permits the easy selection of *S. aureus*, reduces laboratory costs and improves patient care.

Therefore, it would appear that the best clinical and laboratory strategy for *S. aureus* screening from nasal swabs is a combination of direct plating on chromogenic agar, read at 24 h, with an enrichment stage that is followed by plating on chromogenic agar. In this study, 63.5% of *S. aureus* isolates were detected at 24 h, with the remaining 36.5% identified at 48 h. Pre-enrichment of swabs in brain-heart infusion broth, followed by plating on SAID agar, will be used as the strategy of choice for screening the remaining 1046 nasal swabs for *S. aureus* for the larger study designed to determine the carriage rate of MSSA and MRSA and the associated risk factors.

The positivity rate for carriage of *S. aureus* in this study of 204 nasal swabs was 41.7%, which is higher than the rate quoted by CDC; however, the number of swabs examined was small and the true positivity rate will only be determined following completion of the main study.

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Long-term antibiotic treatment of patients with cystic fibrosis: a commensal organism's view

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The long-term use of several classes of antibiotic agent for the prophylaxis, maintenance and treatment of bacterial respiratory pathogens causing chronic chest infections in

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patients with cystic fibrosis (CF) has important consequences for the persistence of commensal flora of the treated patient. In order to survive, such commensal organisms must evolve resistance mechanisms in response to the chronic use of these antibiotic agents. What is not known at present is which resistant mechanisms commensal organisms use, and are these mechanisms potentially transferable to hitherto sensitive pathogens? Therefore, antibiotic resistance in the commensal flora of CF patients may be an important reservoir of genetic material for exacerbating antibiotic resistance in CF pathogens, and this area requires urgent investigation.

Cystic fibrosis patients present a special patient population, in terms of their prolonged use of several antibiotic agents simultaneously throughout life. This will include employment of antibiotics for use in antistaphylococcal prophylaxis during early childhood (<10 years) to prevent the acquisition of *Staphylococcus aureus*, as well as in therapeutic maintenance and intravenous intervention during acute pulmonary exacerbations, mainly for multiresistant and pan-resistant Gram-negative organisms, particularly *Pseudomonas aeruginosa*. Additionally, long-term use of azithromycin (500 mg od/three times per week) is now a common treatment regimen in CF adults, where this agent acts in an immunomodulatory fashion.

In a recently published survey of adherence to European Consensus Guidelines for CF, it was shown that several antipseudomonal antibiotics are used in European centres, depending on the stage of pseudomonal infection (i.e., first detection, intermittent or chronic infection).[1] These included nebulised tobramycin, nebulised colistin, nebulised colistin plus oral ciprofloxacin, oral ciprofloxacin, gentamicin, ceftazidime and others.[1] With co-infection with other CF pathogens, such as *Burkholderia cenocepacia*, the non-tuberculous mycobacteria (particularly *Mycobacterium abscessus*), methicillin-resistant *S. aureus* (MRSA) and other resistant non-fermenting Gram-negative rods, the CF patient is unrivalled in medical microbiology in terms of the complexity of their antibiotic management, the number of antibiotics employed and the long-term use of such agents.

Prolonged use of multiple antibiotics, often used simultaneously in dual or triple therapy, presents an enormous challenge to the commensal flora of the patient and adds great pressure on organisms to adapt in order to survive in their anatomical niche. Previously, Gustafsson and colleagues demonstrated that high antibiotic use selected for commensal organisms with highly increased resistance and a slight increase in mutation frequency.[2] Failure to adapt ultimately will result in their eradication from the body. Therefore, it is in the commensal organism's interest to adapt to a dynamic flux of antibiotic agents, either through development of *de novo* resistance or genetically by acquiring resistance determinants from other organisms that transiently colonise the respiratory tract in a non-pathogenic manner, as well as from true bacterial pathogens that may have either short-term or long-term persistence in this niche. Equally, with their need to maintain resistance to long-term use of antibiotics, does this affect their bacterial fitness?

We now know considerably more about the microbial diversity of the populations of organisms colonising the CF lung, through employment of non-cultural techniques, but more so by molecular techniques, including T-RFLP. The seminal work in this field by Rogers *et al*.[3,4] has demonstrated the presence of many genera, which are not routinely reported from conventional microbiology analyses, either due to an inability of the diagnostic laboratory to detect such organisms, or, alternatively, not reporting the presence of these organisms as they are not believed to be true pathogens and hence are of little or no clinical concern. The work of Rogers *et al*.[3,4] therefore gives us a further insight into the structure of the commensal population within the CF sputum and an appreciation of what genera and species exist in this niche.

Other niches of interest in the CF patient include the skin, the mouth and, most importantly, the gastrointestinal tract, where commensal organisms may have an altered and higher level of resistance than similar commensal flora in a non-CF population not exposed to long-term antibiotic use. The development of multiple, elaborate and simultaneous resistance mechanisms confers an ecological advantage on the commensal flora, whereby it now possess a genetic mechanism to ensure its survival in its own ecological niche, thus maintaining a hostile flora to challenging and potentially colonising pathogens (e.g., *Pseudomonas aeruginosa* and *B. cenocepacia*).

