



30th Anniversary Issue (Part I) – an introduction

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This is the first of two issues of *Culture* which celebrate 30 years of its publication. We have invited four experts to write on four key areas of microbiology and infectious disease. Three of these are devoted to aspects of healthcare associated infection (HCAI), a term used instead of hospital acquired infection in recognition of the changing patterns of healthcare delivery. It is in fact the first time there has been such an emphasis in an issue over these 30 years, and reflects the increasing recognition of the considerable global burden such infections cause. It is interesting to also reflect on what has been covered in the 122 articles published in these 30 years.

Ten papers were devoted to historical aspects of microbiology, although many others have historical sections within them, including useful pictures, which no doubt several of us have borrowed (with due acknowledgement of course)? In this issue, Dr Bill Newsom has pondered on his experiences as an infection control doctor and medical microbiologist over this period and also reminds us of the origins of Oxoid. His experiences will resonate with many readers, depending on their date of professional entry into the field. His conclusion certainly coincides with my own feelings of sadness that it has taken the current situation to show that the warnings of many infection control professionals were correct.

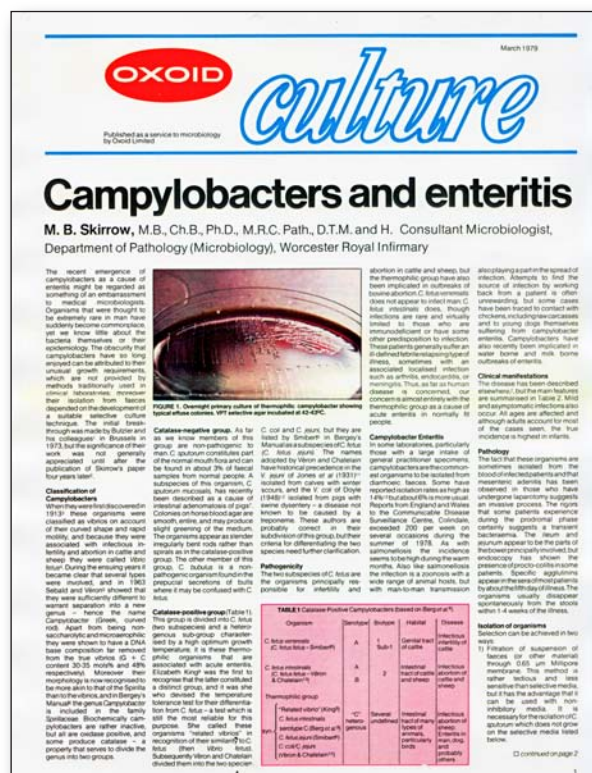
Indeed, it is only in the last few years that politicians and policy makers have realised the importance of HCAI prevention and control. This has been assisted by the high profile of patient safety. Until relatively recently, medical practice has been largely curative-oriented and prevention and control of infectious diseases were not high priorities. While this is improving in the 'developed' world, the developing world is still lagging behind. Dr Michael Borg was thus asked to write an article on the latter, a new area for *Culture*, and one that I was keen to include, having had the privilege of working with Michael and many other experts from the European funded project 'ARMed', which he has referenced in his article. The WHO Global Patient Safety Challenges and activities of the International Federation of Infection Control are also outlined in his important contribution.

Reviews of organisms and the diseases they cause comprise the majority of *Culture's* articles (30%). *Clostridium difficile* has been covered three times previously (second only to *Campylobacter* spp.) and Professor Brendan Wren, in this issue, provides insights into the evolution of this

pathogen. It has just celebrated the 40th anniversary of the clinical human description, and one wonders again about warnings going unheeded when one considers the title of Tom Riley's paper in *Culture* in 1996 ('*Clostridium difficile*: a high-cost nosocomial pathogen').

Biofilms are associated with many HCAs, and it is extraordinary how neglected they are by clinical microbiology. Professor Bill Keevil describes their importance in water systems, and includes some HCAI pathogens e.g. *Legionella* spp. He has kept strictly to his remit, but his group are also exploring the use of biofilm control technologies in the wider field of HCAI prevention and control.

