Corynebacterium and Dolosigranulum: future probiotic candidates for upper respiratory tract infections

Rachael Lappan
The Marshall Centre for Infectious Diseases Research and Training
School of Biomedical Sciences
The University of Western Australia
Perth, WA, Australia
Westfarmers Centre of Vaccines and Infectious Diseases, Telethon Kids Institute
The University of Western Australia
Perth, WA, Australia
Present address: The School of Biological Sciences, 18 Innovation Walk
Monash University
Clayton, Vic. 3800, Australia
Tel: +61 3 9905 5794
Email: Rachael.Lappan@monash.edu

Christopher S Peacock
The Marshall Centre for Infectious Diseases Research and Training
School of Biomedical Sciences
The University of Western Australia
Perth, WA, Australia
Telethon Kids Institute
The University of Western Australia
Perth, WA, Australia
Tel: +61 8 6457 6172
Email: Christopher.Peacock@uwa.edu.au

The presence of the bacterial genera Corynebacterium and Dolosigranulum has consistently been associated with a healthy upper respiratory tract (URT). Commonly occurring together in the nasopharynx of healthy children, the role of these commensal organisms in nasopharyngeal health is unknown, as few studies have sought to determine whether they actively contribute to maintaining a healthy state. We recently identified Corynebacterium pseudodiphtheriticum and Dolosigranulum pigrum as the major nasopharyngeal species associated with resistance to recurrent ear infections, via 16S rRNA gene sequencing and metagenomics. Using in vitro bacterial interference assays, we observed a reduction in the growth of Moraxella catarrhalis – one of the three major otopathogens – in the presence of C. pseudodiphtheriticum. Further in vitro and in vivo studies of the interactions between commensal C. pseudodiphtheriticum and D. pigrum strains, URT pathogens, and the human host will help to clarify their role in

In Focus
nasopharyngeal health. If they play a protective role, these organisms are promising candidates for the development of a probiotic therapy for the treatment or prevention of URT diseases in children.

Human microbiome research has traditionally focused on differences in the composition of the microbial community between healthy and disease states. This research has naturally progressed into ‘mining the microbiota for therapeutics’; the process of identifying members of the microbiota that contribute to health and developing them into therapeutic agents, either in the form of probiotics (live, beneficial microorganisms) or isolated antimicrobial compounds. Several examples mirroring this process have already been demonstrated, where defined bacterial mixtures have successfully treated or prevented infection, or the production of an antimicrobial substance by a commensal organism inhibits a pathogenic close relative.

**Markers of a healthy nasopharynx**

In the microbiome of the upper respiratory tract (URT), the genera *Corynebacterium* and *Dolosigranulum* have been consistently associated with health, particularly in children (Table 1). In these studies, they are frequently reported to co-occur, their presence corresponds with breastfeeding and a lower risk of respiratory infection, and they appear to be negatively impacted by antibiotic use. This suggests that these genera are important characteristics of a healthy nasopharynx. It may be hypothesised that the absence or reduction (by antibiotic use) of *Corynebacterium* and *Dolosigranulum* from the URT microbiota results in respiratory pathogens preferentially colonising the nasopharynx and an increased susceptibility to respiratory disease.

While it is clear that *Corynebacterium* and *Dolosigranulum* are strongly associated with health, little work has been done *in vitro* to characterise how they contribute to health. Do they compete with respiratory pathogens for nutrients and space, or prevent infection via bacterial antagonism? No previous studies appear to have investigated whether these commensal organisms can directly test for such activity. Of those that have investigated *Corynebacterium*, one early study reported *C. pseudodiphtheriticum* binding to pharyngeal cells, competitively excluding *M. catarrhalis*; though no zone of inhibition was observed on blood agar. A more recent study reported that inoculating *C. pseudodiphtheriticum* strain 090104 into mice improved resistance to infection by respiratory syncytial virus and secondary pneumococcal infection, where non-viable 090104 had a weaker effect. Recently, the commensal also demonstrated inhibition of *S. aureus* by exploiting its own virulence components. Other *Corynebacterium* species have shown similar promising antimicrobial activity; *C. propinquum* uses siderophores to restrict the availability of iron to coagulase-negative staphylococci, and *C. accolens* inhibits *S. pneumoniae* via antimicrobial free fatty acids, which *C. accolens* requires for growth.

