OXOID

Volume 30 No 2 September 2009 ISSN 0965-0989

Culture

30th Anniversary Issue (Part II) – an introduction

Barry D Cookson – Guest Editor
Director of the Laboratory of Healthcare Associated Infections, Health Protection Agency, London, UK

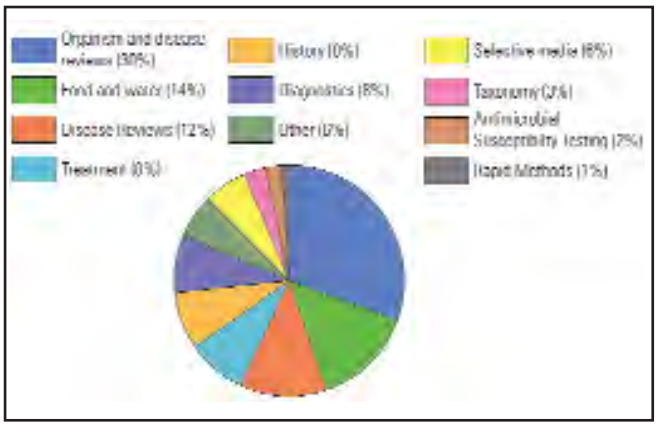
The first 30th Anniversary issue of *Culture* was well received, and I would like to thank the contributing authors and editorial board. This, the second 30th anniversary issue, had a hard act to follow, but I am delighted to say our invited authors have proven to be more than equal to their tasks. Copies of *Culture* are sent to readers in over 75 countries, almost double the number 30 years ago. It is also available on the Oxoid website, at www.oxoid.com, where it is accessed by readers from all around the world. A truly global outreach. It is read by many professional and other groups; the target audience being those working in clinical, as well as industrial and research, microbiological laboratories, from the most junior to the director, from consultant microbiologists to other infection control team members, from under-graduates to post doctorates.

Readers wanted to hear more about the articles published over these thirty years, and I have provided a pie chart to underpin some of the comments made previously. There are often several areas covered in the analysed papers, and I have selected the primary topic of each article to derive these data. In this issue, we see another excellent example from Professor Hugh Pennington of the reflections of an expert on 30 years experience in the field. He covers the fascinating topic of recurrent outbreaks of infection in healthcare settings and the community. I have sat in lecture theatres several times and been shown the quote he refers to by the US Surgeon General, William Stewart. We now know from his article that this is attributed incorrectly. Readers may not be aware that WHO has just launched its third patient safety challenge. The topic is antimicrobial resistance with an emphasis on healthcare settings. This is not the first time WHO has focused on this issue, and it will be interesting to see how successes in their previous two challenges will inform the strategies we will use to address this important issue.

In *Culture*'s first article on bioterrorism, Les Baillie describes some of the incidents that have occurred around the world and the obvious need for new and rapid detection systems. He emphasises the importance of anticipation, preparedness and rehearsing procedures. Fortunately, there has been significant investment into detection systems, and we are now better prepared. Reading his list of pathogens (not all are publicly available; if he told us he would no doubt have to kill us), it is clear that there are some serious new threats, and there is no room for complacency.

Mark Woolhouse describes the discovery and emergence of novel human pathogens over the last 30 years at an average rate of almost 3 per year. There are currently 10 or so new human viruses reported in the literature since 2006 whose status has yet to be confirmed, and it is apparent that many more will be described. More proactive strategies are in place to drill down into the origins of recent pathogens, e.g. the current swine flu pandemic strain. He makes the important point I have also made in another field (disinfectant resistance) of the lack of systematic surveillance and genetic analysis of new pathogens in many parts of the world. There is a prediction of global 'hotspots' for the emergence of novel human pathogens, and, perhaps, any new surveillance efforts should be focused on identifying these rather than attempting to cover the whole globe continuously?

Food and water are the second most popular topics in *Culture*. Melody Greenwood reviews the history and advances made in the investigation of food-related infections over the last 30 years. There have been great strides made in standardisation, validation and accreditation at an international level. The last ten years in particular have seen major advances in molecular, immunological, new culture media and other detection methodologies. We can now be much more confident that outbreaks, which can involve several countries, can be tracked more effectively than ever before. Indeed, in many ways the approaches developed have served as a benchmark for other microbiologists and public health experts to follow.



Topics covered in *Culture* over the last 30 years

Old Lessons Not Learned: recurring infectious outbreaks of the last 30 Years

T H Pennington
Aberdeen
t.h.pennington@abdn.ac.uk

"Culture" first appeared in 1978. With the benefit of hindsight, its foundation marked very interesting microbiological times. MRSA were around – but were not yet epidemic. Sporadic *E. coli* O157 cases had occurred – but so far there had been no outbreaks. *Clostridium difficile* had been identified as a cause of pseudomembranous colitis – but big nosocomial problems were a thing of the future. Many individuals had been infected with HIV – but AIDS had not yet been described. And almost certainly, the first cows to get BSE were already incubating the disease by the end of the decade – but it was 1985 before the first animal fell ill, and 1994 before the first cases of vCJD.

In 1978, however, the news of the day seemed generally to be good. In the UK, *Shigella sonnei* dysentery notifications were declining, and typhoid had ceased to be significant as an indigenous disease – its last big impact had been the 1964 Aberdeen outbreak started by contaminated corned beef imported from Argentina. Tuberculosis in British cattle appeared to be on its last legs. Smallpox had been eradicated. The last naturally occurring case had been in Somalia in October 1977 (although the tragic events in Birmingham in September 1978, when the virus escaped from a research laboratory and caused a fatal case, was a powerful reminder that biosecurity was paramount when handling particularly transmissible, dangerous pathogens). The last influenza pandemic with high excess mortality had been caused by the Hong Kong H3N2 virus in 1968-70. The re-emergence and worldwide spread of an H1N1 virus in 1977 was a much milder event.

So at that time, it seemed right for many health service professionals, deans of medical schools, civil servants (including government scientists) and politicians to go along with the saying that, "the time was ripe to close the book on infectious disease". It has been alleged that this statement was made by the US Surgeon General, William Stewart. However when asked, he could not remember ever saying it. He is supposed to have uttered it in a speech he gave in 1967. But it is not in its published text. Some who quote it say that he said it in 1963, others say 1965, and yet others say 1969 or 1979; none have provided a source that can be checked. It is safe to give Stewart the benefit of the doubt, applaud his very real and very significant achievement as the first to cause health warnings to be put on cigarette packets, and conclude that he was not the guilty party.

That pathogens had not gone away was demonstrated beyond doubt when whooping-cough vaccination became unpopular in the mid-1970s, largely due to the influence of campaigning doctors, Justin Strom in Sweden, Gordon Stewart in the UK, Viera Scheibner in Australia and Galina Chervonskaya in the USSR. They said that because the disease had become relatively uncommon, and because there were potential neurological complications from immunisation, the dangers from the vaccine outweighed its benefits. As a consequence, pertussis immunisation in Sweden and Japan was almost abandoned. However, in Japan in 1979, there was a big epidemic, with 13,000 cases and 41 deaths. In Sweden, the incidence rose to a figure two orders of magnitude greater than in immunised Norway, and diphtheria returned in Russia. In the UK, immunisation acceptance rates also fell. By 1978, only 30% of children under 2 years of age were immunised, and there were big outbreaks in 1978 (65,956 cases, the highest number since 1956) and 1982 (65,810 cases). Another outbreak was predicted for 1986, but immunisation acceptance rates rose to nearly 70%, and the eventual outbreak was smaller than expected, with 36,506 cases. The lesson from these events is that campaigners should ensure, as far as they can, that their work does not have unintended consequences and, in particular, ones that cause more harm than the alleged problem that they are trying to put right. It is beyond reasonable doubt that, as a consequence of the campaigners' publicity, many more children suffered from brain damage caused by infection with *Bordetella pertussis* than were previously being harmed by the vaccine.

It is easy to understand why anti-vaccinators have a ready audience. As Anderson and May[1] have said, "Most people have an intuitive appreciation that the best vaccine programme, from an individual's point of view, is one where almost everyone else is vaccinated while they are not, so that they are indirectly protected without incurring any of the risks or inconvenience associated with direct protection."

On the 28th of February 1998, in its "early report" section, *The Lancet* published the now notorious paper[2] by Andrew Wakefield and twelve other authors. It described 11 boys and one girl (mean age six) who had been referred to a paediatric gastroenterology unit with histories of diarrhoea, abdominal pain and a loss of acquired skills, including language. Ileocolonoscopy and histology of biopsies showed that most had bowel abnormalities. The paper concluded that, "we have identified a chronic enterocolitis in children that may be related to neuropsychiatric dysfunction". Its sting was in its last two sentences: "In most cases, onset of symptoms was after measles, mumps and rubella immunisation. Further investigations are needed to examine this syndrome and its possible relation to this vaccine". A big boost to this message was given by Andrew Wakefield at the press conference associated with publication of the paper, when he advised that measles vaccine should be given separately from the other components of the MMR vaccine. *The Lancet* did its best to mitigate the possible negative effects of the paper by publishing a leader in the same issue by Robert Chen and Frank DeStefano from the US Vaccine Safety and Development National Immunisation Program at the Centers for Disease Control. They wrote: " Vaccine-safety concerns such as that reported by Wakefield and colleagues may snowball into societal tragedies when the media and the public confuse association with causality and shun immunisation. This painful history was shared by the UK (among others) over pertussis in the 1970s … and it is likely to be repeated all too easily over MMR. This would be tragic, because passion would then conquer reason and the facts again in the UK." But history did repeat itself. MMR vaccine uptake fell, from a peak of 92% in1996-7 for children aged two, to 80% in 2003-4, and the number of laboratory confirmed cases rose, from 56 in 1998 to 1370 in 2008. The tragedy was compounded by the poor quality of the evidence that caused it. This was

acknowledged by the retraction of the paper in 2004 by ten of the 13 authors (but not by Andrew Wakefield). They said, "We wish to make it clear that in this paper no causal link was established between MMR vaccine and autism, as the data were insufficient."

It is easy to find British examples of the repetition of tragedies caused by attempts to learn from them, followed by a failure to act appropriately. The classic example is that of safety at football grounds. The Taylor Inquiry into the Hillsborough disaster[3] says, "It is a depressing and chastening fact that mine is the ninth official report covering crowd safety and control at football grounds … it seems astounding that 95 people could die from overcrowding before the very eyes of those controlling the event." In January 1986, Mr Justice Popplewell, whose report[4] following the Bradford Disaster (56 deaths) was the eighth in the series, summarised those of his seven predecessors. He started with the Shortt Report (1924, disorder at the 1923 Cup Final), went on to include those of Moelwyn Hughes (1946, Bolton Wanderers, 33 deaths) and Lord Wheatley (1972, Ibrox Park, 66 deaths), and introduced his summary of them by saying "almost all the matters into which I have been asked to inquire and almost all the solutions I have proposed, have been previously considered in detail by many distinguished Inquiries over a period of 60 years."

