

Antibiotic resistance in microflora inhabiting the oral cavity

by Dr Ian Hart

In order to investigate the problem of resistance to antibiotics in pathogenic bacteria inhabiting the human oral cavity, it is highly advisable to consider the entire community of microflora including commensal species since mobile conjugative transposons coding for antibiotic resistance can be transferred between pathogenic and commensal species. This article discusses the problem in relation to tetracycline resistance, and outlines various lines of research being carried out at University College London's Eastman Dental Institute.

Micro-organisms are widely distributed in the human body, but one of the most extensively colonised regions is the oral cavity, where the number of species present has been estimated to exceed 500 [1]. This is hardly surprising, given both the many ecological niches that exist for micro-organisms in the mouth, as well as the variety of growth substrates, such as host ingestion of food, with which flora come into contact.

Over-prescribing of antibiotics is commonly blamed for the increasing incidence of resistance among human pathogens, but research carried out at University College London's Eastman Dental Institute is now showing that genes for resistance are also present in the normal flora of the oral cavity, and that these genes can be transferred to other, non-commensal species. Research Fellow Adam Roberts and his colleagues have already discovered a number of interesting resistance trends, which they are currently characterising at the molecular level.

The team believes that in order to tackle the problem of resistance, it is necessary to investigate the entire community of micro-organisms inhabiting the oral cavity rather than concentrating only on those which are pathogens. Particular attention has been given to the mobile genetic elements, or transposons, that encode for antibiotic resistance. These have been found in nearly all the bacterial species that have been examined, and they appear to be an integral part of the genome of many different bacterial species. Research indicates that bacteria in the oral cavity not only exchange transposons, but that they may actually interact inside the bacteria to form new and possibly fitter strains.

A particular focus has been the characterisation of the genetic basis of transferable resistance to tetracycline - an antibiotic which is widely used in the treatment of periodontal disease. Mobile conjugative transposons, which are usually located in the genome of bacteria, have been shown to be involved in tetracycline resistance.

An example of one group of broad host range conjugative transposons is Tn916 which was originally isolated from a strain of *Enterococcus faecalis*. Tn916

confers resistance to tetracycline via the ribosomal protection protein Tet(M). Tn916 has been found in, or introduced into, at least 50 different genera of bacteria in many different environments and this number continues to increase. Both Tn916 and its derivatives have been found in the microflora of the oral cavity.

Simulated biofilms

Much of the work carried out at the Dental Institute involves the growth of bacteria in oral biofilms, which are produced in a fermentor and then disrupted. Dilutions of the resulting cells are plated out onto agar and the plates incubated under both aerobic and anaerobic conditions. Any resistant bacteria are then identified, and investigations are carried out to see if this resistance can be transferred to other organisms. It has been found that transfer readily occurs in biofilms as well as in filter mating experiments carried out on agar plates. In order to carry out this work, a wide range of consistent and reliable growth media, supplements and antibiotic discs are utilised, which are obtained from Oxoid Ltd. In addition to brain heart infusion agar and broth, which are used for the biofilm experiments, the team also utilises Mueller-Hinton agar and Iso-Sensitest blood agar plates, as well as the AnaeroGen atmosphere generation system, which provides ideal atmospheric conditions for the growth of obligate anaerobes [Figure 1].

Simulated oral biofilms are created in order to investigate the transfer of conjugative transposons such as Tn916. The biofilms are produced using a constant depth film fermentor which is inoculated with bacteria and an artificial 'saliva' growth medium which allows the growth of mixed microbial consortia to a constant depth. The 'saliva' is made up from 'Lab-lemco' meat extract powder, yeast extract, and proteose peptone from Oxoid, together with hog gastric mucin, sodium chloride, calcium chloride and potassium chloride. Genetic transfer experiments can be carried out as soon as the biofilm is stable in terms of its bacterial composition and population size.

Genetic tools

The conjugative transposons are currently being

genetically modified for use as genetic tools. A method has been developed whereby Tn916 can be used as a vector to introduce recombinant DNA into *Clostridium difficile*. Although not present in the oral cavity, *C. difficile* is a very challenging organism [2], since the DNA within the organism can only be manipulated by introducing recombinant conjugative transposons that have been constructed in the laboratory. The mechanism which enables the organism to become virulent and pathogenic can then be investigated.