**C. pseudodiphtheriticum and D. pigrum in resistance to recurrent ear infections**

Recently, we used 16S rRNA gene sequencing to characterise the nasopharyngeal microbiomes of children with recurrent ear infections (recurrent acute otitis media; rAOM), and healthy rAOM-resistant children. The relative abundance of *Corynebacterium* and *Dolosigranulum* was significantly higher in the rAOM-resistant children, concordant with previous studies comparing the nasopharyngeal microbiota of children with and without OM. Metagenomics further revealed that *C. pseudodiphtheriticum* and *D. pigrum* were the dominant species in the healthy rAOM-resistant nasopharynx with *C. propinquum* and *C. accolens* present to a lesser extent. To investigate whether these commensal organisms have a protective role in the nasopharynx interfering with the growth of pathogens, we conducted bacterial interference assays. *C. pseudodiphtheriticum* and *D. pigrum* were assessed for inhibitory activity against the major otopathogen species (non-typeable *Haemophilus influenzae* – NTHi, *Streptococcus pneumoniae* and *Moraxella catarrhalis*); two strains of each commensal species and seven strains of each otopathogen were tested, including mixtures of both commensals together. Growing the pathogens adjacent to the commensals on agar revealed that neither commensal affected the growth of *S. pneumoniae* or NTHi, but all seven *M. catarrhalis* strains were inhibited by *C. pseudodiphtheriticum* (Figure 1). This effect was not influenced by the presence of *D. pigrum*.

To attempt to identify the production of an antimicrobial compound by *C. pseudodiphtheriticum* as a potential mechanism, we inoculated filter paper discs with cell-free *C. pseudodiphtheriticum* broth culture and cell lysates for a disc diffusion assay on *M. catarrhalis*. No inhibition was observed (Figure 2). It is possible that the inhibitory effect of *C. pseudodiphtheriticum* is not mediated by an antimicrobial substance, for example by competitive acquisition of nutrients. However, it is also likely that such a substance is produced, but was not successfully extracted from *C. pseudodiphtheriticum* culture in a functional state or at sufficient concentrations to produce a zone of inhibition, or its production requires a trigger (like the presence of *M. catarrhalis*). This preliminary work demonstrates that *C. pseudodiphtheriticum* inhibits the growth of *M. catarrhalis*; while this is the least common of the major otopathogens, it may be an important one to target.
given its apparent synergistic interactions with NTHi and *S. pneumoniae*\(^\text{23,24}\). However, the mechanism of interference, the role of *D. pigrum* and whether *Corynebacterium* species can inhibit URT pathogens at a clinically useful degree is yet unknown.

### Towards a potential probiotic therapy using *Corynebacterium* and *Dolosigranulum*

The consistent correlation between *Corynebacterium*, *Dolosigranulum* and upper respiratory health presents a very promising avenue of research towards development of probiotic therapies for the URT. The recent studies demonstrating antimicrobial activity by *C. accolens* against *S. pneumoniae*\(^\text{20}\), *C. pseudodiphtheriticum* against both *S. aureus*\(^\text{20}\) and *M. catarrhalis*\(^\text{22}\), and *C. propinquum* against coagulase-negative staphylococci\(^\text{23}\) suggest it is possible that multiple *Corynebacterium* species are beneficial to the URT, acting via different mechanisms against different pathogens. The role of *D. pigrum* in these interactions remains unknown, however it has been hypothesised that the production of lactic acid by