Being crushed and asphyxiated, or being burned to death, are not the only hazards attendant on the aggregation of people in close proximity. The transmission of many pathogens is optimised, too. Hospitals have always had a bad reputation in this regard. A persistent problem in psychiatric hospitals throughout the nineteenth century and well into the twentieth, was asylum dysentery[5]. A particularly bad year for it in Britain was 1917, when helped by understaffing, overcrowding, and undernutrition, it killed more than 1,000 patients. By 1935, it was much diminished as a problem, but that was the year in which *Clostridium difficile* was first described as a harmless inhabitant of the intestines of infants. The sorry story of its subsequent emergence as a nosocomial pathogen of the highest importance has been told in three inquiry reports, those of the Healthcare Commission investigation into the outbreaks at Stoke Mandeville Hospital (334 cases, with at least 16 deaths in the first outbreak in 2003-4, and 17 in the second in 2004-5)[6] and into the outbreaks at the Maidstone and Tunbridge Wells NHS Trust (more than 500 cases, with about 60 deaths in the two outbreaks in 2005-6)[7], and the report of the Independent Review of *Clostridium difficile*-associated disease at the Vale of Leven Hospital, from December 2007 to June 2008 (55 cases, with 18 deaths)[8]. Features common to all three reports was a custom of frequently moving patients from ward to ward, poor quality hospital buildings with too few single rooms for isolating the infectious patients, inadequate hygiene facilities (too few hand basins, for example), and deficient control of infection procedures by nurses. Three reports in three years addressing the same problem and finding the same deficiencies is bad. Even worse, although the frequency of reports on the causation and prevention of diarrhoea in hospitals has not yet reached Popplewell proportions, excellent ones go much further back in time than the ones in his list. In May 1900, the "Report of Drs. Mott and Durham on Colitis or Asylum Dysentery" was presented to the Asylums Committee of the London County Council. It recommended that accommodation provided for isolation should not be used for other purposes, that patients with a suspicious diarrhoea should be isolated, that, "while recognising the desirability and necessity of the transference of patients from ward to ward for purposes of treatment and administration, great discretion is necessary when diarrhoea, however slight, exists", and that much attention be paid to staff training, disinfection, and handwashing.

Florence Nightingale continues to be the heroine of hospital hygiene because of her work at Scutari. But the long-term impact on military mortality of her assiduous report writing and lobbying was not great. Consider the South African War of 1899-1902. In the Crimea (1853–56), 4,602 British soldiers were killed in action or died of wounds, and 13,497 died from diarrhoeal disease, of which 4,513 were killed by cholera and 2,790 from "continued fever" – probably nearly all typhoid. In the South African War, nearly 50 years later, 7,582 soldiers on the British side were killed in action or died of wounds, and 13,139 died of disease, of which 8,225 were killed by typhoid. The lessons that Nightingale had taught were the wrong ones. When she was at her most influential, she did not believe in germs. Not only was she a miasmatist, she was a firm believer in spontaneous generation[9]. Cleanliness and ventilation were paramount; that diseases had specific causes and could be spread in drinking water were ideas alien to her philosophy. So it will not do just to utter the mantra "lessons must be learned". They must be the right ones.

But that can be very difficult. On the 4th of February 1976, a new recruit to the US Army at Fort Dix in New Jersey developed a respiratory infection[10]. The medical officer told him to go to bed, but he went on a five mile forced march. He collapsed and died before the night was out. Influenza type A/swine was isolated. The virologists were alarmed. At that time, it was thought that a virus of this type had caused the massive pandemic of 1918-19. Half a million were said to have died in the US. The virus specialised in killing young adults; in 1918, at Fort (then called Camp) Dix between the 15th of September and the 6th of October, 6,000 soldiers had influenza and 800 died[11]. The developments in 1976 were rapid. The identity of the virus was confirmed on February the 13th. Work started on a recombinant vaccine on the 17th. There was a press conference on the 19th. The media made the link to 1918 on the 20th. A memo went from the Director of The Centers for Disease Control to the Secretary of Health, Education and Welfare on March the 15th and President Ford was briefed two days later. After meeting with experts, he asked Congress, "to appropriate $135 million, prior to their April recess, for the production of sufficient vaccine to inoculate every man, woman and child in the United States." The first vaccinations were carried out on October the 1st. It had been speculated that a possible vaccine complication could be the Guillain-Barre syndrome. Cases were looked for. Unsurprisingly, they were found, some in November, with more in December. The vaccination programme was suspended on December the 16th. It never started again. The virus did not spread from Fort Dix. The media and the public saw the vaccination programme as a debacle. The Director of CDC was sacked. There was a benefit, nevertheless, because the episode reminded the experts that what appeared to be reasonably certain knowledge about influenza (which underpinned their advice to the President) – such as a 10 year cycle between pandemics, and a swine origin for the 1918 virus – were based on extrapolation and hypothesis rather than hard evidence. The mere passage of time has shown the former to be wrong, and Jeffrey Taubenberger's reconstruction of the genome sequence of the 1918 virus, using in part material from soldiers killed by it, has demonstrated that it had an avian origin[12].

Florence Nightingale's understanding of the aetiology and routes of transmission of infection was rudimentary and incorrect. She was

(Courtesy of Debbie Marshall, University of Aberdeen)

***E. coli* O157**

overconfident in her theories, as were the virologists in 1976, and Andrew Wakefield in 1998. But for me, the most egregious failures are those that lead to a tragedy repeating itself despite scientific understanding of the topic being extensive, inquiry after the first tragedy having been done in depth, explanations of what went wrong being soundly based, and policies to prevent a repetition having been introduced with full public support.

The biggest *E. coli* O157 outbreak in Britain to date occurred in central Scotland, in November and December 1996. About 500 people were infected; 279 cases were confirmed microbiologically and 17 died. The cause of the outbreak was the cross-contamination of meats, many ready-to-eat, occasioned by poor food hygiene practices in the premises of a butcher in Wishaw, John Barr. I chaired an expert group to examine the circumstances that led to the outbreak, to advise on the implications for food safety and to identify lessons to be learned[13]. We recommended that HACCP (hazard-analysis critical control points; at that time already endorsed by the Codex Alimentarius and the World Health Organisation as the system to deliver safe food) should be adopted by all food businesses, and pending implementation, licensing for butchers handling raw meat and ready-to-eat foods should be introduced. In England and Wales, having HACCP was a licensing precondition. Licensing was introduced in 2000. Butcher-associated outbreaks seemed to become less frequent. But in September 2005, another big outbreak occurred in South Wales. There were 157 cases; 118 were confirmed microbiologically, and there was one death. Most affected were children; 44 schools had cases. Ready-to-eat meats supplied to them by a butcher in Bridgend, William Tudor, had been cross-contaminated in his premises because of poor food hygiene practices. I chaired a Public Inquiry into the outbreak[14]. For me, it was a Popplewell moment. The bad practices in the Scottish and in the Welsh butchers' premises were uncannily similar. The Sheriff who conducted the Fatal Accident Inquiry into the deaths in 1996 said that contributory defects were among others: "The failure to devise or enforce … cleaning schedules and equipment … which would have reduced the risk of surfaces being contaminated and to ensure that all staff were given adequate hygiene training, the failure to separate completely within the premises the processes relating to (a) raw meat and (b) cooked meat and in particular to have provided separate … scales and a vacuum packer for each of these separate processes, and the failure on the part of the EHOs prior to the outbreak to identify the food safety hazards inherent in the practices carried out within (the) premises and in particular in relation to the failures identified … above." I quoted these words in the conclusion of my Welsh report because they encapsulated accurately what had gone wrong, not only in Wishaw in 1996, but in Bridgend in 2005. Cross-contamination was the root cause. It was very important in Aberdeen in 1964 (*Salmonella typhi*, 507 cases). There was an inquiry[15]. Sound recommendations were made. Research was done[16]. But the problem persists. Will we ever learn?

I thank *Culture* for providing a powerful platform for me to say that the interruption of transmission of many infectious agents could be done much more effectively if we were much more assiduous in the application of policies based on *what we know now*.

Necessary, but not sufficient, of course. Evolution will see to that. Sooner or later, for example, new enterovirulent *E. coli* will appear, and there will be an influenza pandemic. (Note added in proof – I wrote the first draft of this paper before the new H1N1 influenza had spread to become a pandemic. Evolution hasn't let me down!) But I am confident that, as in the past, the information provided by *Culture* will help us to cope effectively with microbiological challenges to health – both new and old.

References

1. Anderson R.M., and May R.M. (1991) *Infectious Diseases of Humans: Dynamics and Control*. Oxford: O.U.P.
2. Wakefield A.J., Murch S.H., Anthony, A. *et al.* (1998) Ileal-lymphoid hyperplasia, non-specific colitis,and pervasive developmental disorder in children. *Lancet* **351:** 637–641.
3. The Hillsborough Stadium Disaster 15 April 1989 Inquiry by The Rt Hon Lord Justice Taylor, Final Report, 1990. Cmnd 962. HMSO.
4. Inquiry into Crowd Safety at Sports Grounds by Mr Justice Popplewell. Final Report, 1986. Cmnd 9710. HMSO.
5. Gettings H.S. (1913) Dysentery, past and present. *Journal of Mental Science* **59:** 605–621.
6. Investigation into outbreaks of *Clostridium difficile* at Stoke Mandeville Hospital, Buckinghamshire Hospitals NHS Trust, (2006).
7. Investigation into outbreaks of *Clostridium difficile* at Maidstone and Tunbridge Wells NHS Trust (2007).
8. Commission for Healthcare Audit and Inspection, London. Independent Review of *Clostridium difficile* Associated Disease at the Vale of Leven Hospital from December 2007 to June 2008. (2008) University of Aberdeen Department of Public Health (http://www.abdn.ac.uk/public_health/cdad/)
9. Pennington T.H. (2008) Beware Bad Smells. *London Review of Books* **30**(23): 33–34.
10. Pennington T.H. (2003) *When Food Kills*. Oxford: O.U.P.
11. Ireland M.W., Callender, G.R., and Coupal, J.F. (1929) *History of the Medical Department of the United States Army in the World War, XII, Pathology of the Acute Respiratory Diseases, and of Gas Gangrene following War Wounds*. US Government Printing Office, Washington, D.C.
12. Taubenberger J.K., Reid A.H., Lourens R.M., *et al.* (2005) Characterization of the 1918 influenza virus polymerase genes. *Nature* **437:** 889–893.
13. The Pennington Group Report of the Circumstances leading to the 1996 Outbreak of infection with *E. coli* O157 in Scotland, the implications for Food Safety, and the Lessons to be Learned. 1997. The Stationery Office, Edinburgh.
14. Report of the Public Inquiry into the September 2005 Outbreak of *E. coli* O157 in South Wales. Chairman: Professor Hugh Pennington. (2009) Queens Printer and Controller of HMSO. (www.ecoliinquirywales.org).
15. Smith, D.F., Diack H.L., with Pennington T.H., and Russell E.M. (2005) *Food Poisoning, Policy and Politics*. The Boydell Press, Woodbridge.
16. Gilbert R.J., and Maurer I.M. (1969) Cross-contamination by cooked-meat slicing machines and cleaning cloths. *Journal of Hygiene* **67:** 449–453.

