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Science writer **Meriel Jones** takes a look at some recent papers in SGM journals which highlight new and exciting developments in microbiological research.

▲ A hospital hand wash. How effective are alcohol-based hand rubs against *Acinetobacter baumannii*? *Mark Thomas / Science Photo Library*

Alcohol hand rubs increase growth of *Acinetobacter*

Edwards, J., Patel, G. & Wareham, D.W. (2007). Low concentrations of commercial alcohol hand rubs facilitate growth of and secretion of extracellular proteins by multidrug-resistant strains of *Acinetobacter baumannii*. *J Med Microbiol* **56**, 1595–1599.

The medical profession continues to be haunted by serious bacterial infections acquired within hospitals. If the bacteria can survive antibiotics, as in the so-called multidrug-resistant strains, treatment can be very difficult. The classic measure of good hand hygiene can reduce infection rates by 10–50 %, so hospitals have introduced ways for staff to clean their hands frequently without suffering from chapped skin. These include using alcohol-based hand rubs (ABHRs) dispensed from a pump adjacent to patients' beds. These are solutions of ethanol or isopropanol, often in combination with an antiseptic and moisturiser which are less irritating to skin than soap.

Although studies have shown that ABHRs reduce infection rates, they are not effective against all bacteria. One example is the spores of *Clostridium difficile* that can cause very serious gastrointestinal infections. Now, Justin Edwards, Geeta Patel and David Wareham from Barts and The London NHS Trust and the MRC Clincal Sciences Centre of Imperial College London have found evidence that low concentrations of ABHRs enhance *in vitro* growth of *Acinetobacter baumannii*, an opportunistic pathogen of critically ill patients.

A. baumannii has emerged in recent years as an important cause of ventilator-associated pneumonia and blood infections in patients with burns, immunosuppression and critical illness. Many strains also turn out to be resistant to many antibiotics so that treatment is extremely difficult. The bacteria have a remarkable ability to persist on surfaces in the hospital environment and are spread by the hands of hospital staff so that hand hygiene is the key factor in preventing these infections.

The researchers therefore tested whether low concentrations of commercially available ABHRs could affect the growth of multidrug-resistant *A. baumannii*. Depending on the growth medium, the presence of 0.01–1 % ABHR resulted in increased growth of *A. baumannii*. In contrast, a hand-cleaning product that was free of alcohol and relied on strong detergents to kill bacteria did not support the growth of *A. baumannii* at all.

The researchers then investigated the proteins secreted by *A. baumannii* as it grew because previous work has shown that low concentrations of ethanol increase the virulence as well as the numbers of *A. baumannii*. They discovered that OmpA was a major secreted protein, along with another protein with an unknown role. OmpA is well known and may help the cells take up ethanol as a food source when other nutrients are in short supply. It may also help the bacterial cells sense each other and form films on surfaces.

The findings of this paper are certainly interesting, but their clinical significance remains unclear. However, if low concentrations of ABHRs do indeed exist in the clinical environment, this work may have implications for those hospitals currently experiencing outbreaks of *A. baumannii*.

A novel amniotic pathogen

Lawson, P.A., Moore, E. & Falsen, E. (2008). *Prevotella amnii* sp. nov., isolated from human amniotic fluid. *Int J Syst Evol Microbiol* **58**, 89–92.

Researchers from Göteborg, Sweden, and Oklahoma, USA, have identified a novel species of bacterium from human amniotic fluid. The bacteria were first recovered in 1999 from fluid that was described as turbid and ill-smelling. Similar bacteria were found again in amniotic fluid in 2006 and the authors have now worked out exactly what sort of bacteria they are. The cells will only grow in the absence of oxygen, which makes working with them rather difficult. The researchers first tried out the standard biochemical identification tests used with anaerobic bacteria, then commercial bacterial identification kits and finally they analysed the fats within the bacteria and the sequence of one gene.

A comparison of the sequence of the rRNA gene from the two isolates proved they were highly related to each other because the genes were 99.5 % similar. This gene is chosen for taxonomic studies because it changes very slowly over time. As a result, although researchers cannot give a precise correlation between a species definition and rRNA similarity, they consider that any difference over 3 % is significant. The similarity between the two strains and the closest named bacterium was 95.3 %. These bacteria were members of the *Bacteroides–Prevotella–Porphyromonas* group and the two isolates were especially close to members of the genus *Prevotella*. Many species of *Prevotella* have been isolated from people, from the mouth and from both healthy and infected tissues in the pelvic region. The rRNA sequence of the two isolates and the range of sugars and other chemicals that they were able to synthesize were different from all known *Prevotella* species. The closest relative was *Prevotella bivia* which was first identified in the endometrium and has frequently been detected in patients with pelvic inflammatory diseases.

In addition to these two bacterial strains, the researchers realised that several studies that had simply isolated DNA from the vagina of healthy women had recovered the same DNA sequence. It therefore looks like *Prevotella amnii* may be present all the time among the bacteria that inhabit the healthy vagina. However, in some circumstances its numbers increase and it can become a pathogen. The fact that the researchers have now discovered ways to identify this species means that routine hospital laboratories can be more confident in their identification of new opportunistic bacterial pathogens.

Something in the air

Ogata, N. & Shibata, T. (2008). Protective effect of low-concentration chlorine dioxide gas against influenza A virus infection. *J Gen Virol* **89**, 60–67.

