The challenge of endodontic “superbugs” in clinical practice

By Laurence J. Walsh and Basil Athanassiadis

With a range of new methods and technologies to support modern endodontic treatment, it is useful to take stock and consider the prime goals of therapy and how these are best achieved.

Current approaches to treatment are based on instrumentation, irrigation and medicamentation of the root canal system, with the primary goal of eliminating bacteria prior to placing a root canal filling. Molecular methods have demonstrated that bacteria can be detected in the root canal system in virtually all cases of root-filled teeth which have persisting periapical radiolucencies. These bacteria are found not only within the root canal itself, but also in dentinal tubules, accessory canals, canal ramifications, apical deltas, fins and transverse anastomoses - areas which are difficult to access using mechanical instrumentation and irrigation when re-treatment is contemplated.

As we and others have described in recent literature reviews, disinfection of the root canal system is not an easy objective to achieve. Even using potent antimicrobial agents and techniques, achieving reliable and complete disinfection remains a challenge. The published literature indicates that current treatment protocols with rotary NiTi instrumentation and copious antibacterial irrigation will render some 50%-70% of infected canals free of microorganisms by culture-based methods. This does not, however, mean an absence of viable microorganisms.

The biofilm challenge
Biofilms provide a powerful barrier to the diffusion of medicaments, and it is essential that materials and techniques used for endodontics are tested not in plate or broth cultures but rather using biofilm models - since in the latter the microorganisms will be in a metabolically less active state, making them less prone to inactivation by antimicrobial agents.

Resistance of bacterial biofilms to antibiotics can occur due to several factors. Firstly, the polysaccharide matrix of the biofilm retards diffusion of the antibiotic. Secondly, the chemical milieu in the biofilm (low oxygen tension, low pH, waste products, enzymes) can antagonize the effects of the antibiotic. Thirdly, in response to depletion of substrate and/or the accumulation of waste products, bacteria in the biofilm enter a quiescent non-growing state, rendering them safe from agents which target the metabolic activity of bacterial replication. Finally, subpopulations of bacteria in a biofilm may enter a phenotypic state with altered gene expression which is akin to spore formation. Because of these changes, bacteria in biofilms may display a 1000-1500 times greater resistance to antibiotics than when in the freely dispersed planktonic form.

Resistant species
With incomplete disinfection of the root canal, the spectre of selection and dominance of resistant species emerges. In dentistry, it is easy to overlook the fact that Enterococcus faecalis possesses several characteristics akin to the
“superbugs” associated with hospital-based nosocomial infections. Of all the organisms which have been found in root canals, *E. faecalis* has the most well developed (and well deserved) reputation as a difficult target for antibiotic and microbial therapy.

In relation to antibiotics, the family of enterococci have both intrinsic resistance (where the gene for intrinsic resistance resides on the chromosome) and acquired resistance (from mutations in existing DNA or the acquisition of new DNA). They are inherently more resistant to antimicrobial drugs than any other clinically important Gram-positive bacteria encountered in dentistry or medicine.

Enterococci are intrinsically resistant to many common antimicrobial agents, including cephalosporins, clindamycin, penicillinase-resistant penicillins, and vancomycin, and they have acquired resistance to many other classes of antimicrobials, including tetracyclines, erythromycin, chloramphenicol, ciprofloxacin and vancomycin. Because of this heredity, *E. faecalis* has intrinsic resistance to clindamycin, cephalosporins and amnoglycosides. There is strong evidence that most clinical strains of *E. faecalis* isolated from the oral cavity are resistant to both clindamycin and to tetracyclines8-9 - providing a good reason not to use these in root canal medicaments (Table 1).

These resistance patterns explain why clinical microbiological studies using current products show persisting bacterial growth after the use of current inter-visit medicaments. In a recent (2006) study, samples were taken before and after two-visit endodontic treatment from 88 canals with apical periodontitis. All of the canals but one had cultivable growth before treatment. After dressing with Ledermix, Septomixine, or Calasept (calcium hydroxide), the percentages of canals with positive growth on culture were 48% (13 of 27), 31% (8 of 26), and 31% (11 of 35), respectively. In the Ledermix group, 38 strains of bacteria were recovered, with 25 from the Septomixine group, and 25 from the Calasept group.9 A key finding in this study was that Gram-positive facultative anaerobic cocci - such as Enterococci - were the survivors, whereas the Gram-negative obligate anaerobic rods were easily inactivated.