Bioterrorism and detection methodologies for anthrax

Les Baillie
Welsh School of Pharmacy, Cardiff University,
Redwood Building, King Edward VII Avenue,
Cardiff, CF10 3NB, Wales, UK

Introduction

There is no commonly accepted definition of bioterrorism and so, for the purposes of this article, the following version in Wikipedia is as good as any; "Bioterrorism is terrorism by intentional release or dissemination of biological agents (bacteria, viruses or toxins); these may be in a naturally-occurring or in a human-modified form." While capturing the essence of bioterrorism, the description is short on detail. The nature of bioterrorism is such that an aggressor is likely to strike at a time and place calculated to induce maximum terror through a combination of casualties and psychological stress[1]. The motivation for such an attack can range from political, doomsday, religious, economic, ecological or other ideological causes without reference to its moral or political justice.

Indeed, it is concern over the potential use of a biological agent as a weapon of mass destruction which led a recent US Congressional committee to conclude – that a weapon of mass destruction will be used in a terrorist attack somewhere in the world by the end of 2013 and that this weapon is more likely to be a biological weapon than a nuclear weapon[2]. Humans are not the only targets for a biological attack. The destruction of essential foodstuffs, such as rice and domestic animals, could have a devastating effect on the wellbeing of whole populations[3]. One only has to think back to the dramatic effects of the UK foot and mouth (FMD) outbreak in 2001, in which authorities spent 6 months struggling to control a disease outbreak that cost some £14 billion and resulted in the slaughter of 11 million animals. Only a handful of pathogens, all viruses, are considered potential agents of large scale agroterrorism in western nations and they are; FMD in cattle and swine; Rinderpest in cattle; classical swine fever and African swine fever in pigs; avian influenza and Newcastle disease viruses in poultry; and Rift Valley fever virus[3].

In the context of a human bioterrorist attack, which organism should we be concerned about? The majority of bioterrorism research has focused on what the US National Institutes for Health define as category A agents; micro-organisms and toxins which are thought to have been weaponised by various military groups around the world and comprise a mixture of viruses, bacteria and toxins (*Table 1*). Unfortunately, the potential to cause harm with biological material is not restricted to the members of group A, and as a consequence, we now have category B and C agents[4] (*Table 1*). Indeed, prior to the anthrax spore postal attacks in 2001, the only other agent known to have been employed in a bioterrorism incident was a strain of *Salmonella* Typhimurium obtained from a medical supply company in Seattle, US, which was used by members of the Rajneeshee sect in 1984, to infect 751 people, as a means of influencing the results of a local election[5]. The effective use of a "non-traditional" agent highlights the need to avoid tunnel vision and to realise that many naturally occurring infection agents have the potential to be employed in a bioterrorist attack.

A further point to consider is the power of genetic engineering to alter existing organisms and to create new ones. It is now possible to chemically synthesise the DNA molecule equivalent to the poliovirus genome, and it is only a matter of time before the first synthetic bacteria are created[6]. Likewise, the ability to transfer virulence factors and express them in previously harmless micro-organisms raises considerable challenges when seeking to detect the presence of illicitly engineered, pathogenic micro-organisms. For example, the major virulence factors of *Bacillus anthracis*, the causative agent of anthrax and the organism employed in the 2001 bioterror attacks in the US, are located on two mobile plasmids. The ability of these virulence plasmids to naturally transfer to other members of the phenotypically distinct *Bacillus cereus* group has seen the emergence of a strain of *B. cereus* which caused an infection clinically indistinguishable from inhalational anthrax[7]. Given the central role of virulence plasmids in the pathogenicity of anthrax and their ability to move between closely related strains, we should take care in dismissing all clinical isolates of *B. cereus* as environmental contaminants.

Anthrax

There can be little doubt that the spore form of *B. anthracis* deserves its status as the principal biothreat agent (*Figure 1*). In the past, anthrax has been weaponised by a number of states, including Japan, United Kingdom, United States, the former Soviet Union and, more recently, Iraq[8]. By the time of the First Gulf War, Iraq had developed a range of biological warfare munitions which included bombs and SCUD missiles filled with anthrax spores[9]. Unfortunately, the potential of biological weapons to inflict great suffering has now been recognised by terrorist groups such as the AUM Shinrikyo Sect, which carried out the Tokyo nerve gas attacks. Indeed, we are extremely fortunate that the microbiology skills of the sect members were not up to the task, and, as a consequence, they dispersed an anthrax spore vaccine rather than a fully pathogenic strain from the roof of an apartment in a suburb of Tokyo[10]. Unfortunately, the perpetrator of the US mail attacks did have access to the fully pathogenic Ames strain of *B. anthracis*, and as a consequence, 22 cases of anthrax occurred, of which 5 inhalational cases were fatal[11].

Figure 1. Scanning electron microscopy image of *B. anthracis* spores

Table 1. US National Institutes of Health Category A, B, and C Priority Pathogens

Category A

Bacillus anthracis (anthrax)
Clostridium botulinum toxin (botulism)
Yersinia pestis (plague)
Variola major (smallpox) and other related pox viruses
Francisella tularaensis (tularaemia)
Viral haemorrhagic fevers
Arenaviruses
LCM, Junin virus, Machupo virus, Guanarito virus
Lassa Fever
Bunyaviruses
Hantaviruses
Rift Valley Fever
Flaviruses
Dengue
Filoviruses
Ebola
Marburg

Category B

Burkholderia pseudomallei
Coxiella burnetii (Q fever)
Brucella spp. (brucellosis)
Burkholderia mallei (glanders)
Chlamydia psittaci (Psittacosis)
Ricin toxin (from *Ricinus communis*)
Epsilon toxin of *Clostridium perfringens*
Staphylococcus enterotoxin B
Typhus fever (*Rickettsia prowazekii*)
Food- and Waterborne Pathogens
Bacteria
+ Diarrhoeagenic *E. coli*
+ Pathogenic vibrios
+ *Shigella* spp.
+ *Salmonella* spp.
+ *Listeria monocytogenes*
+ *Campylobacter jejuni*
+ *Yersinia enterocolitica*
Viruses (Caliciviruses, Hepatitis A)
Protozoa
+ *Cryptosporidium parvum*
+ *Cyclospora cayatanensis*
+ *Giardia lamblia*
+ *Entamoeba histolytica*
+ Toxoplasma
Fungi
+ Microsporidia
Additional viral encephalitides
+ West Nile Virus
+ LaCrosse
+ California encephalitis
+ VEE
+ EEE
+ WEE
+ Japanese Encephalitis Virus
+ Kyasanur Forest Virus

Category C

Emerging infectious disease threats such as Nipah virus & additional hantaviruses.
NIAID priority areas:

* Tickborne haemorrhagic fever viruses
  - Crimean-Congo Haemorrhagic fever virus
* Tickborne encephalitis viruses
* Yellow fever
* Multi-drug resistant TB
* Influenza
* Other Rickettsias
* Rabies
* Prions
* Chikungunya virus
* Severe acute respiratory syndrome associated coronavirus (SARS-CoV)
* Antimicrobial resistance, excluding sexually transmitted organisms*
  - Research on mechanisms of antimicrobial resistance
  - Studies of the emergence and/or spread of antimicrobial resistance genes within pathogen populations
  - Studies emergence and/or spread of antimicrobial-resistant pathogens in human populations
  - Research on therapeutic approaches that target resistance mechanisms
  - Modification of existing antimicrobials to overcome emergent resistance
* Antimicrobial research, as related to engineered threats and naturally occurring drug-resistant pathogens, focused development broad-spectrum antimicrobials
* Innate immunity, defined as the study of nonadaptive immune mechanisms that recognise, respond to, micro-organisms, microbial products, and antigens
* *Coccidioides immitis* (added February 2008)
* *Coccidioides posadasii* (added February 2008)

* NIAID Category C Antimicrobial Resistance – Sexually Transmitted Excluded Organisms: Bacterial vaginosis, *Chlamydia trachomatis,* Cytomegalovirus, *Granuloma inguinale, Hemophilus ducreyi*, Hepatitis B virus, Hepatitis C virus, Herpes Simplex virus, Human immunodeficiency virus, *Human papillomavirus, Neisseria gonorrhoea, Treponema pallidum, Trichomonas vaginalis*

Although tragic, it is interesting to consider the impact that this series of attacks had beyond the primary victims. They instilled a sense of fear and terror in the public's psyche which threatened their sense of personal and community safety and resulted in the disruption of critical social infrastructure, which, if it had been left unchecked, could have crippled the nation's economy and leadership. In purely economic terms, the anthrax attacks have incurred approximately $1 billion in clean-up costs and the subsequent expenditure of further billions on the development of medical counter-measures, such as vaccines, antibiotics and detection systems. So, what can we do to protect ourselves from the threat of a bioterror attack?

Micro-organisms, such as *B. anthracis,* can take anywhere from 2 to 4 days to cause death, depending on the dose and the route of infection, but providing effective treatment is instigated early, the infected individual will survive. A bioterrorist attack is likely to be covert and thus rapid, and accurate detection and identification will

be a key element in reducing the adverse consequences. It is estimated that an anthrax spore attack against a city the size of New York would result in 1.5 million inhabitants becoming infected, of which 123,000 would still die if antibiotics were administered following diagnosis of the first case. In contrast, if drugs were distributed prior to an attack and were taken upon issue of a warning, the number of deaths would reduce to 50,000[12]. Although still a substantial number, it demonstrates the value of an effective detection system and underscores the reasons why millions of dollars have been invested in the American Biowatch programme.

Environmental monitoring

The function of Biowatch is to detect the release of pathogens into the air, providing warning to the government and public health community of a potential bioterror event[13]. Aerosol samplers situated in 31 American cities collect air, and any agents present are deposited onto a filter, which is subsequently analysed for the presence of potential biological weapon pathogens using polymerase chain reaction (PCR) techniques. The system is thought to detect the causative agents of anthrax, smallpox, plague and tularaemia, but the entire list of pathogens is not publicly available.

The first positive result occurred in Houston, Texas, in 2003, where low levels of the bacterium *Francisella tularensis*, the causative agent of tularaemia, were detected. As a consequence, precautionary measures were taken by the local and state public health agencies, which included increased surveillance for human illness, additional environmental sampling and testing, and an assessment of activities in the area that may have caused the sensors to pick up the organism. It was concluded that the result reflected natural "background" levels of the organism in the environment. The Director of the Houston Department of Health and Human Services stated, "We are investigating to determine if the bacteria were always present or newly present, and if it represents a health threat to the community." This statement encapsulates the major challenge faced when seeking to distinguish a natural event from an attack. How can we differentiate between natural occurrence and the intervention of man? Indeed, in the case of *B. anthracis,* detection is further complicated by its similarity to *B. cereus* and *B. thuringiensis,* both of which are widespread and frequently present in soil, water and air. Analysis of aerosol samples obtained over a 12 month period revealed the presence of *Bacillus* species in 50% or more of samples from eight cities, of which 3-32% belonged to the group containing *B. anthracis*[14].

