# MRSA, New, Yet Old

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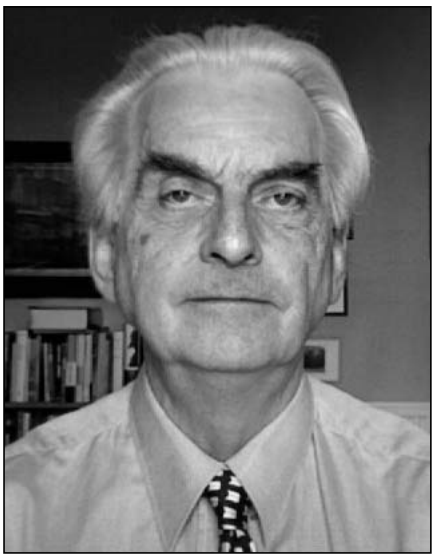

When asked to become President of MRSA Action UK I accepted without hesitation. The decision had nothing to do with my own status as an MSSA carrier (I have been one since my medical student days) but was due to the privilege of becoming formally associated with an organisation typical of the best British special interest groups – ones which exert beneficial effects on policy far outweighing their sparse resources – and because of its aim, which is to prevent the preventable.

It is hard to think of a better example of Hegel's principle – "what experience and history teach is this – that people and governments never have learned anything from history, or acted on principles deduced from it" – than MRSA. Its history also exemplifies another principle – that the relationship between science, practice, and policy is hardly ever simple or straightforward. Perhaps most disappointing of all is that, although the story of MRSA science has been one dominated by British discoveries, we currently languish at the bottom of the international league of success in controlling it in our hospitals.

MRSA stands for methicillin resistant *Staphylococcus aureus*. Medical bacteriology became a science in the late nineteenth century. It was dominated by Germans. They discovered most of the important organisms. The big exception was *S. aureus*, which was first identified and named in 1880 by Alexander Ogston, a surgeon at Aberdeen Royal Infirmary. Ogston was an enthusiastic proponent of the antiseptic methods developed by Joseph Lister in Glasgow in the late 1860s and early 1870s. My estimate is that of all the preventive measures introduced against the staphylococcus, its impact has never since been matched. Before its introduction the *mortality* rate of 'cold' – non-traumatic – orthopaedic operations done by the most experienced surgeons was about 9%. Wound *infection* was virtually universal. In 1884 William Macewen reported his series of 804 antiseptic limb-bone operations at Glasgow Royal Infirmary. Only 8 became infected, and only 3 died, one of pneumonia, one of tuberculosis, and one of diphtheria.

Lister's carbolic worked against the staphylococcus. But it was toxic. Not only did it wreck the hands, it was absorbed through the skin and damaged the kidneys. When a surgeon started to pass black urine it was time for him to take a holiday. Alternative antiseptics came in. Research done in the 1890s showed that hand hygiene with alcohol worked well against *S. aureus*. Its therapeutic index – comparison of its staphylococcal killing power against its ability to cause dermatitis – was good. It was widely adopted. But rubber gloves were introduced and its use fell away.

In the 1930s the standard multi-volume British bacteriology textbook was the Medical Research Council's *System of Bacteriology*. Alexander Fleming wrote the chapter on *Staphylococcus*. Its preparation required him to do some research. It led to the discovery of penicillin in 1928. And the first patient to be treated in its first clinical trial by Howard Florey and his team at Oxford had a staphylococcal infection. Albert Alexander was a policeman. An infection of his face from a rose thorn scratch had spread to his lungs and shoulder. He first received penicillin on 12 February 1941, and improved dramatically. But even with the recycling of penicillin from his urine, the supply ran out, and he relapsed, dying of staphylococcal septicaemia on 15 March.