'Flu, caused by the influenza A virus, is a continuing health problem. It infects around one-fifth of the world's population yearly, causing symptoms that range from very minor to a severe illness. Indeed, 'flu is a factor in the death of around half a million people every year. Some pandemic strains have caused more severe mortality, such as the 1918 pandemic that killed 20–50 million people worldwide. Measures to counteract 'flu include vaccination, which only has short-term and partial success, and antiviral drugs, which all have drawbacks, such as toxicity to the sufferers and the emergence of resistant influenza strains.

The 'flu virus is spread in the air as patients cough and sneeze. Researchers at the Taiko Pharmaceutical Co. Ltd in Japan have been wondering whether there is a way to deal with the virus when it is in this airborne form, so reducing the opportunities for infection. There are many chemicals that are very effective at killing viruses, but most are also toxic to people. The researchers investigated chlorine dioxide (ClO_2) gas, which has a well-known and very effective antimicrobial action when dissolved in water. It has been used to disinfect public supplies of drinking water since the 1950s and is also used in food industries. Air containing low levels of ClO_2 is considered safe to breathe, with the US Occupational Health and Safety Administration setting an upper limit of 0.1 parts per million (p.p.m.) for ClO_2 in the workplace. Norio Ogata and Takashi Shibata wondered whether even this low level could be useful against the 'flu virus.

▶ A crowded public space. Could the addition of chlorine dioxide to the air in public buildings help to prevent the spread of influenza? *Jupiter Images*

They arranged for small groups of mice to inhale a 'flu aerosol for 15 minutes, counted the number of virus particles in the lungs of the mice and recorded their health 16 days later. The air breathed by half of the mice also contained about 0.03 p.p.m. ClO_2 gas. This gas reduced the number of virus particles by over 1000-fold 3 days later and all the mice were alive 16 days later. In contrast, seven out of the 10 mice that had inhaled the viral particles alone had died. The ClO_2 gas had to be present at the same time as the virus in the air; breathing it even 15 minutes later completely cancelled the protective effect. The reason was that the gas damages proteins on the outer surface of the virus that are essential for attachment to mammalian cells to start an infection. The authors suggest that ClO_2 gas could therefore be used to disinfect the air in places such as airports, hotels, offices and schools without the need to close the buildings and thus disrupt the flow of normal life.

◀ A 19th-century tuberculosis ward. This is the Haskoy Hospital for Women in Constantinople (present-day Istanbul), then the capital of the Ottoman Empire. Tuberculosis (TB) is a bacterial infection, usually of the lungs, that was a widespread cause of death before a vaccine was developed in the 20th century. In the late 19th century it was discovered that the disease was contagious, and hospitals were created to quarantine patients and prevent the spread of the disease. This photograph, by the Abdullah Brothers, was taken in the period from 1880–1893.*Library of Congress / Science Photo Library*

Export blockade

McCann, J.R., McDonough, A., Pavelka, M.S. & Braunstein, M. (2007). *β*-Lactamase can function as a reporter of bacterial protein export during *Mycobacterium tuberculosis* infection of host cells. *Microbiology* **153**, 3350–3359.

Tuberculosis continues to kill millions of people slowly every year. It is caused by the bacterium *Mycobacterium tuberculosis*. Few drugs are effective against this species and the bacteria have very effective strategies to thrive within the body. For example, they colonize macrophages, a type of human cell that usually destroys invading bacteria. To do this the bacterium secretes proteins to ensure survival through interference with the body's defence systems. *M. tuberculosis* has at least four systems to export proteins from the cells. The one that is probably responsible for most protein secretion is the general secretion pathway (Sec), but the twin-arginine translocation pathway (Tat) is also important. The pathways recognize signals on the proteins to identify which ones to export, but also appear to export proteins that lack conventional signals. *M. tuberculosis* secretes proteins when grown alone in laboratory cultures, but a different set appears to be made and exported during infection of the host. Researchers think that vaccines or drugs to prevent export of these proteins could be very effective therapies. Unfortunately, it has proved difficult to even identify them since it is difficult to collect bacterial proteins from infected human tissues.

Researchers in the USA have now devised a system to identify and study these elusive proteins by turning an antibiotic resistance mechanism against the bacterial cells. The trick is to use a test where the exported protein, in this case *β*-lactamase, is essential for the bacteria to survive. *β*-Lactamase is an enzyme that many bacteria use as protection against the antibiotic penicillin. Since penicillin interferes with synthesis of the bacterial cell wall, the *β*-lactamase must be outside the cell to provide protection. *M. tuberculosis* is normally resistant to penicillin so the researchers created a sensitive strain by removing the protective gene, called *blaC*. They then linked the *β*-lactamase gene to any gene where they suspected that the encoded protein might be exported. Mammalian cell cultures were infected with the bacteria and penicillin was added. If the cells really did export the protein, the *β*-lactamase was exported as well and protected the bacteria from the antibiotic. If the protein was not exported, the bacteria died.

The idea was tested with several *β*-lactamase genes using proteins known to be exported by the Tat and Sec pathways to come up with a reliable system. Shortened versions of the *Escherichia coli* TEM-1 *β*-lactamase gene as well as *M. tuberculosis* BlaC worked well with several export signals. The cultured mammalian cells were very like macrophages, so the researchers think that they now have a way to identify the most interesting category of proteins, namely those that are exported by *M. tuberculosis* during intracellular growth.