I have thoroughly enjoyed helping the editorial team to compile this anniversary issue and hope you will have gained as much from reading the reviews of our experts as I have done. In our second anniversary edition we will address other topics that *Culture* has covered over the years: discovery of new pathogens since the first edition, regulations in the food industry, bioterrorism and detection methodologies and an article reflecting on lessons that have not been learned from recurring infections of the last 30 years.



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Campylobacters and enteritis

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The recent emergence of campylobacters as a cause of enteritis might be regarded as something of an embarrassment to medical microbiologists. Organisms that were thought to be extremely rare in man have suddenly become common pathogens and all know little about the factors themselves or the epidemiology. The difficulty that campylobacters have in being associated with enteritis is that their usual growth requirements, which are not provided by methods traditionally used in clinical laboratories, prevent their isolation from faeces deposited on and subsequently in a suitable selective culture technique. The initial breakthrough was made by Butler and his colleagues in Boston, in 1973, but it was not until the work was published in the journal of the American Society for Microbiology in 1975 that the world became aware of the existence of these organisms.

Classification of Campylobacters

The genus *Campylobacter* (1913) these organisms were described as vibrios on account of their curved shape and rapid motility, and because they were associated with infectious enteritis and abortion in calves. They were called vibrios until 1973 when they were renamed campylobacters. During the early years of the 1970s, the genus was divided into three species, *C. jejuni*, *C. coli*, and *C. fetus*, and in 1982 a fourth species, *C. lari*, was described. These four species were sufficiently different to be placed in four different genera, hence the name *Campylobacter*. The genus *Campylobacter* is now divided into two subgenera, *Campylobacter* and *Campylobacter*. The subgenus *Campylobacter* is further divided into two species, *C. jejuni* and *C. coli*. The subgenus *Campylobacter* is further divided into two species, *C. fetus* and *C. lari*.

Campylobacter-positive group (Table 1). This group is divided into *C. jejuni*, *C. coli*, and *C. fetus*. The subgenus *Campylobacter* is further divided into two species, *C. jejuni* and *C. coli*. The subgenus *Campylobacter* is further divided into two species, *C. fetus* and *C. lari*.

Campylobacter-negative group (Table 1). This group is divided into *C. fetus*, *C. lari*, and *C. coli*. The subgenus *Campylobacter* is further divided into two species, *C. fetus* and *C. lari*. The subgenus *Campylobacter* is further divided into two species, *C. coli* and *C. coli*.

Pathogenicity

The genus *Campylobacter* is a pathogenic organism. It is a gram-negative, motile, and fastidious organism. It is a common cause of enteritis in man and animals. It is a common cause of enteritis in man and animals. It is a common cause of enteritis in man and animals.

Isolation of organisms

The isolation of campylobacters is a difficult task. It requires the use of a selective culture medium. The medium should be able to support the growth of campylobacters and inhibit the growth of other organisms. The medium should be able to support the growth of campylobacters and inhibit the growth of other organisms. The medium should be able to support the growth of campylobacters and inhibit the growth of other organisms.

Table 1. Causes of Positive Campylobacteriosis (after Skirrow et al.)

Organism	Species	Host	Enteritis	Abortion	Isolation of organisms
<i>C. jejuni</i>	<i>C. jejuni</i>	Human	Enteritis	Abortion	Isolation of organisms
<i>C. coli</i>	<i>C. coli</i>	Human	Enteritis	Abortion	Isolation of organisms
<i>C. fetus</i>	<i>C. fetus</i>	Human	Enteritis	Abortion	Isolation of organisms
<i>C. lari</i>	<i>C. lari</i>	Human	Enteritis	Abortion	Isolation of organisms

Hospital-Acquired Infections – a retrospective

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Introduction

In 1861, Sir James Young Simpson, Professor of Obstetrics in Edinburgh, wrote: 'The man laid on the operating table of one of our surgical hospitals is exposed to more chances of death than the English soldier on the fields of Waterloo'. He used the word 'hospitalism' to describe the problem. Soon afterwards, Florence Nightingale started her *Notes on Hospitals* with: 'It may be a strange principle to enunciate as the first requirement in a hospital that it shall do the sick no harm¹'.