Fleming discovered the first naturally-occurring penicillin-resistant staphylococci in 1942. Then they were uncommon. However, important research by the bacteriologist Mary Barber at the Hammersmith Hospital in London showed that not only did they increase proportionately soon after the introduction of penicillin

(from 12.5% in April-November 1946 to 38% by February-June 1947) but that the rise was not caused by the organisms becoming resistant while patients were being treated. It was due to the spread of a resistant strain in the hospital. Such strains made penicillinase, a penicillin-destroying enzyme. In response a penicillin derivative resistant to the enzyme, methicillin, was developed by the Beecham Research Laboratories in Surrey. It was thought that penicillinase production was the only way for a staphylococcus to become resistant to penicillin, so resistance to methicillin would not develop. But within a year such strains appeared, at Guildford. The first MRSA outbreak occurred two years later, in 1963, at Queen Mary's Hospital for Children at Carshalton. Eight wards were affected; thirty-seven patients were infected and one died. Gordon Stewart was its bacteriologist at the time. He closed his account of the outbreak with prescient words: "Lastly, and most important, patients harbouring these rare strains must be isolated, vigorously treated, and preferably should be sent out of hospital as soon as possible." The organism continued to cause problems, however, and bacteriologists to warn.

A 1985 account of a two-year outbreak at the Royal Free Hospital concluded "Several authors have reported failure to contain MRSA infection without an isolation unit; hospitals without such facilities or, as at this hospital, unable to finance the staffing of a unit, may find that this epidemic MRSA will pose a considerable threat to their clinical practice."

MRSA are antibiotic resistant because they have acquired a gene, *mec* A, that allows them to build cell walls (a process blocked by penicillin antibiotics) in the presence of methicillin. At least eleven different MRSA have evolved independently in different parts of the world. A turning point for the UK was the appearance of two epidemic strains, EMRSA 15 and 16. EMRSA 16 was first seen in Kettering in 1992.It spread quickly. In 1994 it was causing problems in 21 London hospitals. By 2000 it was common throughout Britain, and was spreading internationally. The voluntary reports to the Health Protection Agency (and its predecessor, the Public Health Laboratory Service) of staphylococcal bloodstream infections in England, Wales and Northern Ireland are informative. In 1992 116 isolates were resistant and 4462 sensitive. In 2003, 6085 were resistant and 8560 sensitive. A simple way of monitoring the scale of the problem is to measure the ratio of the two. It is reasonably accurate because it automatically takes account of changes in hospital practice that affect staphylococcal infections as a whole. Resistant strains became commoner. By 1999 they accounted for 40% of *S.aureus* isolates. It is still the same today. But in the Netherlands it is about 1%. Why is this?

The Dutch and Scandinavian success in controlling MRSA has been due to their policy of "search and destroy". Key elements are the treatment of MRSA carriers in single rooms with barrier precautions, the screening and precautionary isolation of high-risk patients (eg those from endemic places like the UK) until negative test results come, the vigorous investigation of all patients and healthcare workers in a ward if any patient becomes a carrier, and the closure of a ward to new patients if there is evidence of the transmission of infection. Hand disinfection is not mentioned in Dutch guidelines because it is already being done assiduously. Using mathematical modelling the Dutch have concluded that their success has been due to their combined approach – no single measure will work on its own – and that if applied to the UK it would bring our MRSA levels to theirs within 6 to 12 years.

During the first three decades of their evolution UK MRSA caused local outbreaks. A degree of complacency developed; "search and destroy" was deemed to be too expensive. When EMRSA 15 and 16 appeared they were not taken seriously enough. Old habits die hard; policy makers have only just begun to give isolation the attention it needs.

Staphylococci grow well on agar plates. But saying that exhausts virtually all that is straightforward about them. All attempts to develop vaccines have failed. We do not know why some people carry *S.aureus* for life and others not, neither do we understand why EMRSA are such successful nosocomial pathogens. For the overwhelming majority of patients infected in hospital, the precise route of transmission is never established. Is aerial transmission important? We do not know. Will the new community MRSA strains establish themselves in hospitals? We can only guess.

Some complain that MRSA have become political. Their analysis is right, but their judgment wrong. All infections have a political dimension. Consider foot and mouth disease. Even before it ceased to be endemic in Britain, in 1889, the Government had a vigorous stamping out policy – search and destroy. It has spent billions. If only we had had the same for MRSA!

