and K files without the use of chemical solvent under irrigation with sterile saline. The root canal length was measured with electronic apex locator and a small sterile K file wherever possible without use of any irrigant. After that a 2ml disposable syringe with 21 gauge needle was taken. Sterile normal saline was drawn into canal. The solution inside the canal was absorbed onto 2 or 3 sterile paper points for 30 seconds.

For aerobic culture 50% of the samples were studied for identification of isolates by gram stains and culture growth on nutrient, blood and MacConkey agar. Colonies from nutrient agar was used for biochemical tests and antibiotic sensitivity. The remaining 50% of the samples was placed into the BacT/ALERT aerobic transport medium bottle and sent for culture and sensitivity by using VITEK-2 automated analyser. For anaerobic culture, sample was placed into BacT/ALERT Anaerobic transport medium bottle which was immediately sent to the microbiological laboratory. In the laboratory, the BacT/ALERT automated culture instrument was used for routine culture. The predominant isolate obtained after culture was evaluated for routine microbial identification and susceptibility testing using the VITEK-2 automated analyser (Bio Merieux, UK).

Result

46 cases showed growth of microorganisms out of 51 cases. In 5 cases there was no growth. The predominant isolate bacteria was analyzed in each case and total of 18 types of bacteria were isolated and among them 17.3% cases reported *Enterococcus faecalis* and another 15.2% cases reported *Enterobacter cloacae* complex. *Pseudomonas aeruginosa* accounted for 8.7% while 6.5% constituted of *Enterococcus faecium*, *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus hominis*. *Acinobacter baumannii*, *Ralstonia pickettii*, *Staphylococcus aureus* and *streptococcus salivarius* accounted for 4.3%. *Candida albicans*, *Citrobacter freundii*, *Morganella morganii* species, *Pantoea* species, *Pseudomonas putida*, *Staphylococcus epidermidis*, *Staphylococcus lentus* accounted for 2.1% each.

In analyzing the prevalence of bacterial genera in failed root canal *Enterococcus* accounted for 23.9% of cases, *Enterobacter* and *Staphylococcus* 15.2% of the cases, *Pseudomonas* 10.8%, *Echerichia* and *Klebsiella* 6.5%, *Acinetobacter*, *Ralstonia* and *Streptococcus* 4.3%, *Candida*, *Citrobacter*, *Morganella* and *Pantoea* 2.1% of the cases.

Further more in analyzing the bacteria present in failed root canal cases on gram staining, it was found that 55.5% cases were gram negative and 44.4% were gram positive.

Facultative anaerobic bacteria were more prevalent with 88.8% and obligate aerobes were constituted 11.1%.

Discussion

In the present study, *Enterococcus faecalis* was the most commonly found microorganism. The common recovery of *E. faecalis* from canals where the treatment has failed suggests that it is an opportunistic pathogen whose persistence in the root canal presents a significant therapeutic problem^[7]. In this study, they were isolated from 17.3% of the canals with bacterial growth.

This finding agrees with those reported by Molander *et al.*^[8] and Sundquist *et al.*^[9], who respectively found *E. faecalis* in 47 and 38% of previously root treated canals with positive culture. Peciuliene *et al.*^[10, 11] reported (in two studies) a higher isolation frequency of this microorganism: 70 and 64%. According to Hung-Chih Chien *et al.* 83 of 111 (74.7%) teeth demonstrated a positive culture, in which viridans group streptococci were most abundant, with an isolation rate of 54.1% (60 of 111 specimen). The isolation rate of *Enterococcus* was 12.6% (13/111), while both *F. nucleatum* and *Pre. intermedia* were found in 8.1% (9/111) of isolates. Other microorganisms including *Pep. micros* and *Pre. Endodontalis*, were also identified and isolation rates for these 2 organisms were 7.2% (8/111) and 4.5% (5/111), respectively^[12].

Our result showed that all 8 *E. faecalis* strains were sensitive to linezolid, teicoplanin and vancomycin, 6 (75%) were sensitive penicillin, chloramphenicol, ampicillin and amoxyclav, 5 (62.5%) were sensitive to tetracycline, 3 (37.5%) were sensitive clindamycin and erythromycin. All were resistant to gentamycin.

Susceptibility and antibiotic sensitivity of different bacteria were analyzed and linezolid, teicoplanin & vancomycin were the most effective antimicrobial against gram positive bacteria and Amikacin, Gentamycin, Cefotaxime, Cefepime, Cefoperazone, Piperacillin/tazobactam, Imipenem and Meropenam were the most effective antimicrobial against the gram negative bacteria for root canal failure.