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Table 1. Studies observing the presence of *Corynebacterium* and *Dolosigranulum* in the URT microbiota and their association with a healthy state.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Major <em>Corynebacterium</em> and <em>Dolosigranulum</em> findings</th>
<th>Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bogaert et al. (2011)(^5)</td>
<td>• Both identified as common genera in the nasopharynx of healthy children</td>
<td>96 healthy children</td>
</tr>
<tr>
<td>Lauf et al. (2011)(^6)</td>
<td>• Both genera associated with decreased risk of pneumococcal colonisation and diagnosis of otitis media (OM)</td>
<td>108 children with upper respiratory tract infection symptoms</td>
</tr>
<tr>
<td>Pettigrew et al. (2012)(^7)</td>
<td>• Both genera associated with decreased risk of acute OM (AOM) when Streptococcus and Haemophilus abundance was low and no antibiotics used in the previous 6 months. • Levels of <em>Dolosigranulum</em> were lower in children receiving antibiotics (mostly (\beta)-lactams) in the previous 6 months</td>
<td>73 healthy children, 95 children with upper respiratory infection (URI), and 72 children with both URI and AOM</td>
</tr>
<tr>
<td>Biesbroek et al. (2014)(^8)</td>
<td>• Increased presence and abundance of both genera in breastfed children at 6 weeks of age. • <em>Dolosigranulum</em> negatively associated with wheezing and number of respiratory tract infections • <em>Corynebacterium</em> operational taxonomic unit highly homologous to <em>C. pseudodiphtheriticum</em> and <em>C. propinquum</em></td>
<td>101 breastfed infants and 101 formula-fed infants</td>
</tr>
<tr>
<td>Biesbroek et al. (2014)(^9)</td>
<td>• Both genera associated with breastfeeding and lower rates of respiratory infections. • Infants at 1.5 months of age dominated by <em>Corynebacterium</em> and <em>Dolosigranulum</em> shifted to <em>Moraxella</em> and <em>Dolosigranulum</em> at 6 months of age. These profiles were more stable than those dominated by <em>Haemophilus</em> or <em>Streptococcus</em></td>
<td>60 healthy children</td>
</tr>
<tr>
<td>Bomar et al. (2016)(^10)</td>
<td>• Both genera associated with the absence of <em>S. pneumoniae</em> colonisation • Demonstrated in vitro that <em>C. accolens</em> inhibits <em>S. pneumoniae</em> with antipneumococcal free fatty acids</td>
<td>9 children colonised with <em>S. pneumoniae</em> and 18 children without</td>
</tr>
<tr>
<td>Prevaes et al. (2016)(^11)</td>
<td>• Both genera associated with healthy infants compared to those with cystic fibrosis • Antibiotic treatment (predominantly co-trimoxazole) related to a reduction in <em>Corynebacterium</em> and <em>Dolosigranulum</em> in both groups</td>
<td>20 infants with cystic fibrosis and 45 healthy infants</td>
</tr>
<tr>
<td>Bosch et al. (2017)(^12)</td>
<td>• Prolonged reduction of <em>Corynebacterium</em> and <em>Dolosigranulum</em> associated with a higher number of respiratory tract infections • Both genera significantly reduced in abundance after antibiotic usage</td>
<td>112 healthy infants</td>
</tr>
<tr>
<td>Chonnairtree et al. (2017)(^13)</td>
<td>• <em>Corynebacterium</em> found to be associated with infants without AOM • Antibiotics (most commonly amoxicillin) did not affect otopathogen genera but significantly decreased <em>Corynebacterium</em> and <em>Dolosigranulum</em></td>
<td>65 infants with AOM and 74 infants without AOM</td>
</tr>
<tr>
<td>Hasegawa et al. (2017)(^14)</td>
<td>• Both genera associated with low likelihood of severe bronchiolitis</td>
<td>40 hospitalised infants with bronchiolitis and 110 healthy infants</td>
</tr>
<tr>
<td>Kelly et al. (2017)(^15)</td>
<td>• Both genera associated with healthy children compared to those with pneumonia • HIV-infected children with pneumonia had a near complete absence of <em>Dolosigranulum</em></td>
<td>204 children with pneumonia (14 with HIV infection) and 60 healthy children</td>
</tr>
<tr>
<td>Copeland et al. (2018)(^16)</td>
<td>• Both genera associated with healthy adults compared to those with chronic rhinosinusitis</td>
<td>21 adults with chronic rhinosinusitis vs 12 healthy adults</td>
</tr>
<tr>
<td>Lappan et al. (2018)(^17)</td>
<td>• Both genera associated with children resistant to recurrent AOM (rAOM)</td>
<td>86 children with rAOM and 98 rAOM-resistant children</td>
</tr>
</tbody>
</table>
D. pigrum lowers the pH of the local environment, selecting for the growth of Corynebacterium species. This is highly plausible, as several species of Lactobacillus inhibit M. catarrhalis via lactic acid, which also exhibits antimicrobial and anti-inflammatory properties in the human vagina. A niche-specific probiotic therapy containing multiple species with complementary effects may be an effective approach for the URT, especially for restoration after antibiotic use, which has been demonstrated to reduce Corynebacterium and Dolosigranulum populations (Table 1). Previous clinical trials using nasal spray probiotics for children with rAOM have suggested that pre-treatment with antibiotics allows re-establishment of the healthy microbiota, preventing the re-emergence of pathogens.