### Table 1. Antibiotic Resistance in Enterococci

<table>
<thead>
<tr>
<th>Antibiotic family</th>
<th>Type of resistance</th>
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<tbody>
<tr>
<td>β-Lactams (penicillins, cephalosporins)</td>
<td>Intrinsic resistance. Can also acquire Beta lactamase production.</td>
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<tr>
<td>Tetracyclines</td>
<td>Acquired</td>
</tr>
<tr>
<td>Lincosamides (clindamycin)</td>
<td>Low level intrinsic resistance.</td>
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<tr>
<td>Macrolides (erythromycin, etc)</td>
<td>Acquired</td>
</tr>
<tr>
<td>Aminoglycosides (gentamycin, etc)</td>
<td>Low level intrinsic resistance.</td>
</tr>
<tr>
<td>Glycopeptides (vancomycin, etc)</td>
<td>Acquired</td>
</tr>
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Based on Refs 3,7,8,10 and 11

### Table 2. Survival factors for *E. faecalis* and related non-cultivable Gram positive facultative anaerobes

- Natural resistance to many antibiotics
- Low susceptibility to many biocides
- Can live and persist in the poor nutrient environment of endodontically-treated teeth
- Can survive in a quiescent non-cultivable phase with low metabolic activity for extended periods of time
- Can tolerate a broad range of pH values (from 4-11)
- Forms dense biofilms
- Can invade dentinal tubules up to 300 microns
- Can tolerate calcium hydroxide

### The great unknown?

Adding to this is the issue of non-cultivable flora present in root canals and other sites within the oral cavity - of the more than 700 different species which can be detected by molecular methods, 50% are non-cultivable, and thus our understanding of endodontic infections is not absolute. In all likelihood, there are other species, yet to be cultivated, which may share characteristics of *E. faecalis* (Table 2).

Studies using bacterial cultivation methods have shown that infected necrotic pulps, and pulpless, infected teeth (i.e. teeth without any previous endodontic treatment, have a polymicrobial flora with 4-7 species present, mostly strict anaerobes, with approximately equal proportions of gram-negative and gram-positive organisms.12,13 In contrast, previously root-filled teeth with apical periodontitis have 1-2 cultivable species, and these are dominated by facultative anaerobic Gram-positive bacteria such as *E. faecalis*.14-16 Using molecular methods, which recover the non-cultivable species, gives a rather different picture, with 1-5 species present in well treated cases with sound coronal restorations, and 2-11 species in teeth with defective coronal restorations.16-17 Based on the published literature, a reasonable estimate would place the number of bacterial species in infected root canals between 10 and 30 - a much greater challenge to disinfection than previously thought. In fact, the argument could be made that endodontic procedures, if done poorly, select for the more robust and resilient organisms (such as *E. faecalis*), because the more susceptible Gram-negative anaerobes are relatively easily eliminated. Data from culture and molecular studies indicate that *E. faecalis* may be present in from 29% to 70% of root-filled teeth with periapical lesions.18,19
Taking this on board and looking at the range of agents currently used as inter-appointment medicaments, a range of problems can be identified (Tables 3 and 4). Despite recent trends to use other antibiotics in these medicaments, from first principles, in a polymicrobial infection the decision to use a single antibiotic can be questioned. It is more likely a combination of agents would be needed to address the diverse flora encountered. If a combination of antibiotics were used, this would decrease the likelihood of resistant strains developing. The typical antibiotic-related long term problems of resistance and sensitization would remain, however.

**Clues from the past**

Given these problems, it may be timely to revisit other strategies for dealing with persistent bacteria which do not rely only upon inactivating a specific biochemical pathway (as do antibiotics), but instead taking a wide approach by using biocides. Because biocides have a broader spectrum of activity, working on multiple target sites within microorganisms, bacterial resistance to biocides does not develop, although it should be recognized that some bacteria are naturally resistant to certain biocides because of their cell wall structure.