According to Hung Chih Chien aminopenicillins exhibited good activity against the viridans group of streptococci and preendodontalis, new tetracycline derivatives, minocycline and doxycycline expressed very pronounced antimicrobial activity, metronidazole had less effect on facultatives such as viridans group streptococci and *Enterococcus*, clindamycin was inhibitory against most of the anaerobic and facultative organisms, of all the cephalosporins tested, 30% species except *pre intermedia* were resistant to cephalexin, furthermore, 15.4% of *Enterococcus* and *F. nucleatum* were resistant to cefixime, 40% of organisms were resistant to erythromycin. Among the tested antibiotics trovafloxacin, a third generation fluoroquinolone showed the highest activity against all 6 species^[12].

According to study conducted by Heena Sadiq *et al.* it was found that the bacteria isolated from the root canals were commonly resistant to ampicillin, Erythromycin was relatively unaffected according to the results and clindamycin had shown to be effective against the pathogens of root canal in the study^[13].



Fig 1: BacT/Alert Culture Bottle (Anaerobic & Aerobic)



Fig 2: Sample Collection



Fig 5: Bacterial & Ast Culture Template of Gram Negative Bacteria



Fig 3: Loading Aerobic & Anaerobic Culture Bottle In BacT/Alert



Fig 6: Culture Plate of E.Coli



Fig 4: Bacterial & Ast Culture Template Of Gram Positive Bacteria



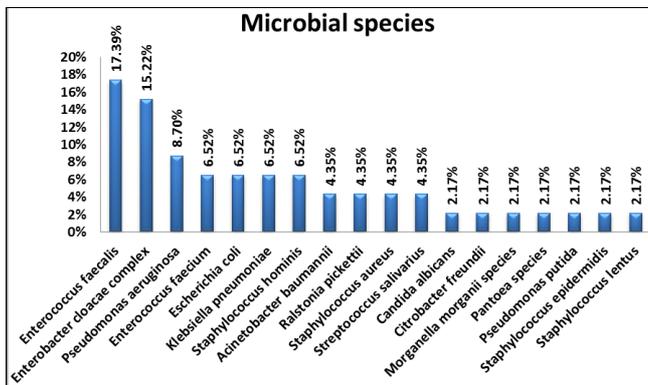
Fig 7: Culture Plate of E. Faecalis



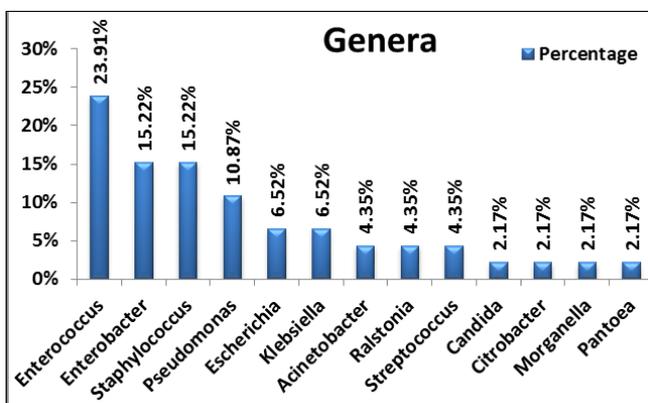
Fig 8: Antibiotic Susceptibility Testing of E.Coli



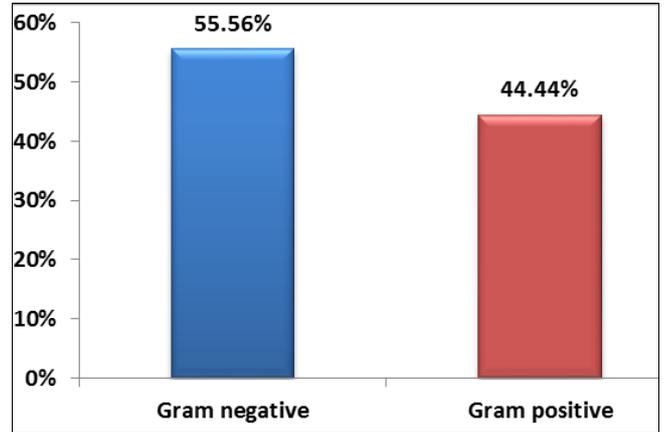
Fig 9: Antibiotic Susceptibility Testing Of *E.Faecalis*