While the system is designed to detect the presence of biothreat agents in the atmosphere, it still requires a remote laboratory to perform the PCR and to confirm the validity of the result, particularly as a positive could have major health, social and political implications. A further point to consider is that the efficiency of a detector depends on its location relative to an attack, thus an aerosol testing system would not detect a covert attack via the water or food chains. Indeed, it is likely that the arrival of sick people at a medical facility would be the first indication that an attack has occurred. The first victim of the anthrax mail attacks was identified following admission to hospital and subsequent clinical and laboratory investigation. Thus diagnostic laboratories have a key role to play in detecting potential biothreat agents.

Many people may be surprised to know that the UK Health Protection Agency can trace its history back to the Emergency Public Health Laboratory Service established in 1939 to monitor the threat of microbiological warfare. A similar capability has recently been established in America, to ensure an effective laboratory response to bioterrorism. The Laboratory Response Network for Bioterrorism (LRN) comprises a nationwide network of local, state and federal government laboratories that provide confirmatory testing of potential bioterrorism pathogens using consensus protocols. As part of this mission, they support the Biowatch programme. The network is designed as a pyramid. At the bottom are the front line sentinel laboratories, such as hospital clinical laboratories, which provide routine diagnostic services, such as Gram staining, culture and motility, which enable them to make presumptive diagnoses of biothreat agents. The next level in the pyramid comprises more than 140 state and local public health, military, international, veterinary, agriculture, food and water testing laboratories. These facilities are equipped with biosafety level 3 laboratories and are responsible for confirming the identity of samples referred to them by the sentinel laboratories, using approaches such as PCR and immunoassays. At the top of the pyramid are national laboratories which are responsible for specialised strain characterisation, bioforensics, select agent activity and handling highly infectious biological agents[15]. In the UK, the standard method for the detection and identification of *B. anthracis* is based on microscopic examination of smears with 1% McFadyean's polychrome methylene blue stain, followed by culture[15]. Confirmatory tests include motility testing, susceptibility to penicillin and diagnostic phage, and finally PCR.

Culture

The somewhat surprising experience of the 2001 anthrax postal attacks was that traditional culture, although relatively time-consuming compared to rapid PCR or antibody-based assays, was more sensitive, required less technical training and was able to detect viable organisms, unlike the other assays[17]. The bacterium grows well on a variety of culture media. Characteristic colonies, when grown on sheep blood agar, appear rough, grey and nonhaemolytic when incubated aerobically, and often show a classic 'medusa edge'. When incubated in the presence of 5% CO_2, fully virulent isolates of *B. anthracis* produce mucoid, wet-looking colonies, as a consequence of capsule formation. Thus culture from clinical samples is relatively simple, given the ability of the organism to grow on routine laboratory media and the likely absence of other members of the closely related *B. cereus* group, which are ubiquitous in the environment and can be misidentified as *B. anthracis* based on these morphological criteria.

For this reason, the culture of samples obtained from environmental sources necessitate the use of some form of selection. Polymyxin lysozyme-EDTA thallous acetate (PLET) agar is used traditionally as selective growth medium for *B. anthracis*. However, PLET agar contains highly toxic thallium acetate at levels which are considered unsafe in some countries[19]. Alternative selective media exploit the ability of the bacterium to grow in the presence of low concentrations of polymyxin B and incorporate some form of reporter system based on carbohydrate utilisation (mannitol), lecithinase production or hydrolysis of a chromogenic substrate[18,19]. While these media are capable of inhibiting the growth of closely related species, it must be remembered that they also have an inhibitory effect on the target organism, and, as a consequence, care must be taken if the organism

is likely to be present in small numbers. On these occasions, it is prudent to include either nonselective media or a broth-based enrichment step, such as brain heart infusion broth with polymyxin.

The ability to quantify the number of viable organisms present in a contaminated environment is an important factor in assessing the risk to human and animal health, mapping the point of agent release and the extent of contamination, and determining the effectiveness of decontamination strategies. Current decontamination approaches require an environment to be cleaned to a level at which no viable spores can be detected.

Currently, there are no validated assays with which to quantify the number of viable *B. anthracis* in an environmental sample. Culture-based approaches adopted to date consist primarily of a combination of swabbing or air sampling and direct agar plating, and are reported to have a sensitivity of ~10 organisms/gram of sample[17,20]. Given that the infective dose of anthrax for humans can be as low as 10 spores (fatal case in Connecticut), a more sensitive assay is required[11].

Once a colony has been presumptively identified, a range of confirmatory tests are performed which include; Gram stain, motility and susceptibility to penicillin and diagnostic phage. If available, PCR and antibody-based assays can also be used which target unique signatures, such as the major virulence factors (toxins and capsule), chromosome, vegetative cell wall (*Figure 2*) and spore surface. Indeed, a plethora of rapid antibody and PCR assays have been developed which claim to be able to directly detect and confirm the presence of *B. anthracis* in environmental samples. Unfortunately, there are no independent means of confirming the accuracy of many of these claims. This is a particular cause for concern as an erroneous result could have profound consequences, particularly if the assay is used by first responders at the scene of a bio-agent release. To address this issue, the US Department of Homeland Security recently instituted a testing and evaluation programme to determine the efficacy of individual assays.

Conclusions

Over the course of human evolution, infectious disease has been an ever present cause for concern. While our ability to access clean water, food and modern drugs has resulted in a society in which the fear of infection has been significantly reduced, it has not been eliminated. The media regularly runs stories about the threat of pandemics, in part because the fear of disease sells newspapers! Given that the aim of the terrorist is to instil fear and gain the oxygen of publicity, the illicit use of biological material may present an attractive option. In the event that an attack occurs, anticipation, preparedness, rehearsed procedures and rapid detection will be key, and it is to be hoped that the current massive investment in environmental and real-time detection systems will leave us better prepared.

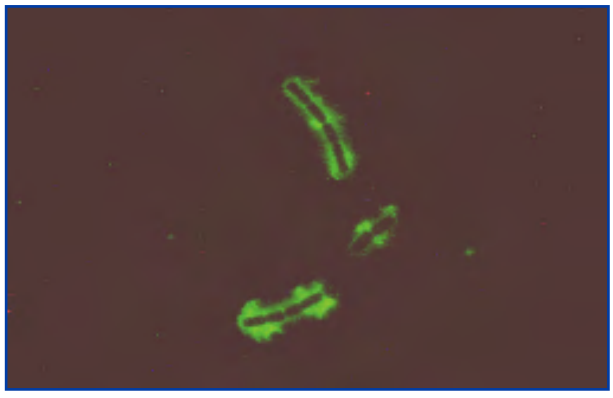


Figure 2. Vegetative bacteria stained with a fluorescent monoclonal antibody against *B. anthracis* cell wall galactose/N-acetylgluosamine polysaccharide

References

1. Hall M.J., Norwood A.E., Ursano R.J., and Fullerton C.S. (2003) The psychological impacts of bioterrorism. *Biosecur. Bioterror.* **1**(2): 139–144.
2. Commission on the prevention of weapons of mass destruction proliferation and Terrorism – The report of the commission of the prevention of WMD proliferation and Terrorism, 2008.
3. Breeze, R. (2004) Agroterrorism: Betting Far More than the Farm. *Biosecur. Bioterror.* **2**(4): 1–14.
4. (http://www3.niaid.nih.gov/topics/BiodefenseRelated/Biodefense/research/CatA.htm)
5. Carus W.S. (2001) *The Illicit Use of Biological Agents Since 1900.* Center for Counterproliferation Research, National Defense University, Washington DC.
6. Cello J., Paul A.V., and Wimmer E. (2002) Chemical synthesis of poliovirus cDNA: generation of infectious virus in the absence of natural template. *Science* **297**(5583): 1016–1018.
7. Hoffmaster A.R., Ravel J., Rasko D.A., *et al.* (2004) Identification of anthrax toxin genes in a *Bacillus cereus* associated with an illness resembling inhalation anthrax. *Proc. Natl. Acad. Sci. USA.* 2004 Jun 1; **101**(22): 8449–8454.
8. Inglesby T.V., O'Toole T., Henderson D.A., *et al.* (2002) Anthrax as a biological weapon, 2002: updated recommendations for management. *J.A.M.A.* **287**(17): 2236–2252.
9. Zilinskas R.A. (1997) Iraq's biological weapons, the past as future? *J.A.M.A.* **278:** 418–424.
10. Takahashi H., Keim P., Kaufmann A.F., *et al.* (2004) *Bacillus anthracis* incident, Kameido, Tokyo, 1993. *Emerg. Infect. Dis.* **10**(1): 117–120.
11. Jernigan D.B., Raghunathan P.L., Bell B.P., *et al.* (2002) Investigation of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. *Emerg. Infect. Dis.* URL: http://www.cdc.gov/ncidod/EID/vol8no10/02-0353.htm
12. Wein L.M., Craft D.L., and Kaplan E.H. (2003) Emergency response to an anthrax attack. *Proc Natl Acad Sci USA.* **100**(7): 4346–4351.
13. Shea D.A., and Lister S.A. (2003) The BioWatch Program: Detection of Bioterrorism Congressional Research Service Report No. RL 32152.
14. Merrill L., Dunbar J., Richardson J., and Kuske C.R. (2006) Composition of bacillus species in aerosols from 11 US cities. *J Forensic Sci.* 2006 May; **51**(3): 559–565.
15. Morse A.S., Kellogg R.B., Perry S., *et al.* (2003) Detecting Biothreat Agents: the Laboratory Response Network *ASM News* **69**(9): 433–437.
16. UK National harmonised procedure for the culture and identification of *Bacillus anthracis* from field cases. Issue no: 2, Issue date: 26- 07-07 Issued by: Veterinary and Public Health Test Standardisation Group on behalf of SGDIA
17. Beecher D.J. (2006) Forensic application of microbiological culture analysis to identify mail intentionally contaminated with *Bacillus anthracis* spores. *Appl. and Environ. Microbiol.*, **72**(8): 5304–5310.
18. Tomaso H., Bartling C., Al Dahouk, S., *et al.* (2006) Growth characteristics of *Bacillus anthracis* compared to other *Bacillus* spp. on the selective nutrient media Anthrax Blood Agar® and Cereus Ident Agar® *Syst. Appl. Microbiol.* **29**(1): 24–28.
19. Luna V.A., Peak K.K., Veguilla W.A., *et al.* (2005) Use of two selective media and a broth motility test can aid in identification or exclusion of *Bacillus anthracis. J. Clin. Microbiol.*, **43**(9): 4336–4341.
20. Baillie L.W.J. *Bacillus anthracis.* In The Encyclopaedia of Food Microbiology. Eds: Batt C.A., Robinson R.K., and Patel P.D. Academic Press (2000), **1:** 129–135.