Both quotations preceded the realisation that microbes were at the root of the problem. Not until the 1880s was medical microbiology put on a firm footing by Robert Koch and his colleagues. Colonies of some bacteria grew on the cut surface of a potato, but this would not support growth of pathogens. Instead, Koch used an agar medium with a nutrient – usually a meat extract. Meat extract was available in Germany, thanks to Justus Von Liebig, but it was expensive. So a large scale manufacture was set up in Uruguay by the *Liebig's Extract of Meat Company*, which was registered in the UK. The product was called LEMCO (work it out). LEMCO was primarily used for drinks (OXO®). Starting with Lab-LEMCO, Oxoid grew from this into a company now serving a wide range of products to microbiology laboratories worldwide. It has always been a user-oriented company, providing data sheets and manuals, and these were complemented by *Culture*, which now celebrates its 30th anniversary. It is a great privilege to be part of the celebration and to be able to give a very personal view of the last eventful 30 years.

Thirty years ago

I had just taken charge of infection control in our new university hospital, in addition to running the hospital laboratory and providing a clinical service. Infection control in Cambridge was relatively advanced. The Infection Control Committee was well established and met regularly, and we had a full-time infection control nurse. However, her remit covered five hospitals in Cambridge and three in adjoining towns. Each morning, we would spend an hour together looking through the laboratory results; isolates of staphylococci were recorded in the 'staph book', and positive blood cultures in the 'bacteraemia book' – no computers then! Apart from this on-going surveillance – the main activity was as a reactive 'fire brigade'.

By now, many of the staphylococcal problems of the 1950s described by Williams *et al.*² had been addressed. Operating theatres were properly ventilated, and isolation facilities were being provided in new hospitals. The introduction of β -lactamase resistant penicillins (meticillin, flucloxacillin), and aminoglycosides (gentamicin) meant that infections could be treated. In 1972, rifampicin (the film *Rififi* was showing in Milan the week it was named) meant that drug-resistant tuberculosis could be treated. One of our patients was

cured after four years in hospital but was almost impossible to rehabilitate. Cephalosporins and then quinolones arrived. Isolation facilities were used for non-infectious reasons – leukaemia units or private patients, and around 1980, a colleague said, 'Nice job we used to have Bill!' How wrong we were!

My first outbreak in Cambridge – in 1977, was one of 'winter vomiting disease' – it cut through a mixed ophthalmology/dermatology ward like a knife through butter. Even a doctor entering the ward for the first time that evening was vomiting the next day. We closed the ward to admissions, sent the patients home and cleaned the empty ward. No more outbreaks occurred in 1977, but the last time I went for a hearing-aid service, I was handed a leaflet warning me not to think of entering the hospital if I felt sick or had diarrhoea.

One Saturday afternoon, we discovered typhoid bacilli in the blood of our hospital head cook. She had just returned from a holiday in Egypt. A major outbreak plan was initiated, but fortunately there were no secondary cases.

Salmonella remained on the agenda throughout the 1970s, with reports on infections from thyroid tablets and pancreatin, as well as food. The real pressure on UK infection control services came from the Stanley Royd outbreak in 1984. This occurred over the August bank holiday, and affected half of the 788 patients, and 106 of 980 staff in this mental hospital; nineteen patients died. An official enquiry³ failed to find the source of the outbreak, caused by *Salmonella* Typhimurium, but poultry was suspected. The organism was cultured from drains in the kitchen floor, and the lack of adequate refrigeration was noted. The report highlighted the failure of infection control policies and staff, finally established the infection control nurse, and introduced a community physician responsible for control of communicable disease in the UK, who was later supplemented by the regional epidemiologist.

Endemic or small point-source outbreaks were largely due to enterobacteria and pseudomonas strains (resistant to available antimicrobials), which, in those pre-disposable days, often related to domestic items such as mops and mop buckets, urinal bottles, bedpans/washers, washbasins, contaminated disinfectants, or artificial ventilation in intensive care wards with associated humidifiers and tracheal suction.