# "difficile" by name, "difficile" by nature

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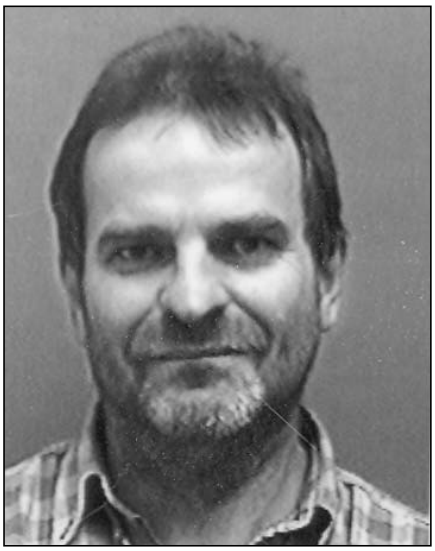

The bacterial genus *Clostridium* is an ancient grouping, which evolved on this planet long before there was an atmosphere. To them oxygen in the air we breathe is a poison. They are 'anaerobes', and thrive in oxygen-free environments such as our digestive tracts. Partly as a means to survive exposure to the air, they produce a specialised structure called an endospore. Compared to normal bacterial cells, spores are extremely resistant to all manner of chemical and physical agents, surviving exposure to heat, drying, certain disinfectants and low energy radiation.

The antics of a few give this large genus a bad name, just 12 species cause over 90% of clinical disease. The vast majority are entirely benign. Indeed, many species are of great value to mankind. *Clostridium acetobutylicum*, the forerunner of the modern biotechnology industry, is able to ferment renewable carbon neutral biomass into butanol – a biofuel superior to ethanol as a petrol substitute. The neurotoxin of *Clostridium botulinum*, more popularly associated with BoTox and cosmetics, has tremendous therapeutic uses (eg the treatment of squints), while the spores of harmless clostridial species have great potential as tumour delivery systems for treating cancer.

*C. difficile* is a black sheep of the family. The organism is part of the 'normal' gut flora in 3% of healthy adults, although this percentage increases with age. Problems occur when the good bacteria in the gut are disrupted, most usually through their obliteration by prescription of antibiotics. Under these conditions, *C. difficile* proliferates to cause *Clostridium difficile*-associated disease (CDAD). Clinical severity ranges from a self-limiting diarrhoea, through acute and severe diarrhoea to the potentially fatal pseudomembranous colitis. The bacterial factors responsible for disease, so-called virulence factors, are two large toxins (Toxin A and B). Spores are pivotal in disease transmission, but while other factors must play a role, their identity currently remains little more than conjecture.

Since the turn of the new millennium there has been a dramatic rise in the incidence of *C. difficile*. Cases of CDAD in England and Wales have increased year on year from 19,600 cases in 2000 to 55,620 in 2006, a 184% increase. As a result, 2005 saw 3807 death certificates on which *C. difficile* was either directly or indirectly attributed as the cause of death; more than twice that of MRSA. A number of reasons have been suggested for this increase, ranging from improvements in reporting procedures, the increasing age of the population and therefore the number at risk, increased antibiotic resistance, lower standards of hygiene and overcrowding in hospitals. A further significant factor has been the emergence of so-called 'hypervirulent' strains.

Reports on the emergence of more virulent strains in Canada first began to appear in the scientific literature in 2003. These documented an increase in incidence (5-fold the historical average); more severe disease (complications rising from 7.1% to 18.2%); higher relapse rates (increased from 20.8% to 47.2%); increased mortality (from 4.7% to 13.8%) and great antibiotic resistance (most notably to fluoroquinolone antibiotics). Characterisation of the strains involved indicated that they were all of one particular type (type 027 of the 150-plus recognised ribotypes), that they all produced a relatively rare toxin (CDT) in addition to toxins A and B and carried a mutation in a gene *(tcdC)* that leads to the production of increased levels of toxins. By June 2006, type 027 strains had been reported in 7 Canadian provinces, and by October 2007 had been isolated in 37 US states. The scientific community at large and the public alike became generally aware of similar problems in the UK in June 2005 with The Independent front page headline 'New Superbug threatening Britain's hospitals'. It referred to two outbreaks at Stoke Mandeville hospital between October 2003 and June 2004, and again between October 2004 and June 2005. Over this period some 334 patients were affected with 38 mortalities. Since this date numerous

UK hospitals have been affected, and 027 strains have now been isolated from 16 European states and Switzerland.