Further research has recently been carried out involving the generation of an erythromycin-sensitive derivative of *C. difficile* strain 630. It has been demonstrated that Tn916 enters the genome of this strain at multiple sites, indicating its potential utility as a tool for insertional mutagenesis [3]. A transposon insertion library consisting of over 3,700 independent transconjugants has now been produced, from which three isolates with no cytopathic effect on human cells have been identified.

New mobile genetic elements

Also under investigation are novel mobile genetic elements, some of which have an extremely interesting structure because they are completely mosaic. A strain of *Enterococcus faecium*, which is resistant to tetracycline, streptomycin and the mercury in dental amalgam [4], has been isolated. The streptomycin and mercury resistance genes are present on a large conjugative plasmid, but the tetracycline resistance gene is a separate entity, present on a novel mobile element. Results indicate that it is composed of portions of both Tn916 and another conjugative transposon, Tn5397, as well as portions of a large conjugative, multi-resistant plasmid. There are also genes present on this element that are related to the genes present at a virulence-related locus of *Dichelobacter nodosus*, the causative agent of foot rot.

Another novel genetic element which has been characterised contains *tet(W)* - a recently discovered tetracycline resistance gene. This was originally isolated from *Butyrivibrio fibrisolvens*, a rumen bacterium, but it can also be isolated from the oral cavity and is the second most common tetracycline



Figure 1. AnaeroGen allows the growth of anaerobes present in the orthodontic environment.

resistance gene to be found within the mouth after *tet(M)*. The *tet(W)* gene is linked to a gene which confers erythromycin resistance as well as to insertion sequences.

Resistant endodontic bacteria

A further study isolated cultivable microflora from patients undergoing endodontic treatment at the Eastman Dental Institute, and the isolates were screened for resistance to tetracycline. Approximately half of the tetracycline-resistant bacteria were shown to possess the *tet(M)* gene, with some of these genes being present on Tn916-like elements, demonstrating that these elements are common in endodontical-derived bacteria. Transfer assays were carried out with Tn916-like positive bacteria and it was shown that tetracycline resistance could be transferred to other bacteria. The endodontic environment is relatively self-contained in relation to the rest of the oral cavity, which facilitates the transfer of genetic material between the microflora colonising it. Conjugative transposons are prevalent in the endodontic environment; this is an important finding given that tetracycline is used to treat periodontitis.

Potential reservoirs of resistance

The increasing problem of resistance to antibiotics has also led to investigations into other potential reservoirs of bacteria with which humans come into contact. At the Eastman Dental Institute research into bacterial transmission between canines and humans was undertaken as, given the close contact between dogs and their owners, it was thought that the risk of bacterial transfer and potential subsequent transfer of any resistant genes from dogs to humans may be high. Resistance to five different antibiotics in the total cultivable oral flora of dogs was studied. However, results

indicate that the risk of antibiotic resistance gene transfer to humans from this source is low. Using a cohort of nine dogs, the minimum inhibitory concentrations (MICs) for five antibiotics were determined for each bacterial isolate, and only low level resistance was found.

Despite this reassuring result, the fact remains that oral bacteria can be readily transferred between humans. Oral microflora do not necessarily stay in the oral cavity, but may pass through the digestive tract. Although they may not survive the conditions in the gut, their genes, which have been found in the external environment, could still be transferred to other bacteria. Clearly more research on this topic is necessary.

References

1. Paster BJ *et al.* Bacterial diversity in human subgingival plaque. *J Bacteriol* 2001; 183: 3770-3783.
2. Roberts AP *et al.* Development of an integrative vector for the expression of antisense RNA in *Clostridium difficile*. *J Microbiol Methods* 2003; 55: 617-624.
3. Hussain HA *et al.* Generation of an erythromycin-sensitive derivative of *Clostridium difficile* strain 630DE and demonstration that conjugative transposon Tn916 enters the genome of this strain at multiple sites. *J Med Microbiol* 2005; 54: 137-141.
4. Davis IJ *et al.* Linkage of a novel mercury resistance operon with streptomycin resistance on a conjugative plasmid in *Enterococcus faecium*. *Plasmid* 2005; 54: 26-28.

The author

Ian Hart, Ph.D.,
Diagnostics Marketing Manager,
Oxoid Ltd.,
Basingstoke, UK.