The Devonport Incident⁴, which affected many post-operative patients and killed five of these, involved transfusions of contaminated dextrose and illustrated the danger of trusting labels (e.g. sterile) in those days. The company autoclaves were faulty – and the subsequent 'Clothier Report' to the Medicines Commission laid the groundwork for the code of 'Good Manufacturing Practise' still in use today⁵. A smaller incident at Papworth⁶ involved post-operative infections due to patients on ventilators being given contaminated ice to suck – the ice machine was plumbed in incorrectly and had backflow from the drains (*Figure 1*).

Hand hygiene

Since Semmelweis, hand hygiene has been the basis for infection

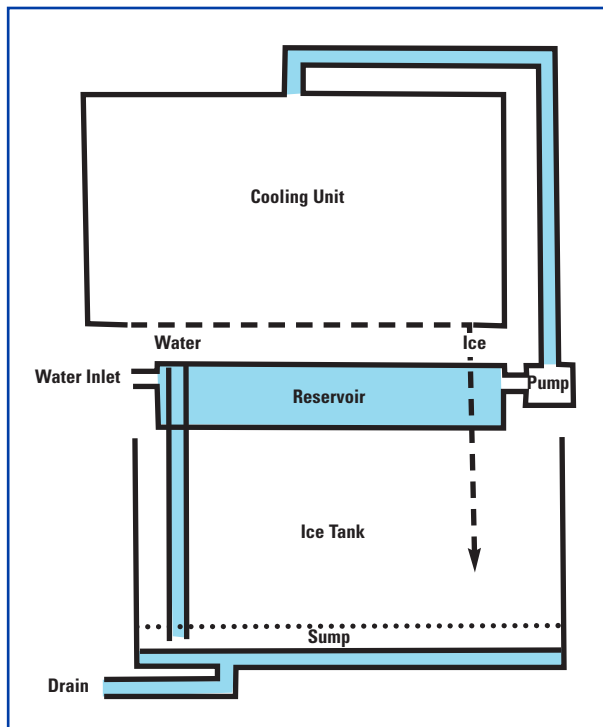


Figure 1. Plan of the ice machine with recycling and direct connection to drain

prevention (Figure 2). However, apart from the constant challenge of user non-compliance, we encountered several other problems. A small outbreak of post-operative *Pseudomonas aeruginosa* eye infections resulted from storage of scrubbing brushes (now banned) in a disinfectant that was neutralised by soap. Ojarvi noted that some handwash preparations created skin problems, which not only deterred users, but also increased their load of skin micro-organisms⁷. A local hospital had antiseptic soap containers fixed to the walls that were refilled but never washed out – until they received a batch of contaminated soap, when the system had to be abandoned. Finally – can you trust a name? ‘Phisohehex’ was used as a surgical scrub – it contained hexachlorophane. However, 3% hexachlorophane dusting powder was found to be toxic to babies. Phisohehex continued to be sold, but the active ingredient was

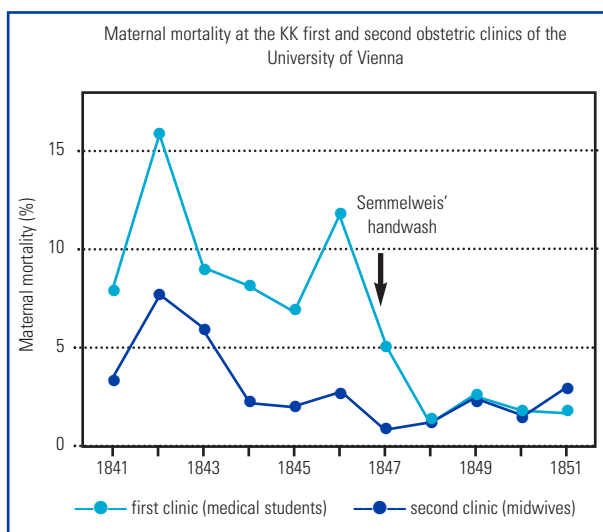


Figure 2. The effect of Semmelweis' handwashing (from Manfred Rotter)

changed to chlorhexidine. European standards for hand care products owe a lot to the work of Professor Manfred Rotter, using n-propanol as a reference agent⁸. However, ‘alcohol’ is a generic term and users/suppliers may still not be aware that the different alcohols have varying activity.