The discovery and emergence of novel human pathogens

Mark Woolhouse
Centre for Infectious Diseases,
University of Edinburgh,
Edinburgh, UK

Introduction

In the past 30 years, 87 new species of human pathogen have been identified, at an average rate of almost 3 per year. The list is a mixture of several different kinds of event:

1 the recognition of pathogens that have existed in humans for a long time, but have only recently been detected (e.g. hepatitis C);
2 pathogens that have existed for a long time but have only recently had the opportunity to infect humans (e.g. baboon cytomegalovirus);
3 newly evolved human pathogens that did not previously exist (e.g. HIV-1).

A full list of the 87 species can be found on the *Culture* website at www.oxoid.com. All of these are important and interesting, but in different ways. Here, the focus is mainly on the 'emerging' pathogens, which includes both (2) and (3) but excludes (1). To put it another way, I will concentrate less on pathogens that we have recently discovered and more on pathogens that have recently discovered us.

What is a pathogen species?

First, we need to define 'pathogen'. A reasonable working definition is: a microbial or parasite species that can infect and is capable of causing disease in humans under natural transmission conditions[1]. A key word here is 'natural': there is a remarkably long list of pathogens for which the only known instances of human infection were due to deliberate exposure in the laboratory. These are not regarded as natural pathogens, though they may have the potential to become so.

Next, we need to address a long standing problem of taxonomy, especially microbial taxonomy, of what constitutes a species. For immediate purposes, a pragmatic answer is that a species is what the taxonomists say it is (based on resources such as the National Center for Biotechnology Information (NCBI) and the International Committee on Taxonomy of Viruses (ICTV)). On that basis, according to the most recently published estimate, there are approximately 1400 species of human pathogen currently recognised[2]. These are mostly bacteria (including rickettsia), fungi (including microsporidia) and helminths, plus some viruses and protozoa and two agents of prion diseases (see *Figure 1*).

However, in this context, the 'species' is an inadequate taxonomic unit, for two different reasons. First, taxonomy is not a constant: one taxonomist's species can be another's subspecies, species complex or genus, and classifications are continually tinkered with and revised. Second, it masks important differences within species. These are most succinctly illustrated by reference to two pathogens species, *Escherichia coli* and influenza A, both of which contain variants with very distinct pathogenicities in humans, such as *E. coli* O157 or H5N1 influenza A. Indeed, although the latter can both be meaningfully described as new human pathogens, because they are not new 'species' they are not included in the new species of the last 30 years.

Novel human pathogens

The 87 species of human pathogens discovered since 1980 thus makes up just 6% of the total. But they are not representative: in contrast to the make-up of all human pathogens (see *Figure 1*), this list is dominated by viruses (see *Figure 2*). The taxonomic rules for constructing *Figure 1* and *Figure 2* are the same, so the contrast between them is robust to any differences in how species are defined within the major groupings. Relative to what we had discovered before, we are now discovering many more viruses than other kinds of pathogen.

In contrast, although viruses, as a whole, are over-represented, these are not any particular kinds of virus. Novel viruses are distributed across families in much the same way as the known viruses (with the exception of the Retroviridae, which were not recognised at all 30 years ago). Nor is there any over-representation of RNA versus DNA viruses. In contrast, of the relatively few new bacteria species discovered, most are rickettsia, and of the fungi, most are microsporidia.

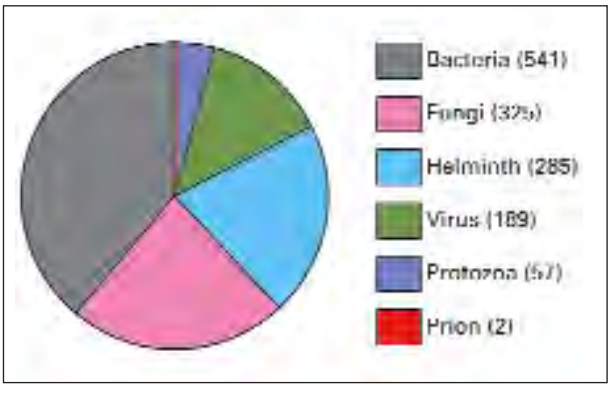


Figure 1. Pie chart of the main taxonomic groups of human pathogens, showing the number of species in each (of a total of 1399 species).

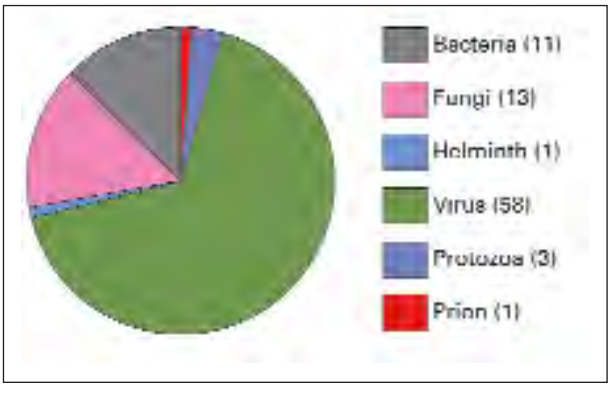


Figure 2. Pie chart of the main taxonomic groups of human pathogens discovered since 1980, showing the number of species in each (of a total of 87 species).

However, if we look at finer taxonomic scales, the picture changes. Although there have been relatively few new species of bacteria reported as human pathogens in the past 30 years, there has been a large number of important variants discovered. One example is the verocytotoxigenic *E. coli*, and some authors also include antibiotic resistant strains as 'new' pathogens. Taking this approach, one study reported that, of disease outbreaks associated with novel pathogens (strains as well as species) in the past few decades, approximately half were caused by pathogenic bacteria, with a third of those involving antibiotic resistant strains[3].

There are three possible explanations for patterns in the kinds of new pathogens that are being discovered:

1 improvements in pathogen detection technology for certain kinds of pathogen (e.g. the use of modern sequencing technologies to detect viruses);
2 direction of discovery effort at certain kinds of pathogen (e.g. the detection of other new human coronaviruses shortly after the appearance of SARS, or the recent clusters of discoveries of rickettsia and microsporidia); or
3 some kinds of pathogen are currently entering the human population at higher rates than others (e.g. the rapid emergence of antimicrobial resistant strains).

I will return to these possibilities later on, but first I will discuss the origins of novel pathogens.

Zoonoses

Zoonoses are infectious diseases which are naturally transmitted between other vertebrates and humans. Approximately 60% of human pathogen species are known to be zoonotic, and the percentage is even greater among those that have recently been discovered[2]. Indeed, over much longer time scales, it is likely that many of the infectious diseases we currently think of as largely or exclusively human (such as falciparum malaria, measles or diphtheria) have animal origins[4].

A survey of the kinds of animal reservoirs that are most important reveals some interesting trends[5]. Zoonotic pathogens are most commonly associated with ungulates, rodents and carnivores, to a lesser extent with other mammals, some with birds, and very few with other vertebrates. The reservoirs associated with newly emerging pathogens show much the same pattern. In other words, our new pathogens are coming from much the same sources that they always have.

A noteworthy consequence of the animal origins of human pathogens is that the veterinary community often has more knowledge initially of a novel human pathogen than does the medical community. Retroviruses, coronaviruses, ehrlichias, papillomaviruses, rotaviruses, lentiviruses, transmissible spongiform encephalopathies (TSEs) and infectious cancers were all known in animals before they were recognised in humans[6].

Pathogen pyramid

The emergence of a novel human pathogen can be thought of in terms of a series of levels, represented by the pathogen pyramid (see *Figure 3*). The first level is exposure of the human population often, though by no means exclusively, from an animal source. Not all exposures result in a successful human infection (we will consider how many, shortly). Of those that can infect humans, not all will be able to transmit (by whatever route) from an infected human (which normally requires that the pathogen access specific regions of the body, notably the lower gut, upper respiratory tract, urinogenital tract or blood). Of those that can transmit, only a fraction will be able to do so at a high enough rate to establish themselves in the human population (a concept expressed by a quantity known as the basic reproduction number[7]).

Progress up the pyramid can be halted by two kinds of barriers, biological and ecological. Biological barriers imply that the pathogen lacks the molecular machinery to access host cells or tissues and/or to evade host defences. Ecological barriers imply that the pathogen has the biological capability but lacks the opportunity. This distinction is important: biological barriers can only be overcome by evolution of the pathogen, whereas ecological barriers can be overcome by changes in the host or the host-pathogen environment. The types of changes involved are discussed in more detail below.

The estimated numbers of human pathogen species at each level (see *Figure 3*) indicate that the pyramid analogy is appropriate. Although we have no good estimates of how many pathogens humans have been exposed to, there are good data on the zoonotic potential of pathogens of domestic animals[8]. These are of interest because we can be certain that humans have been exposed to these pathogens due to their close contact with their hosts. It turns out that almost half the known species of pathogen of domestic ungulates and carnivores are zoonotic, implying that the barriers to pathogens crossing between species are not as profound as might have been assumed[9]. Clearly, many pathogens can infect multiple hosts[10], and it has been suggested that a broad host range is a predictor of likely emergence in humans[5].

A perfect storm

There have been several attempts to relate pathogen emergence (or re-emergence) to specific drivers. While it is possible to suggest the kinds of drivers that may be important in general[5,11], specific examples of the emergence of novel human pathogens tend to have multiple rather than single causes. These might include any or all of the following: urbanisation, conditions in hospitals, poor governance, globalisation of travel and trade, population displacement, immunocompromising effects of AIDS or malnutrition, deforestation, intensification of agriculture, use and misuse of antimicrobials, trade in exotic pets or bush meat, climate change and numerous others. Many of these drivers are as active in the early 21st century as at any time in human history, prompting the US Institute of Medicine to warn that, 'a transcendent moment nears upon the world for a microbial perfect storm'[11]. According to this viewpoint, we have to be prepared for the continued emergence of novel human pathogens in the immediate future.

An alternative way of understanding the conditions required for pathogen emergence is to take the pathogen's perspective. For any pathogen or potential pathogen, 'emergence' is simply taking advantage of an opportunity to invade a new habitat: us. For this reason, emerging pathogens have been likened to weeds[12], and they are characterised by having sufficient biological and epidemiological

flexibility to take advantage of favourable new circumstances that a rapidly changing environment continually provides. Pathogens are so numerous and diverse – not only taxonomically but in terms of their biologies and ecologies – that it is possible to imagine that almost any change in the human environment, and particularly in our relationship with other animals both domestic and wild, might provide an opportunity for some pathogen somewhere. There are numerous examples to support this view: the complex association between Nipah virus emergence and changes to Malaysian pig farming practices; or the emergence of BSE/vCJD due to changes in cattle feed production practices; or the indirect transmission of SARS coronavirus from (probably) bats to humans via farmed civets in so-called 'wet' markets.