Between 1990 and 2003, laboratory reports from England and Wales collected at the Anaerobe Reference centre by Jon Brazier demonstrated that the most common UK 'epidemic' strains belonged to ribotype 001 (55%). The second most common strain was type 106 (10%). By 2005, when a random survey was undertaken over a 1 week period, 001 had declined to just over 25%, type 106 had risen to nearly 26%, and 027 had burst on to the scene representing almost 25% of all isolates. A similar survey is currently ongoing, and while the results are not finalised, 001 seems to have fallen away further, while 106 and 027 remain neck and neck (Jon Brazier, personal communication). These newly common strains are more resistant to antibiotics than other strains so that the once dominant strain of the 1990s, type 001, is being replaced by "fitter" strains that have advantages in adapting to and overcoming the changing selective pressures of our healthcare environment.

Currently many UK hospitals and elderly nursing homes have high levels of contamination with *C. difficile* spores, with increasing numbers of susceptible, antibiotic-treated patients propagating the organism. If infection rates are to be controlled, a number of measures need to be followed. These include: regular surveillance; isolation or barrier nursing; maintenance of high standards of personal hygiene, and; intensive cleaning of affected wards to remove the bacterial spores. These measures need to be mindful of the fact that spores of *C. difficile* are resistant to alcohol-based antiseptics (alcohol hand-washing gels are ineffective), and chlorine-based disinfectants can be only partially effective. To minimise outbreaks and spread of the organism, adherence to strict antibiotic policies is required. The use of oral cephalosporins and clindamycin, which are known to precipitate the disease, needs to be restricted. Additionally fluoroquinolones, not previously associated with the disease, now seem to be selecting for hypervirulent strains such as type 027 strains and need careful use. Future research must concentrate on: developing improved diagnostic methods; increasing our knowledge of the mechanisms by which the host becomes resistant/susceptible to infection; developing new therapies; improving knowledge of transmission mechanisms; developing disinfecting/cleaning methods that remove the spores from the patient environment, and; increasing our understanding of what makes a strain virulent.

The Clostridial Research Group, within Nottingham's Centre for Healthcare Associated Infections (CHAI), is focused on a number of issues. We are particularly interested in determining how the organism causes disease and why certain strains have become hypervirulent. If we are to make progress, we need to identify the *C. difficile* determinants that are required for infection and disease progression. Insight into possible mechanisms has arisen following the determination of the genome sequence of a representative strain. However, such a genetic blueprint tells us there are 4,000 or so individual genes, but it doesn't tell us what they do. In biology, you never really know what a gene does until it isn't there. Thus, to prove that any gene product contributes to disease we need to inactivate the gene and compare the virulence of the mutant generated to the non-mutated organism. Until recently this was not possible in *C. difficile*, as the methods available for making mutants were ineffective. A technological breakthrough at Nottingham has removed this bottleneck with the development of the ClosTron gene targeting system. It enables the rapid and reproducible creation of mutants, and has led to a 5 year MRC project (initiated October 2007) in collaboration with UCL (Peter Mullany) which seeks to inactivate all those genes previously hypothesised as being involved in virulence and assessing the effect on the capacity of the strain to cause disease. If we understand how the bug causes disease, we can develop rational countermeasures.

Equally important is the need to be able to rapidly diagnose CDAD. Symptoms alone are not enough to diagnose the condition. Toxin assays can reveal the presence of *C. difficile* in the patient's faecal sample, but can result in false negatives if concentrations are too low. Culturing the organism is more sensitive if methods are carried out correctly, but can give false positives, as some people are asymptomatic carriers. New, more rapid methods are required, particularly to identify the new hypervirulent strains. Towards this target, Nottingham is part of a European consortium, lead by Dr Ed Kuijper (Leiden University Medical Centre, NL), which seeks to develop appropriate diagnostic tests. Their development will enable clinicians and infection control teams to mount more immediate and effective countermeasures.