To fully investigate the possibility that these commensal organisms work synergistically to provide resistance to respiratory pathogens, and the potential for this to be translated into a therapy, there is an array of possibilities for future research. First, it remains likely that C. pseudodiphtheriticum produces an antimicrobial substance that inhibits M. catarrhalis. To isolate it, a range of commensal nasopharyngeal strains could be screened for optimal antimicrobial
activity, and optimisation of culture conditions or enrichment of proteins may be required, similar to the approach taken by Latham et al. (2017) for the isolation of an antimicrobial substance from *Haemophilus haemolyticus*. The commensal *Corynebacterium* genomes are not well characterised; a very recent sequencing effort by Stubbendieck et al. (2019) has improved the available genomic information for *C. pseudodiphteriticum* and *C. propinquum*, but these draft genome assemblies are still incomplete as of August 2019 (NCBI Genome Assembly and Annotation reports). There is now one strain of *D. pigrum* with a complete assembly (83VPs-KBS) available. Mining the genomes of multiple commensal strains for putative antimicrobial genes may provide further evidence for protective factors. Additionally, epithelial cell models have previously been successful in understanding the competition between *H. haemolyticus* and NTHi in the URT and would also be useful to obtain a deeper insight into the competitive mechanisms and host responses, to see if colonisation with these organisms in infants has the potential to treat or prevent a range of upper respiratory illnesses in children.

**Conflicts of interest**

The authors declare no conflicts of interest.

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**References**


Pathogen adaptation to vaccination: the Australian Bordetella pertussis story

Laurence Don Wai Luu
School of Biotechnology and Biomolecular Sciences, University of New South Wales, Sydney, NSW, Australia
Tel: +61 2 9385 4063
Fax: +61 2 9385 1483
Email: laurence.luu@unsw.edu.au

Christopher Peacock, BSc Hon Biological Sciences (1985), Fellow of the Institute of Medical Laboratory Sciences (1988), University of London, PhD Human Genetics on ‘the Susceptibility to Visceral Leishmaniasis’ (1998), University of Cambridge. After running a research and service histology laboratory at the London School of Tropical Medicine and Hygiene, he spent one year on a successful HIV project in Abidjan, West Africa followed by two years in the Amazon region of Brazil working on genetic susceptibility to TB, Leprosy and Leishmaniasis. Having completed his PhD in 1998, he undertook a position as a postdoctoral research associate continuing the work on human susceptibility to infectious diseases followed by a role as a senior computational biologist at the Wellcome Trust Sanger Institute leading to publication of the first Leishmania genome as part a Special edition of Science in 2005 and the publication of a comparative Leishmania genome paper in 2007 published in Nature Genetics. In 2007, he moved to Australia to help set up a Division of Genetics and Health in infectious diseases at the Telethon Institute of Child Health Research and in 2009 took up a senior lecturer position at the University of Western Australia. Shortly afterwards he was awarded one of the inaugural ARC Future Fellowships. In addition to neglected tropical diseases, his research interests now encompass metagenomics, and novel parasitic infections in indigenous wildlife.

Whooping cough (pertussis) is a highly contagious vaccine preventable respiratory disease caused by the Gram-negative bacterium Bordetella pertussis. Despite high level vaccination coverage over the past 20 years, Australia has one of the highest per capita burdens of pertussis globally. One of the primary factors associated with the re-emergence of pertussis is pathogen adaptation of B. pertussis to the current acellular vaccines used. This article will focus on the genomic and proteomic changes that have occurred in the Australian B. pertussis population, the significance of these adaptive changes on fitness in a vaccinated environment and what we can do to reduce the significant burden of pertussis in the future.

The rising incidence of B. pertussis in Australia

Pertussis vaccinations were first introduced in Australia in 1953 using a whole cell vaccine (WCV), which contained dead B. pertussis cells. This led to a dramatic reduction in the number of pertussis notifications from 767 cases per 100 000 in the 1930s...