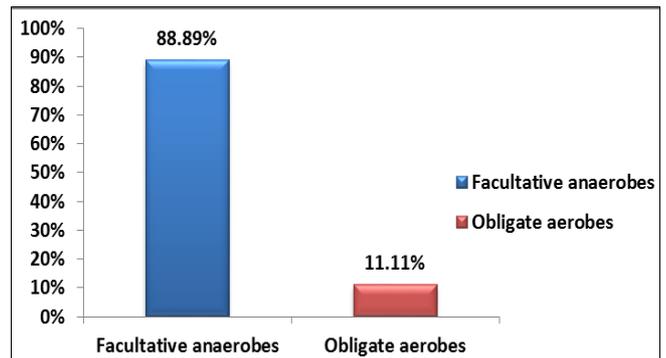
Graph 1: Microorganisms found in root canals of failed endodontic patients (n=46)



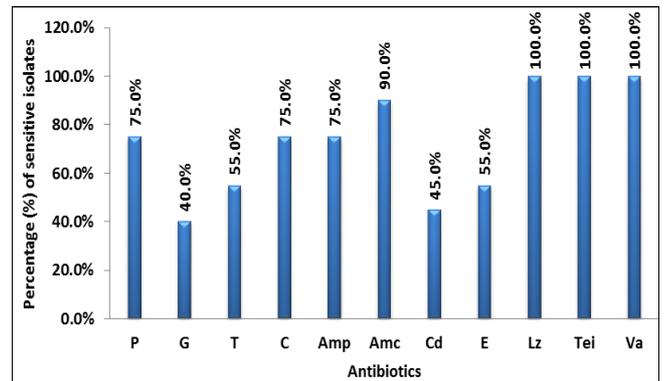
Graph 2: Prevalence of bacterial genera found



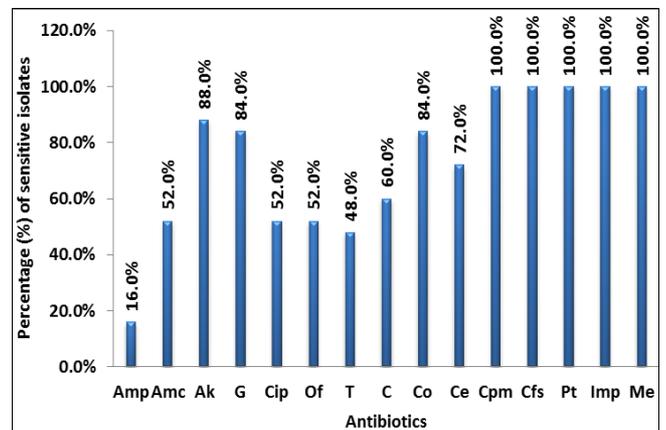
Graph 3: Prevalence of gram positive and gram negative bacteria



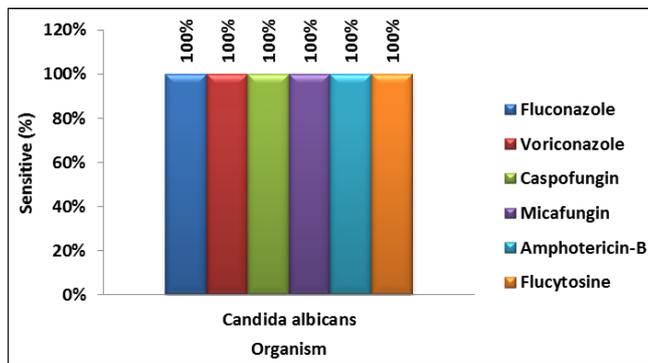
Graph 4: Prevalence of facultative anaerobes and obligate aerobes bacteria



Graph 5: Antibiotic sensitivity pattern of Gram positive organisms



Graph 6: Antibiotic sensitivity pattern of Gram negative organisms.



Graph 7: Antibiotic sensitivity pattern of Candida

Conclusion

The purpose of this study was to investigate and analyse the microbiological flora of root canals in endodontic failure cases and to find out the antibiotic susceptibility of these microorganisms. The fact that *Enterococcus* spp. was found in majority of cases indicates that *Enterococcus* is the predominant bacteria responsible for endodontic failure in a particular sub-population. Susceptibility and antibiotic sensitivity of different bacteria were analyzed and it was found that linezolid, teicoplanin & vancomycin were the most effective antimicrobial against gram positive bacteria and Amikacin, Gentamycin, Cefotaxime, Cefepime, Cefoperazone, Piperacillin/tazobactam, Imipenem and Meropenam were the most effective antimicrobial against the gram negative bacteria for root canal failure.

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