Another kind of change is change in the pathogen itself. This is most apparent, and extremely important, when pathogens evolve resistance to drugs or when vaccine-escape mutants arise. However, even in the absence of drugs or vaccines, pathogens are continually evolving. This is especially true of the RNA viruses; these have very labile genomes and therefore show very high levels of genetic variation and rates of evolution (DNA viruses may not be far behind). Reflecting this, one hypothesis for the high rate of emergence of novel human viruses from animal populations is that human infective variants are continually being thrown up as part of their natural population dynamics in the animal host[13]. If they do enter a human population, most of these will cause only minor (and perhaps completely undetected) outbreaks; a few will adapt to their new host quickly enough to survive and perhaps go on to cause much more major public health problems. The early history of the human immunodeficiency viruses illustrates this in practice. HIV-1 and -2 have entered the human population independently on many different occasions[14]. But just one lineage has gone on to cause the AIDS pandemic; most of the remainder have been restricted to minor, self-limiting outbreaks. This hypothesis also suggests that viruses are especially likely to generate novel human pathogens and therefore that the over-representation of viruses in the list of recently discovered pathogen species (*Figure 2*) is not simply an artefact of advances in detection technologies but is a real biological phenomenon.

Future trends and the need for surveillance

We can and should anticipate the continued discovery and emergence of novel human pathogens in the immediate future. A recent analysis[15] found that the rate of discovery of novel human viruses has been more or less constant since the 1950s, with little indication that we are nearing the end of the process (in contrast to other groups, such as helminths, where new species are now very rarely reported). In addition, as modern, more powerful sequencing technologies are used for virus detection on larger and larger scales, there is every prospect that the rate of virus discovery will increase. Indeed, there are currently 10 or so new human viruses reported in the literature since 2006 whose status has yet to be confirmed by the ICTV (and so are not included in *Figure 2*).

The prospects for further discoveries of viruses and other kinds of pathogen are reinforced by the observation that, worldwide, for only a small fraction of patients admitted to hospital with suspected infections is a specific pathogen identified. The recent discovery of very widespread pathogens, such as metapneumovirus (associated with childhood respiratory disease[16]), confirms that we are still identifying the aetiological agents of common conditions. Clearly, there is a case for large scale, systematic investigation of pathogens associated with clinical illness.

In the first instance, we might expect that such surveys will discover mainly long established aetiological agents of existing conditions, and that these will eventually all be identified (accepting that this could

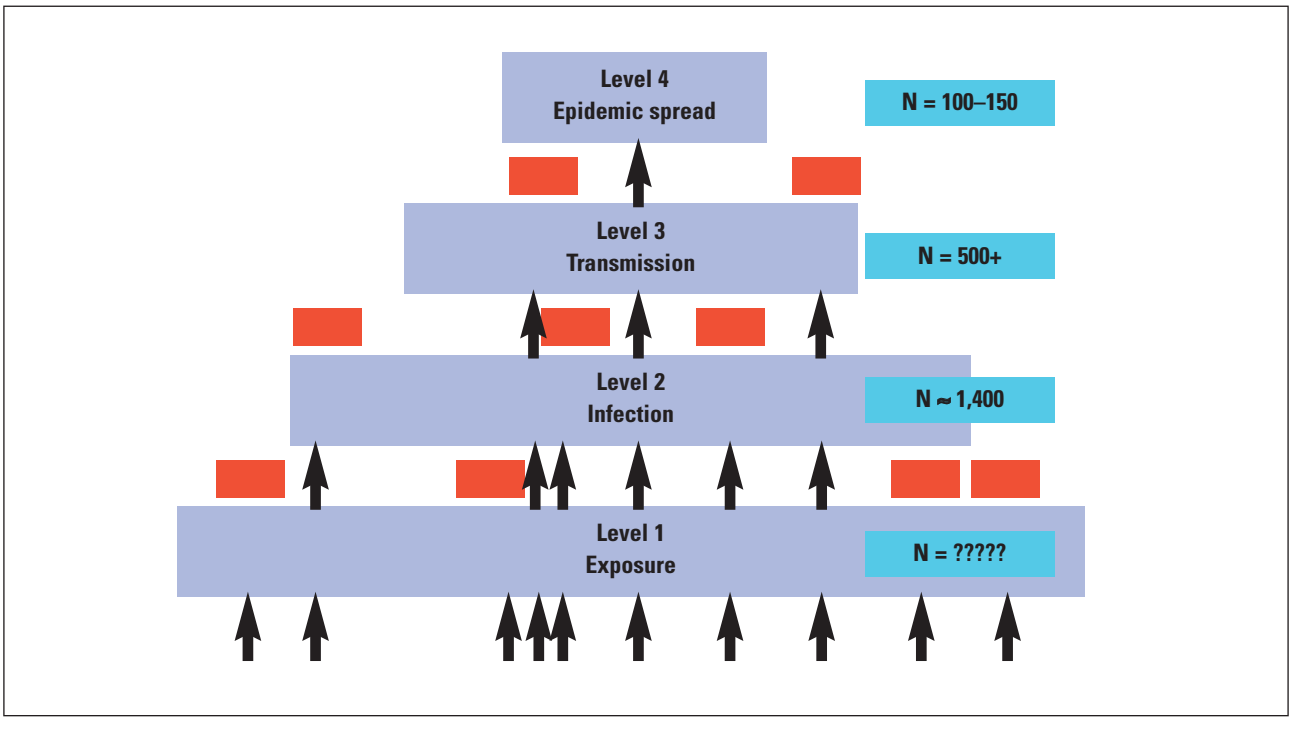


Figure 3. The pathogen pyramid. Four different levels of pathogen emergence are distinguished (see text). Arrows represent successful transitions to the next level, bars represent barriers (either biological or ecological) to the next level. Insets represent the estimate number of human pathogen species (N) at leach level.

take a considerable time). However, if the hypothesis is correct that pathogen (and particularly virus) variants capable of infecting humans are continually being produced as a side effect of their natural population dynamics then, of course, the process of discovering new human pathogens will effectively be never ending. In such circumstances, clinical observation is likely to remain at the front line of surveillance for the foreseeable future; many novel pathogens will first be detected by unusual clinical presentations or pathology, recent examples being the SARS coronavirus and variant CJD.

The realisation that the majority of novel human pathogens have animal origins implies that there may be considerable benefits to carrying out surveillance for human pathogens or potential human pathogens in animal populations as well as human. This topic has recently been reviewed in depth by the US National Academy of Sciences, and their report is in preparation[17]. Our current understanding of the process of pathogen emergence, while very incomplete, does provide some pointers on how to direct a surveillance effort for potential novel human pathogens in animal reservoirs; in particular, we are most concerned with mammal populations and, to a lesser extent, birds, but wildlife are potentially as important as domestic animals (noting that our knowledge of wildlife pathogens remains, in general, extremely poor).

There are obvious challenges in implementing large scale pathogen surveillance in animal populations (especially wildlife); for example, potentially serious human pathogens, such as *E. coli* O157, may show no clinical signs in their reservoirs, and so passive surveillance is ineffective, active surveillance is required. Nevertheless, there are obvious benefits in switching from the current state of affairs – where humans are essentially acting as sentinels for novel and emerging zoonoses – to a more pro-active approach. This argument is nicely illustrated by an initial phylogenetic analysis of 2009 swine-origin influenza A[18]. That study suggests that, although the origins of the current pandemic strain in humans go back only as far as early 2009, the strain itself may have existed for up to two decades, circulating in an unidentified host population. That host is likely to have been pigs (but could conceivably have been humans or another host species); the uncertainty arises directly from a lack of systematic surveillance and genetic analysis of influenza viruses in pigs. In other words, we were not aware of this new strain of influenza until it began to cause illness and deaths in the human residents of Mexico.

Part of the challenge is the global nature of the problem; in the past 30 years, novel human pathogens have been reported from every continent[2]. However, in practice, the largest number of disease outbreaks involving new kinds of pathogens (species or strains) has been first reported from the USA[3]. This almost certainly reflects reporting bias: the next most prolific country is the UK, while countries such as China, India, Egypt and Mexico have strikingly low numbers of reports of novel pathogens[19]. If reporting bias can be corrected, then other predictors of the emergence of new kinds of human pathogen can be identified. Such an exercise has been attempted[3] and has led to the prediction of global 'hotspots' for the likely emergence of novel human pathogens. Further development of this approach is warranted as the notion of hotspots is clearly important for the targeting of surveillance efforts. What is already apparent, however, is that there is a massive dearth of surveillance capacity in various regions of the world, including potential hotspot areas. It has been argued that, given the global nature of many infectious outbreaks (e.g. SARS or swine-origin influenza A) and the very high impact of even relatively minor public health problems on travel and trade (e.g. H5N1 influenza A or BSE), strengthening surveillance capacity in selected regions would be a global common good and should be actively supported by the international community[20].

Acknowledgement

I thank numerous colleagues and co-authors for stimulating discussions, especially Rustom Antia, Nathan Wolfe, Peter Daszak and Andy Dobson.

References

1. Taylor, L.H., Latham, S.M. and Woolhouse, M.E.J. (2001). Risk factors for human disease emergence. *Phil. Trans. R. Soc. B, Biolog. Sci.* **356:** 983–989.
2. Woolhouse, M. and Gaunt, E. (2007). Ecological origins of novel human pathogens. *Crit. Rev. Microbiol.* **33:** 231–242.
3. Jones, K.E., Patel, N.G., Levy, M.A., Storeygard, A., Balk, D., Gittleman, J.L. and Daszak, P. (2008). Global trends in emerging infectious diseases. *Nature* **451:** 990–993.
4. Wolfe, N.D., Dunavan, C.P. and Diamond, J. (2007). Origins of major human infectious diseases. *Nature* **447:** 279–283.
5. Woolhouse, M.E.J. and Gowtage-Sequeria, S. (2005). Host range and emerging and re-emerging pathogens. *Emerg. Infect. Dis.* **11:** 1842–1847.
6. Palmarini, M. (2007). A veterinary twist on pathogen biology. *PLoS Pathogens* **3:** e12.
7. Woolhouse, M.E.J., Haydon, D.T. and Antia, R. (2005). Emerging pathogens: the epidemiology and evolution of species jumps. *Trends in Ecology and Evolution* **20:** 238–244.
8. Cleaveland, S., Laurenson, M.K. and Taylor, L.H. (2001). Diseases of humans and their domestic mammals: pathogen characteristics, host range and the risk of emergence. *Phil. Trans. R. Soc. B, Biolog. Sci.* **356:** 991–999.
9. Webby, R., Hoffman, E. and Webster, R. (2004). Molecular constraints to interspecies transmission of viral pathogens. *Nature Medicine* **10:** S77–S81.
10. Woolhouse, M.E.J., Taylor, L.H. and Haydon, D.T. (2001). Population biology of multi-host pathogens. *Science* **292:** 1109–1112.
11. Institute of Medicine (2003). Microbial threats to health: emergence, detection, and response. Washington: National Academy Press.
12. Dobson, A. and Foufopoulos, J. (2001). Emerging infectious pathogens of wildlife. *Phil. Trans. R. Soc. B, Biolog. Sci.* **356:** 1001–1012.
13. Woolhouse, M. and Antia, R. (2008). Emergence of new infectious diseases. In Evolution in Health and Disease (2nd ed.) (Stearns, S.C. and Koella, J.K., eds.), Chapter 16, pp215–228. Oxford University Press, Oxford, UK.
14. Van Heuverswyn, F. *et al.* (2006). Human immunodeficiency viruses: SIV infection in wild gorillas. *Nature* **444:** 164.
15. Woolhouse, M.E.J., Howey, R., Gaunt, E., Reilly, L., Chase-Topping, M. and Savill, N. (2008). Temporal trends in the discovery of human viruses. *Proceedings of the Royal Society of London, Series B* **275:** 2111–2115.
16. Osterhaus, A. and R. Fouchier (2003). Human metapneumovirus in the community. *The Lancet* **361:** 890–891.
17. National Academy of Sciences (in preparation). Achieving sustainable global capacity for surveillance and response to emerging diseases of zoonotic origin.
18. Smith, G.J.D., Vijaykrishna, D., Bahl, J., Lycett, S.J. *et al.* (2009). Origins and evolutionary genomics of the 2009 swine-origin H1N1 influenza A epidemic. *Nature* **459:** 1122–1125.
19. Woolhouse, M.E.J. (2008). Emerging diseases go global. *Nature* **451:** 898–899.
20. King, D.A., Peckham, C., Waage, J.K., Brownlie, J. and Woolhouse, M.E.J. (2006). Infectious diseases: preparing for the future. *Science* **313:** 1392–1393.