At Nottingham we have initiated programmes of work which should eventually lead to more effective means of controlling CDAD. In the mean time, the UK has one of the worse, if not the worst, rates of *C. difficile* infection in the developed world. We can clearly do better, and there is a collective responsibility from all those concerned (politicians, funding agencies, healthcare professionals, research scientists) to deliver a safer environment to the UK public.

# HIV Vaccines

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More than 20 new virus infection threats have emerged in the last 30 years. Of these, Human Immunodeficiency Virus (HIV) has dominated, although avian influenza has the potential to be even more catastrophic. HIV emerged in central sub-Saharan Africa by transfer from Chimpanzees to humans. Chimpanzees are widely infected with a very similar virus, the closest to HIV is found in animals in south eastern Cameroon. The transfer most likely occurred by biting or contact with Chimpanzee blood. Such transfers could well have occurred sporadically for centuries but the virus infection that 'took off' in humans probably happened between 1930 and 1950. Since then, HIV has spread and radiated around the world; it now infects more than 25 million people. The mortality rate is close to 100% without treatment.

The pharmaceutical industry, building on basic research in academic laboratories, has been highly successful in discovering more than 20 anti-HIV drugs. When used in combinations of three or more, they can very successfully suppress the virus. The modern drugs have fewer side effects and are relatively easy to take. They can control the virus indefinitely and have reduced mortality from AIDS substantially in developed countries, a major success story for late 20th century medicine. However, the drugs do not eliminate the virus and have to be taken for life. They are expensive and their correct and safe use needs substantial medical infrastructure. Although there are ambitious roll-out programmes for HIV drug treatment in Africa, fewer than a quarter of those who need therapy are receiving it.

The alternative to complex and expensive life-long treatment programmes should be a vaccine. However, HIV vaccine development has been extraordinarily difficult. Soon after discovery of the virus in 1983, it was thought that it would be straightforward to generate virus envelope protein by genetic engineering techniques (it was) and to make a vaccine. After several years one such vaccine progressed to advanced clinical trials to test its ability to prevent infection and it completely failed. The reasons for this are now becoming clearer, thanks to detailed high quality research on the structure of the virus envelope protein which has led to an understanding of how the virus can take advantage of mutations to evade immune responses. The virus envelope protein mediates attachment to the two protein receptors on the surface of human T lymphocytes (called CD4 and CCR5) and then causes fusion of the virus and cell membranes enabling the virus to invade the cell. This process involves complex changes in the shape of the envelope protein, first as a consequence of binding to CD4 to expose the site on the envelope that binds to CCR5 and then major shape change in the stalk of the molecule to cause membrane fusion. This flexibility in shape makes it very hard for antibodies to bind sufficiently well to stop the process. Furthermore, the envelope protein is coated in sugar which protects it from antibody attack. A very extensive search for parts of the envelope that can bind protective antibodies has revealed just four 'Achilles heels', but infected humans and vaccinated humans only very rarely make antibodies to these sites and even then the antibodies are made in quantities too low to be protective. So the trick is going to be to find synthetic molecules that strongly stimulate these antibodies when put into vaccines, much easier said than done and not yet achieved after years of effort.

These difficulties led to another approach, using a vaccine to stimulate killer T cells. Killer T lymphocytes are not infectable by HIV, because they lack CD4 on their surface, and their natural role is to clear up virus infections by killing virus infected cells in the interval between virus entry and production of virus progeny – a time window of around 24 hours. Normally this is a very effective way of controlling a virus infection and there is very good scientific evidence that these T lymphocytes control the chronic phase of HIV infection, helping the patient to delay progression to AIDS, often for more than 10 years in the absence of treatment. Extensive studies in monkeys showed that vaccines that stimulate killer T lymphocytes could influence the course of infections with simian immunodeficiency virus (SIV), a very close relative of HIV. Vaccinated animals did become infected – the killer T cells can only act after cells have become infected – but they controlled the virus better and survived longer. Given these results,

there was considerable optimism that this approach might be useful in humans. Although HIV can escape from killer T cells by mutating the parts of the virus seen by the T cells, these are in relatively invariable proteins of the virus, so it was expected that the T cell stimulating vaccines would be able to cope with much of the virus variability.