Infection control services

First on the scene was the ‘infection control nurse’ (ICN). In 1959, Torbay hospital, in the West of England, was serviced from the laboratory in Exeter, some 20 miles away. On-site policies, monitoring and advice were needed by the Torbay surgeons, and with matron’s permission, they appointed an ICN. The outcome was successful enough to justify publication in the *The Lancet*⁹. The name of Brendan Moore, the Exeter microbiologist, has passed into infection control ‘lore’. In 1963, Stanford University appointed Kathryn Wenzel as the first ICN in the USA. The Communicable Diseases Center (CDC), Atlanta, instituted training courses for ICNs in the 1960s, and in 1969, US hospitals required an ICN for accreditation. By 1970, there were enough ICNs in the UK to justify a national association – the Infection Control Nurses Association (now the Infection Prevention Society), and this was joined in 1972 by the Association for Professionals in Infection Control and Epidemiology (APIC) in the USA. In 1980, the nurses were joined by the microbiologists – the Hospital Infection Society (HIS) in the UK, and the Society for Healthcare Epidemiology of America. All these societies provide opportunities for discussion, meetings, guidelines, educational courses and journals. In 1987, the International Federation for Infection Control was formed to provide a point of reference for national societies and to encourage the infection control movement throughout the world. The current chairman is Dr Michael Borg – also writing in this issue.

Surveillance

The value of prospective surveys of infection was illustrated by Cruse and Foord from Calgary in their five year study of surgical site infections¹⁰. They reported outcomes on 23,649 wounds. They compared clean, clean contaminated, and dirty operations, and made particular note of length of pre-operative stay in hospital and effect of skin preparations, together with surgical hand preparation and types of drapes used. A short pre-operative stay and no shaving of the operation site were recommended. They found increased rates of infection in the elderly where drains were used and after long operations.

The CDC ‘Study of the efficacy of nosocomial infection control’ (SENIC project) masterminded by Robert Haley *et al.*¹¹ showed that, in 1975-1976, the nationwide hospital infection rate among 6,449 acute-care US hospitals was 5.7% – and implied that over four million cases occurred annually. This gave strong support to an infection control programme which included surveillance, and recommended an ICN per 250 acute beds. However, Haley points out that surveillance is useless unless the results are acted upon (personal communication, 2008).

A UK national ‘point prevalence survey’ was carried out in 1980 as one of the first ventures of HIS. The results¹² showed that approximately 10% of 18,186 patients in 43 hospitals (including Cambridge) had a nosocomial infection. A second survey (Emmerson *et al.*, 1996), in 1993-4, involving 37,111 patients, in 157 centres (including the Republic of Ireland), showed a 9% infection rate¹³.

This was despite significant improvement in infection control services. However, this survey included patients in high dependency units, and reflected the increasing complexity of medical care. Surgical wound infections had dropped, but lengths of hospital stay had dropped dramatically and, with no post-discharge surveillance in place, the true occurrence was unknown. One or two 'straws in the wind' predicted future problems. Bacteraemia rates had increased from 18 to 235 cases, and gastro-intestinal infections from 23 to 189 – the authors suggested ward outbreaks of *C. difficile* could have accounted for this.

The July 2008 issue of the *Journal of Hospital Infection* was devoted to Surveillance and dedicated to Professor Michael Emmerson, whose obituary it contained. The third survey (UK/EIRE)¹⁴ had shown an overall 7.6% prevalence of newly named Healthcare-associated infections (HCAI), and now gastro-intestinal infections were top of the list, the number of bacteraemias had increased noticeably. Indwelling devices were noted as risk factors; their use has steadily increased over the last 30 years. Significantly, this survey received Government backing. However, a more sinister aspect of political interest was the threat on both sides of the Atlantic to restrict funding for hospitals with high infection rates.