Equally, with the ability to survive intense and prolonged antibiotic pressure, such commensal flora are dangerously poised to become potential pathogens if (i) there is a downward shift in the immune status of the patient (e.g., following lung transplantation, (ii) where such commensals are genetically promiscuous in acquiring virulence determinants from co-habiting true pathogens, and (iii) where horizontal gene transfer events occur, leading to the acquisition of antibiotic resistance determinants by newly colonising pathogens. For example, Kanj *et al*.[5] previously reported post-transplant bacteraemia infections in the CF population, with commensal flora in addition to the normal CF pre-transplant Gram-negative respiratory flora. Coagulase-negative staphylococci caused bacteraemia in three patients on days 16, 132 and 169, respectively, following lung transplantation. Enterococcal infection was described in two patients on days 24 and 41, respectively, post-transplantation, as well as another infection with *Nocardia asteroides* at day 478. Hence, careful attention must be given to the potential development of a highly resistant commensal flora, particularly if the patient is likely to become immunocompromised.

The acquisition of virulence determinants is also a significant cause for concern in antibiotic-resistant commensal organisms, and where such commensal flora dominate. One reason for their success is the relative plasticity of their genomes to adapt to varying host immune responses, as well as selective antibiotic pressure. With this genomic plasticity and the ability to naturally transform, commensal organisms have the ability to take up virulence determinants, which then potentially could transform their status from commensal organism to opportunistic pathogen to true pathogen. A good example of this has been *Escherichia coli* and the several pathogenic subtypes of *E. coli*, including enteropathogenic *E. coli* and verocytotoxigenic *E. coli* (VTEC). Within the Gram-positive organisms, *Streptococcus agalactiae* recently has been shown to have recombinational ability, leading to the replacement of a locus of several genes or the allelic exchange of the internal part of the gene.[6] Hence, this organism, which is primarily a commensal organism colonising the gastrointestinal and genitourinary

tracts of up to 50% of healthy adults, now has the ability to become pathogenic to the host.

Recently, we have shown that long-term use of azithromycin in adult CF patients has led to a macrolide-resistant population of viridans group streptococci (VGS) isolated from patients' sputum, in comparison to similar VGS populations originating from non-CF patients not treated with azithromycin for long periods of time.[7] In addition, complete gene homology in the macrolide resistance determinants, particularly *erm*(B) and *mef*(A) is also shared with other closely related genera, including *Gemella, Enterococcus* and *Granulicatella*. Presence of multiple macrolide-resistance determinants occurring at high frequency in VGS commensal organisms is of potential importance to the CF patient, other CF patients and the non-CF population. The presence of bacterial pathogens in CF sputum generally reflects those bacterial genera commonly associated with CF lung disease, including *P. aeruginosa* and *B. cenocepacia*. Normally, these pathogens do not constitute an infection risk to the healthy non-CF individual. However, the presence of such macrolide-resistance determinants in VGS organisms may be problematic for the healthy non-CF individual, as these may act as a reservoir of resistance determinants for other respiratory pathogens, particularly *Streptococcus pneumoniae*, where these commensal flora are transmitted from the CF patient to non-CF individuals (e.g., between CF and non-CF siblings within a household). Furthermore, the existence of macrolide-resistance determinants has been described in environmental waters (GenBank accession number: EU168331), farm animals (GenBank accession number: EU168331) and domestic animals, highlighting the important ecological evolution and transmission of these genes globally.

Finally, the presence of highly resistant commensal organisms and their genetic resistant determinants is of potential interest to infection control in the hospital setting, particularly in relation to CF and non-CF patients (i.e., are CF patients an important reservoir of resistance determinants?) For instance, given the amount of β-lactams taken by CF patients during their lifetime, we do not known at present whether or not their intestinal flora react by becoming extended-spectrum β-lactamase (ESBL) producers. Therefore, more research is required urgently to assess what genetic mutations and resistance determinants are being selected naturally by the commensal flora of the CF patient and whether or not these mutations are important in terms of their transmissibility to hitherto sensitive pathogens.

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Hb Owari associated with α-thalassaemia-1 in a Taiwanese subject

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An abnormal haemoglobin, Hb Owari (*HBA2*:c,364 G>A; or HBA1) was first described in Japan by Hrano *et al*. in 1986.[1] It is a non-pathological α-chain variant characterised by a mutation at the α^{121} position that changes valine to methionine (121[H4]Val>Met). It produces a neutral-to-neutral amino acid substitution in the α-chain. The site of amino acid substitution (α121) can be determined by the chymotryptic digest fingerprinting of the core fraction of the α-chain, with the oxidised counterpart of the abnormal peptide (α118–22) easily found as an extra spot. The clinical presentation of heterozygous Hb Owari is normal, and the proportion of abnormal HbX is 12.7–19% of total haemoglobin. However, the compound heterozygote with other haemoglobinopathies had previously not been reported. This study presents a case of a compound heterozygote of Hb Owari with α-thalassaemia-1.

A 25-year-old Taiwanese visited the haematology out-patient department as microcytic anaemia was noted at regular check-up in June 2009. Peripheral blood examination showed a microcytic hypochromic anaemia, and the red cells showed mild microcytosis and hypochromasia. The haemogram was as follows: Hb 13.4 g/dL, RBC 5.81×10^6/μL, MCV 72.5 fL, MCH 23.1 pg and MCHC 31.9 g/dL. White blood cells and platelets were within the normal range. Serum iron and ferritin levels (92 μg/dL and 95.3 ng/mL, respectively) were within the normal range. High-performance liquid chromatography (HPLC; Primus CLC 385, Primus, Kansas City, USA) showed an abnormal HbX peak (36.2%) at a retention time of 4.76 min (Fig. 1).

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