The vaccine that looked the most promising, because it had stimulated the strongest killer T cell responses in HIV-uninfected people in early clinical trials, was the Merck vaccine. This was based on a common cold virus, adenovirus-5, into which was inserted three HIV genes. Although many people had some pre-existing immunity to adenovirus, it was shown that this did not reduce the immune response to the HIV genes. Therefore a large trial to test the efficacy of the vaccine was set up in volunteers who had a relatively high risk of HIV infection. After two years, in September 2007, the trial was terminated because an interim data analysis showed that the vaccine had no protective effect. Worse, there was a trend towards more infections in people who had pre-existing immunity to the adenovirus in people who received the vaccine, compared to those who had a placebo vaccine (saline). This has caused much alarm and despondency. Merck has pulled out of HIV vaccine research and other major pharmaceutical companies have followed suit.

A debate is ongoing as to what went wrong in the Merck trial. It is quite possible that none of the safety questions raised would hold up in a longer term study with more people tested, but reasonably no-one wants to take any risk of causing harm. It does look as if the vaccine failed to reduce the virus level in those infected, the primary goal, but it could be that the type of T cells stimulated were not strong enough and that there were not enough of them. It is also possible that the vaccine and the infecting virus differed too much for the T cells to be effective. It is also possible that the newly infecting HIV causes so much immunosuppression that it overwhelms even a vaccine prepared immune responses. All these ideas are being examined at the moment.

So where do we go from here? There is still an urgent global need for an HIV vaccine. This is less pressing in the developed world because drug treatment can do so much, though without a vaccine the number of people infected will steadily increase. The burden for further HIV vaccine development is now wholly on the non-commercial funding agencies and the academic world. Both major approaches have now hit brick walls, but for the antibody field this led to a boost in top quality science aimed at really understanding the problem in depth, which could in the future lead to real discoveries in vaccine design. The T cell field needs the same kind of reassessment and redirection. The National Institutes of Health (NIH) in the USA, with remarkable foresight and well before the result of the Merck vaccine trial was known, set up a $350m international consortium, that includes UK laboratories in Oxford and London, to examine more closely how very early HIV infection is controlled and to what extent genetic and pre-exposure natural immunity influences the outcome. The aim is to better understand why some people respond to HIV infection better than others, a very few completely controlling the virus without any drug treatment. A full understanding of the 'correlates of protection' has a good chance of helping the better design of vaccines.

In conclusion, this is a difficult time for HIV vaccine development. We are all looking for new leads after recent disappointments. What is constant is the need for the vaccine and it remains a high priority to attract the best young scientists into the field with the chance to be truly innovative in contributing to the effort.

*In discussion the following points were made:*

Alcohol is a marvellous cleaning agent and was widely used until the recent introduction of rubber gloves. Money was not available in South Africa from the Government but came from international sources. Disagreement was expressed on the efficacy of gels on C.diff. The evidence base is unreliable however, and obtaining scientific and clinical proof of efficacy is very difficult, especially in South Africa where no work was undertaken on the problem and evidence is lacking. In response to the charge that hospitals have retreated with respect to challenges to hygiene it was pointed out that there were no hip replacements in hospitals 50 years ago, and there were no strains of bacteria resistant to penicillin then, hence the risk from either of these did not exist. The negative impact of the RAE has resulted in less expenditure in this area and the number of medical scientists funded is very small resulting in no doctors going into microbiology. In contrast, Alexander Fleming put all his personal income arising from research on penicillin back into the subject.

Surveillance was raised as an issue. Are we up to scratch with surveillance with Blue Tongue, Avian Flu and SARS hovering on the horizon? In the US, the CDC and in the UK, the WHO do pick things up. Academics could also do more to make it their business too, especially concerning Avian Flu, where links need to be established between surveillance and diagnostic tools, but it all comes down to money. National surveillance undertaken in real time differs in the private sector which does not report, and the NHS which does, but is very important in picking up issues such as the Cadbury chocolate contamination incident for example. Comparison was made between the highly regulated conditions of abattoir slaughtermen on the one hand and an unregulated hospital culture where nurses pay for their own uniforms to be laundered at home. A change in human attitudes and behaviour is necessary but it is not clear how we should do it. Perhaps variable resistance of people to HIV could form the basis for natural selection in the future?