Emerging infections

Hepatitis

Hepatitis has long been known as a risk to staff and patients, from blood. The outbreak in the renal dialysis centre in Edinburgh described in the Rosenheim report in 1972¹⁵ demonstrated the need to ensure blood for transfusion was safe by excluding infected donors. Even so, there remained an 'undetectable window' period in the newly infected donor. Hepatitis C emerged from the non-A-non-B group in the late 1980s. An estimated 170 million people suffer from Hepatitis C, and because of the chronicity of the disease, blood transfusion has been a significant source of infection, especially for groups such as haemophiliacs. According to WHO, 70 million units of blood are collected worldwide, and at least 13 million are incompletely screened for viral infection.

Legionella

The celebration of the bicentenary of American Independence by the American Legion in Philadelphia, in July 1976, was marked by the 'Broad Street' outbreak of pneumonia, involving at least 221 people, with 34 deaths. Not all were legionnaires, some had just walked past the Bellevue Stratford Hotel. Despite an investigation involving 100 CDC scientists, the outbreak remained a mystery. Joseph McDade was asked to look for *Coxiella*. He inoculated guinea pigs, and noted a rise in temperature, but when he sub-cultured material into eggs nothing grew. Quoting from *Trauma*¹⁶ – 'On December 27th, for reasons he was never clear about, McDade had another look at tissue from his infected guinea pigs. He could see intracellular bacteria.' He then succeeded in growing the legionella in eggs. The reason for his initial failure was that he had used antibiotics in his media!

Legionella infections cause both epidemic and endemic problems in hospitals – associated with aerosols from water – either in the water supply, or in air conditioning units. Wet cooling has now disappeared from UK hospitals. My experience related to sporadic cases of pneumonia. At Papworth, a heart transplant patient was involved. Examination of the water supply revealed a 'blind loop' in

CEL BENIN
REGD. TRADE MARK BRL1241
Sodium 6—(2,6—dimethoxybenzamido)
penicillanate monohydrate

“ A new penicillin has been prepared which is active against the usual penicillin-sensitive microorganisms and yet resists staphylococcal penicillinase. This is a major event in chemotherapy. From the information given in the British Medical Journal last week and in the three papers appearing in our present issue there is good reason to hope that the new BRL 1241 ('Celbenin', Beecham Research Laboratories) will be a means of controlling the staphylococcal infections which have plagued hospitals throughout the world during the past ten years. ”

EDITORIAL (1960) LANCET, ii, 585

Figure 3. Announcing meticillin, in 1960

the shape of a heat exchanger that had been shut down but remained connected to the water supply. In Cambridge, a renal transplant patient was affected. I had to collect a water sample from a tap in the basement of the ten storey building. While collecting it, I was showered from the tap, and was later horrified to find the water contained 10^5 legionella/ml. However, neither I nor any other patient was affected. In Papworth, the water system was repaired and cleaned, and then, in both hospitals, the water was circulated at 80°C during the night (with due warning to staff and patients) and then hyperchlorinated. No further problems occurred.

Clostridium difficile

In the 1960s, we noted that stools from patients with antibiotic-associated diarrhoea stained Gram-positive – with what appeared to be sheets of staphylococci. Treatment was the newly available vancomycin. With hindsight, this could have been caused by *C. difficile* (or perhaps it disappeared with the advent of anti-staphylococcal penicillins). *Clostridium difficile* had been discovered in 1935, and found in the stools of neonates – but regarded as non-pathogenic. The use of broad spectrum antibiotics grew; lincomycin and clindamycin appeared and were soon associated with 'pseudomembranous colitis'. The seminal paper by Bartlett *et al.* in 1978¹⁷ associated this with the *C. difficile* toxins. During the 1980s, we screened stools for *C. difficile* toxin. Three points emerged – it was found in patients who were not ill (20% of the elderly hospitalised population) could be carriers, and even after treatment with vancomycin or metronidazole – relapses were common. Finally – the organism persisted in the environment exceedingly well. Guinea pigs treated with penicillin succumb very easily unless kept in a germ-free environment. It seems that widespread use of antibiotics has allowed a significant load of *C. difficile* to build up in humans. The