Developments in food microbiology - the last 30 years

Melody Greenwood
Microtech Services (Wessex) Ltd
Wallisdown, Bournemouth, Dorset, UK

Introduction

Thirty years ago, most food laboratories used conventional culture methods for all microbiological tests. Some automation of the laboratory testing was available, including gravimetric diluters, spiral platers, laser and TV camera-based colony counters, but these pieces of apparatus were very expensive and few laboratories could aspire to their purchase. Even fixed or variable volume pipettors were relatively rare. Laboratories produced their own culture media, mainly from commercially available dehydrated powders, but it was still quite common to prepare media formulae from individual ingredients. Performance testing of the culture media by the user laboratory was rare, and poor performance of culture media was detected more by accident than by good quality assurance procedures.

By the end of the 1970s, academic microbiologists had a good understanding of factors affecting growth and recovery of target bacteria, but few methods were standardised. The concept of stress affecting the cultural recovery of organisms had not yet percolated into most food testing laboratories, and it was not until the early 1980s that a pre-enrichment step for *Salmonella* spp. isolation became a normal part of the procedure. Development of enrichment media for the detection of *Campylobacter* spp. in food was in its infancy, and food-borne illness associated with *Listeria* spp. or *Escherichia coli* O157 had not yet been widely recognised.

Most of the enumeration methods in food microbiology in use today are the same or similar to those in use 30 years ago. Conventional pathogen testing, even now, takes several days to obtain a definitive answer; at least three days is required to obtain a negative result for *Salmonella* spp. and five days for *Listeria* spp., using internationally standardised procedures. Several more days may then elapse if suspect colonies are detected and need confirmation. If a positive release system is in place following production, this entails significant storage costs and reduction in available shelf life for many products of limited durability.

The following article describes some of the developments that have taken place over the last 30 years that have had a significant impact on the daily work of the food microbiologist.

The effect of food-borne outbreaks

During the early 1980s, a series of outbreaks due to the consumption of food contaminated with *Listeria monocytogenes* were reported. A variety of food types, including coleslaw, raw vegetables and soft, ripened cheese, were implicated. This organism is unusual in its ability to grow at refrigeration temperatures and also due to the significant mortality associated with infection (around 30%). Routine surveillance testing carried out towards the end of the 1980s indicated that many ready-to-eat products and ready meals were contaminated with this organism, suggesting widespread contamination in production plants.

Further outbreaks of infection in the late 1980s were associated with pâté that had received inadequate heat treatment to ensure that the centre of the pâté block had reached a listericidal temperature[1]. As a result of these outbreaks, the food industry began its long and continuing struggle to ensure that its ready-to-eat products and factory environments were free of contamination. This gave rise to extensive food and environmental sampling programmes designed to demonstrate the absence of the organism and identify any contamination problems in a timely way.

The late 1980s also saw a rapid rise in the number of *Salmonella* spp. cases, mainly but not entirely due to contamination of hens' eggs with *S.* Enteritidis, burgeoning rates of *Campylobacter* spp. infection (see *Figure 1*) and the occurrence of outbreaks of verocytotoxin-producing *Escherichia coli* (VTEC) in the United States. The general alarm over food hygiene issues was further exacerbated by the Bovine Spongiform Encephalopathy (BSE) outbreak associated with the consumption of beef. All these food scares had significant implications for food producers, resulting in increased levels of ingredient, finished product and environmental sampling for the food laboratories.

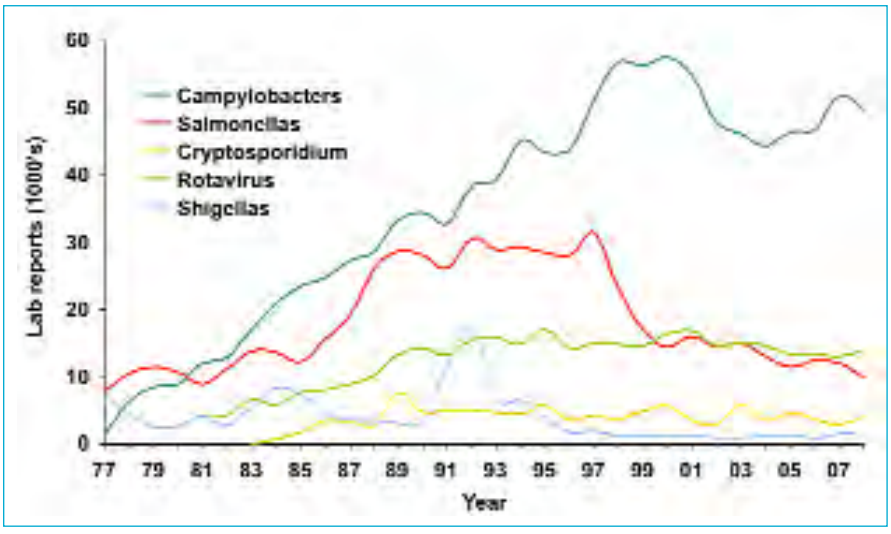
Laboratory accreditation

Thirty years ago, food laboratories did not operate under well-controlled management systems. Tests were usually performed following documented, in-house methods that were relatively non-specific in their requirements for performance. This resulted in considerable variation in the performance of the same method both within a single laboratory and between laboratories. Auditing of procedures in the laboratory was rare, and problems were dealt with on a firefighting basis.

In the 1980s, it was recognised that this situation needed improvement in order to provide reliable microbiological test results that helped to ensure the production of safe food. Schemes for assessing and accrediting food microbiology laboratories were developed in order to address this inherent variation. Over the following years, there became an increasing requirement, both industry-driven and legislatively, for food microbiology laboratories to achieve external accreditation and to participate in a proficiency testing scheme. During the 1990s, many food laboratories went through the accreditation process, which involved demonstrating that they worked to a sound, documented quality system that described both the managerial processes and the technical procedures used to ensure consistency and reliability of performance with full traceability. This included the demonstration that staff were adequately trained and competent to perform the work they carried out and auditing of the various aspects of the quality system. Hand in hand with this, proficiency testing schemes were developed commercially in which the food laboratories were expected to take part.

Nowadays, most commercial and government laboratories are accredited to ISO 17025[2], which contains the general requirements that laboratories have to meet in order to demonstrate that they operate an appropriate management system to ensure technical competence and validity of results. Auditing, both internal and external, by accreditation bodies and by customers is now a routine part of the food microbiology laboratory's daily life. Participation in

external proficiency testing schemes as well as internal quality assurance testing schemes to demonstrate and maintain appropriate competence and well documented in-house training programmes are also a significant part of present day requirements. Laboratories are also required to perform statistical analysis of these test results for estimates of measurement uncertainty, performance characteristics and detection of trend and bias.



Information courtesy of Health Protection Agency

Figure 1. Selected GI Pathogens (England and Wales)

Method standardisation

The requirement for laboratory accreditation, coupled with the increasing requirement to use internationally standardised methods or methods shown to be equivalent in performance to these, help to assure that the same test for a target organism, such as detection of *Salmonella* spp. or enumeration of *Staphylococcus aureus,* would result in the same outcome in different laboratories throughout the world (intrinsic variability of microbiological testing not taken into account). The first international standard published by ISO for detection of *Salmonella* spp. was published in 1981[3]. Many more standard methods for detection and enumeration of food-borne pathogens and hygiene indicator organisms have been published since then, including molecular methods. These published methods are reviewed and updated on a regular basis, and new methods elaborated as the need arises. When standards are revised, the laboratory needs to evaluate the changes and is usually expected to adopt them. These standardised test methods are underpinned by other normative standards describing general rules for microbiological examinations (ISO 7218[4]), preparation of sample homogenate and dilutions (ISO 6887[5]) and performance testing of culture media (ISO 11133[6]); accredited laboratories are expected to take account of these requirements and guidance. This process of harmonisation and standardisation is highly beneficial for both domestic and international trade of food commodities, as the importing country or purchaser can have confidence in the results of the laboratory first performing the tests.

Culture media and performance testing

Over the last 30 years, the quality of the ingredients used in the media formulations has been optimised by the commercial culture media producers, in particular the inherently variable biological ingredients, such as bile salts and blood, resulting in products of good consistency, productivity and selectivity. Many of the agar media in use today for detection and enumeration of food-borne microbial contaminants were also in use by 1980. Although these isolation media are relatively specific for their target organisms, further confirmation of identity is usually needed by means of biochemical tests. Galleries of biochemical substrates with accompanying databases for interpretation of results were commercially available for confirmation and identification tests during the 1970s, but most food testing laboratories relied on conventional media produced in-house. Now the majority of laboratories rely on such kits almost exclusively for their identification requirements.

Because of accreditation requirements, there has been an increasing requirement for laboratories producing their own culture media to demonstrate that they are fit for purpose, in terms of productivity and selectivity, as well as physical properties. While 10 years ago it may have been acceptable to streak or inoculate positive and negative control organisms onto or into culture media, more quantitative procedures are now required. Laboratories are increasingly expected to adopt the principles of performance testing described in ISO 11133 in order to demonstrate the good quality of their media. This is quite a time-consuming activity for the modern food laboratory; however, what is the point of testing a food product if the quality of the culture media is so poor that levels are significantly underestimated or target pathogens not recovered when present? Partly as a result of these quality assurance requirements, there is an increasing reliance on commercially produced pre-poured plates, pre-prepared diluents and culture media, and agar bases that just need melting and supplementing.

Developments in culture media

One of the more simple innovations to have a significant impact on the ease of recognition of target colonies was the incorporation of enzyme-based detection systems such as fluorescent and chromogenic substrates in the 1990s. The first chromogenic medium to become widely available commercially was tryptone bile glucuronide agar (TBX), which was developed by a team of scientists in the Public Health Laboratory Service (now part of the Health Protection Agency) in the UK. The chromogenic agent, 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide, is hydrolysed by the enzyme glucuronidase to form an insoluble chromophore which accumulates in the cells, producing blue-green colonies. Strains of *Escherichia coli* possess the enzyme, glucuronidase; when the medium is incubated at 44°C, the reaction is highly specific for this organism, rendering further confirmation or characterisation unnecessary. Since then, a wide range of different chromogenic media based on similar principles have been developed, which allow target organisms such as *Salmonella* spp., *Listeria* spp., *Campylobacter* spp., *Escherichia coli* O157, *Cronobacter* spp. and *Staphylococcus aureus* to be easily detected in the presence of competing background flora.