Biocide activity is affected by several factors - notably their concentration, period of contact, pH, and temperature. The presence of organic matter is a major issue, since this can interfere with the antimicrobial effects exerted by some biocides - with chlorhexidine being a good example. Conversely, the activity of biocides can be enhanced by the use of chemical agents such as EDTA which increase the permeability of bacterial cell membranes.

Whilst there are many biocides which could be used, chlorhexidine (CHX) would rate well amongst other candidates in the field, despite some of its issues. Slow release forms of CHX and mixtures of CHX with other agents (such as calcium hydroxide) appear promising in terms of penetration into dentinal tubules and antibacterial effects against E. faecalis.

**Back to the future**

Manipulating the physical, ionic, and metabolic factors which modulate the properties of the biofilm may provide a new approach to dealing with endodontic pathogens - as an adjunct to effective antimicrobial medicaments. These approaches are now being explored for dental caries. Biofilm properties may be manipulated by influencing cell to cell signaling within the biofilm. Blocking this “quorum sensing” would reduce the ability of the biofilm to tolerate stresses such as reductions in nutrients or assault by external chemical agents (such as biocides). Slowing the biofilm accumulation rate may be possible using agents which affect quorum sensing. “Magic bullet” and “smart bomb” therapies are of ongoing interest. Using photosensitization via endogenous (protoporphyrin) or exogenous (sensitizer) molecules in biofilms with light as the vector may overcome the penetration problem, and remains an important area for further investigation.

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### Table 3. Issues with contemporary endodontic medicaments

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<thead>
<tr>
<th>Medicine</th>
<th>Effects</th>
<th>Inactivation mechanism</th>
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<tbody>
<tr>
<td>Calcium hydroxide pastes</td>
<td>Effects are limited by buffering of alkaline pH by dentine proteins, particularly in the apical third of the canal. Final pH and hydroxyl ion release vary according to the vehicle used in the paste. Limited if any penetration into dentine tubules. Only limited inactivation of E. faecalis.</td>
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<tr>
<td>Ledermix (Lederle), Endopaste (ADM)</td>
<td>Based on 3.2% Demeclocycline hydrochloride. This tetracycline has a bacteriostatic rather than bactericidal action. Effects are short-lived because of diffusion. Demineralizes and complexes with dentine, resulting in staining, a process accelerated by light. Limited inactivation of E. faecalis because of natural resistance to tetracyclines.</td>
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<tr>
<td>Odontopaste (ADM)</td>
<td>Based on 5% Clindamycin. Does not have the dentine staining issue of tetracyclines. Limited inactivation of E. faecalis because of natural (intrinsic) low-level resistance to lincosamides, which is increased by acquired resistance in clinical conditions.</td>
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<tr>
<td>Septomixine Forte (Septodont)</td>
<td>Contains neomycin, polymyxin B sulphate, and tyrothricin. Issues with limited spectra of activity, since neomycin is bactericidal against Gram-negative bacilli but is ineffective against Bacteriodes spp, while polymyxin B is ineffective against Gram-positive bacteria. Little or no inactivation of E. faecalis.</td>
<td></td>
</tr>
<tr>
<td>Chlorhexidine pastes</td>
<td>Inactivated by residues of sodium hypochlorite, forming para-chloro-aniline as a by-product. Binds to the surface and has substantivity, but does not penetrate into tubules. Inactivated by high organic loads. Partly inactivated by serum, dentine proteins and hydroxypatite. Moderately effective against E. faecalis but only as an irritant.</td>
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### Table 4. Factors which reduce the effectiveness of topical antimicrobial agents

- Resistance
- Poor diffusion
- Inadequate exposure (dose and time)
- Poor topical delivery system (bio-availability)
- Reservoirs of re-infection such as foreign bodies
- Agent is not active (e.g. because of pH)
- Inactivation by host or bacterial factors
References


About the authors
Professor Laurence J. Walsh is the technology editor of Australasian Dental Practice magazine. He is also a noted commentator on and user of new technologies and is the Head of The University of Queensland School of Dentistry.

Basil Athanassiadis is a general dental practitioner in suburban Brisbane who has had a long standing interest in dental materials and endodontics. He recently completed a research masters degree on the antibacterial properties of endodontic medicaments which was supervised by Professors Paul Abbott and Laurie Walsh.