Alternative or rapid methods

Over the years, more automated methods have been developed in an attempt to reduce the time taken to obtain a result and also to provide more labour saving ways of testing. These are generally known as alternative or rapid methods. Although these automated systems help to reduce the duration of testing for enteric pathogens, their lower limit of detection is still generally quite high, making it necessary to ensure that the target pathogen is at a level of 10^4–10^5 cfu/ml before running the automated test. This usually means that at least a pre-enrichment stage and often a secondary enrichment stage is required before running the automated test.

Immunological methods

Before the 1980s, manual enzyme-linked immunosorbent assays (ELISA) were available for use following conventional enrichment culture for detection of some food-borne pathogens and microbial toxins. Following heat treatment of the enrichment culture to release the antigens, tests were performed in microtitre trays, with all stages of inoculation, mixing, washing and reagent addition being performed by a technician, and the result read using a plate reader or spectrophotometer. Furthermore, the reagent solutions usually required preparation immediately before running the test.

Subsequently, various semi-automated commercial procedures were developed using the microtitre plate format in which the capture antibody linked to the enzyme was bound to the well. Separate pieces of equipment (shaker, washer, reader) were required to perform the test, so there was still significant technician involvement. However, this proved a useful screening tool for identifying negative cultures for pathogens, such as *Salmonella* spp., within 48 hours, particularly for companies dealing with large numbers of samples consisting of a limited number of food substrate types. The procedure effectively replaced the plating stage of conventional culture, but the sensitivity was relatively low as 10^4–10^6 organisms per ml were required. Over the following twenty years, the equipment has become increasingly automated, and systems are now available that just require the technician to inoculate the heated culture into a reagent strip, start the process run and check the printouts at the end of the run. A number of different ELISA-based formats are available that use chemiluminescence or fluorescence as well as colour reactions for detection, and there are also one-step sandwich immunoassays and dipstick-based systems coated with reagents in which the result is visualised by immunoprecipitation lines.

Immunomagnetic separation

The development of immunomagnetic beads by Dynal in the 1980s resulted in a significant improvement in sensitivity of both conventional cultural methods and those linked to automated "alternative" methods, while further reducing the time to a negative result. These small paramagnetic beads (<100µm) are coated with antibody to the target organism, and for use, are placed in a capped tube to which 1ml of enrichment broth is added. After a short period of mixing to allow binding of the target organism to the antibodies on the magnetic particles, a magnet is applied to the outside of the tube. This immobilises the beads against the side of the tube, thus allowing removal of the liquid. After washing, the bead suspension can either be plated directly onto an appropriate selective agar or used in an alternative method. This technique is particularly useful for recovery of pathogens from raw foods in the presence of high levels of competing flora and is routinely used for detection of *Escherichia coli* O157. It is also used for detection of parasites in food. The immunomagnetic process can either be done manually, using a mixer and magnetic particle concentrator, or using a fully automated instrument.

During the last decade, a further development of this technique (the Pathatrix® System) enables simultaneous culture of the entire 25g sample suspension with beads by heating to the appropriate temperature while repeatedly circulating the suspension through a capture phase where the antibody-coated magnetic beads are immobilised. The captured beads are then eluted, collected and concentrated, and can be used for plating on selective agar or coupled with an ELISA-based or other rapid method for detection. This immunomagnetic separation principle has enabled a significant reduction in the time to obtain a negative result which is now possible in 24-30 hours when coupled with a rapid detection method.

Molecular techniques

Although the polymerase chain reaction (PCR) was developed in the mid-1980s, tests applicable to food microbiology laboratories were slow to become available. The stringent requirements for the physical separation of the various stages of the PCR process (sample handling and preparation, reagent preparation, nucleic acid extraction and amplification) to prevent cross-contamination and the occurrence of false positive results precluded its use except in specialist facilities. Interference and inhibition of the PCR reaction by the food matrix also proved to be a major obstacle, and specific sample preparation protocols tailored to different food matrices had to be developed. Stringent validation of the procedure to show equivalence with conventional techniques was also required, as well as demonstration of the specificity for the DNA of viable target organisms as opposed to DNA from dead cells.

These problems have now been resolved and commercially prepared reagents combine the primers, polymerases and nucleotides into a single pre-packaged tablet, thus eliminating multiple liquid transfers and the potential for operator error. The last decade has seen the slow adoption of PCR-based screening methods, including standard, real-time and reverse-transcriptase PCR protocols, such as those provided by the automated BAX® System, by food industry laboratories. However, the PCR technique for pathogen detection still requires a pre-enrichment or enrichment culture rather than using the sample itself, as a level of 10^4 cells/ml of target organism in the suspension is often required to ensure consistent detection. This requirement may be reduced by the use of immunomagnetic separation to concentrate the target organisms and attain this level more quickly.

Phage based system

A system using bacteriophages to detect food-borne pathogens was developed by Alaska Food Technology during the late 1990s, which shows considerable promise in significantly reducing the detection time of the major food-borne pathogens. Only viable bacteria are detected, because they must be actively growing to be infected by the phage. Because the procedure is capable of detecting as few as 100 cells per gram, a shorter time is needed for enrichment (8h) and results can be obtained within 12h of commencement. After immunomagnetic separation of the enrichment culture to concentrate the target cells, infecting phage is added, which binds to specific components of the cell surface. Enzymes in the phages' tails make

holes in the cell wall, and the phage nucleic acid is injected. Phage replication occurs within the bacterium, and enzymes are produced that disrupt the cell wall, releasing new phages and the enzyme, adenylate kinase (AK). This enzyme, catalyses the conversion of ADP to ATP, resulting in an approximate 40-fold amplification of ATP in the bacterial cell. After addition of the enzyme, firefly luciferase, the luminescence can be read using a luminometer.

Method validation

With the introduction of these more automated methods for pathogen detection, it became clear that independent validation was required to show equivalence with conventional methods. This was already happening in the United States in the 1980s under the auspices of the Association of Official Analytical Chemists (AOAC), but with the exception of AFNOR in France, was not happening in a cohesive way elsewhere. A Eureka project, started in Europe in 1993, resulted in the formation of MicroVal, a European network for the validation and certification of alternative microbiological methods. This organisation played a key role in elaborating the technical rules for validation that were later standardised in 2003 as ISO 16140[7]. It is essential these days for manufacturers to obtain evidence of appropriate validation from a recognised certification body.

If accredited food laboratories wish to add a test method that uses a proprietary kit or piece of equipment or add an additional conventional culture test to their scope of accreditation, they are required to provide an evaluation of its use in their hands and for their sample types. Current EU legislation ([EC] 2073/2005[8] as amended by [EC] 1441/2007[9]) for food specifies certain ISO and EN microbiological testing methods but some flexibility is allowed regarding alternative methods so long as they have been appropriately validated against the method specified and demonstrated to be equivalent.

All food microbiology laboratories need to consider whether their customers are affected by this legislation, and to use the methods specified or validated alternatives. While certification by an outside body that demonstrates equivalence to a standard method of detection or enumeration is of immense value to the laboratory, it is important that the contents of the collaborative trial are carefully reviewed to determine the relevance to the products they will be testing. Once this has been done, they can then plan their own investigations taking this information into account. This evaluation can be an onerous task, but careful design can help to minimise the amount of work required.

Conclusion

Over the last decade in particular, automated procedures have become an increasing feature of modern food laboratories. The use of Laboratory Information Management Systems (LIMS) has become the norm rather than the exception, facilitating data handling and analysis. In the larger contract laboratories, automated sample handling systems have been introduced that provide reduced technical input and a high degree of traceability throughout the sample handling procedure, including the media preparation component. Quality systems are in place to help ensure that microbiological test results are reliable, and compliance with accreditation requirements has become routine, although this requires a considerable amount of resource. Further efforts are still needed to reduce the turnaround time for pathogen detection; however the building blocks now seem to be in place to make these advances, and "next day" results for pathogens are becoming a reality instead of a dream. This will help to reduce storage costs due to more rapid identification of negative results, extend shelf life duration at the retail point and help to provide an earlier warning of potential food safety issues. However, it is likely that conventional microbiological culture methods will co-exist alongside more rapid "alternative" methods for the foreseeable future.

From a personal point of view, it has been a very interesting and highly stimulating 30 years with many challenges, both microbiological and managerial.

References

1. McLauchlin J.S., Hall S., Velani S.K. and Gilbert R.J. Human listeriosis and pâté. *Br. Med. J.* 1991; **303:** 773–775.
2. ISO/IEC 17025. General requirements for the competence of testing and calibration laboratories 2005. Geneva: International Organisation for Standardization.
3. ISO 6579. Microbiology – General guidance on methods for the detection of Salmonella 1981. Geneva: International Organisation for Standardization.
4. ISO 7218. Microbiology of food and animal feeding stuffs – General requirements and guidance for microbiological examinations 2007. Geneva: International Organisation for Standardization.
5. ISO 6887. Microbiology of food and animal feeding stuffs – preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Geneva: International Organisation for Standardization.
   Part 1: General rules for the preparation of the initial suspension and decimal dilutions 1999.
   Part 2: Specific rules for the preparation of meat and meat products 2003.
   Part 3: Specific rules for the preparation of fish and fishery products 2003.
   Part 4: Specific rules for the preparation of products other than milk and milk products, meat and meat products, and fish and fishery products 2003.
6. ISO/TS 11133 Microbiology of food and animal feeding stuffs – Guidelines on preparation and production of culture media. Geneva: International Organisation for Standardization.
   Part 1:2009. General guidance on quality assurance for the preparation of culture media in the laboratory.
   Part 2:2003. Practical guidelines on performance testing of culture media.
7. Commission of the European Communities. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union* 2005; **L338:** 1–26.
8. Commission of the European Communities. Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Off. J. Eur. Union* 2007; **L322:** 12–29.
9. ISO 16140 Microbiology of food and animal feeding stuffs – Protocol for the validation of alternative methods 2003. Geneva: International Organisation for Standardization.

Once again, the Culture editorial board would like to express its gratitude to the guest editor, Professor Barry Cookson. Both 2009 issues of Culture have attained the high standard befitting its 30th anniversary, and this has been in large part due to Barry's contribution. It has been a delight working with him.

Published by Oxoid as a service to Microbiology.

Our thanks go to the Culture editorial board: Professor Grahame W Gould, Dr David Petts, Mr David E Post, Dr Peter Stephens

OXOID

DEDICATED TO MICROBIOLOGY

Oxoid, Wade Road, Basingstoke, Hants, RG24 8PW, UK.

Tel: +44 (0) 1256 841144
Fax: +44 (0) 1256 329728
Email: oxoid.info@thermofisher.com

wwww.oxoid.com
www.thermofisher.com