third UK prevalence survey showed that 1.72% of the hospital population had *C. difficile* infection, and 1.2% had community-acquired infection, a total of 1,304/75,694 surveyed¹⁴. Recent work has shown that some clones of *C. difficile* are more dangerous (see Professor Brendan Wren's article), and clearly the last word remains to be said. Major outbreaks, such as that in Tunbridge Wells, have demanded extreme measures, and the recommendation of Erichsen, the UCH surgeon from 1874, in his book, *Hospitalism* springs to mind: 'To close each surgical ward once a year for a month, during which time it should be whitewashed and cleaned'.

HIV/Tuberculosis

The pandemic of HIV, which commenced in the 1980s, has had major repercussions on HCAI, because it reinforced the need for universal precautions, ensured a population of highly susceptible patients and fed the resurgence of tuberculosis (TB). WHO estimates that there were 9.2 million new cases of TB in 2006, with 1.6 million deaths, including 200,000 in AIDS patients. These figures appear to have stabilised, although the problems of Multiple and of Extended Drug-resistant TB remain. While much of this spread is outside hospitals, outbreaks of resistant tuberculosis in hospital staff have created concerns. Competition for isolation facilities meant that MRSA patients could no longer be isolated in some US hospitals, and the MRSA bacteraemia rates rose.

MRSA

Dimethoxybenzpenicillin was introduced by Beecham, in 1960. The methoxy groups effectively blocked the action of staphylococcal β -lactamase, and provided an anti-staphylococcal drug that Beecham's called 'methicillin' (Figure 3). It had relatively low activity and acid instability – so large intravenous doses were required – and was soon replaced by the isoxazolyl penicillins: oxacillin, cloxacillin and flucloxacillin, which were more potent and could be given orally. Methicillin remained on sale purely for laboratory susceptibility tests, but eventually production ceased. The recent change of name to 'meticillin' comes as a surprise (and has not been universally adopted by all journals e.g. *Journal of Antimicrobial Chemotherapy*). The issue of *The Lancet*, announcing the arrival of meticillin, contained a paper from St George's Hospital, London, on its successful use for 'fogging' the nursery air to eliminate staphylococcal infection in neonates¹⁸. It was also sprayed in the surgical wards in Melbourne in the 1960s. Was it a coincidence that the rise in meticillin-resistant staphylococci was first noted in Australia? Some of the first reported strains of MRSA were isolated from patients who had not been treated with meticillin, and indeed some came from Poland, where the agent was not on sale. Today, MRSA vies with *C. difficile* as the number one problem in the UK. Endless papers are published (Figure 4) and controversies abound. From a historical perspective, the recent Wellcome publication: *Superbugs and Superdrugs* provides an excellent summary¹⁹.

Beta-lactamases

Soon after the introduction of carbenicillin, the first anti-pseudomonas penicillin, I isolated a highly resistant strain of *Pseudomonas aeruginosa* from a patient who had not received the drug. It produced a novel β -lactamase²⁰, now called PSE-4, originally thought to be coded by a chromosomal gene rather than the transferable plasmid, TEM, already causing problems in other strains of pseudomonas and enterobacteria. Since then, many new β -lactam antibiotics and β -lactamase inhibitors have been marketed, but the

bacteria have kept up – with the introduction of the 'Extended spectrum β -lactamase producing enterobacteria', which provide an increasing problem of both endemic and epidemic proportions in both hospital and community^{21, 22}.

Vancomycin-resistance

The original vancomycin powder from the 1950s had only a 50% antimicrobial activity and was so brown it was called 'Mississippi mud'. However, it was a life saver and was licensed by the FDA within two years of discovery. The arrival of methicillin meant it took a back seat for many years, although it had been purified and remained a last-ditch option. By the 1990s, however, treatment of MRSA and *C. difficile* meant that vancomycin topped the list for antibiotic expenditure in our university hospital. The related drug, avoparcin, was much used in European animal husbandry. The result has been the emergence of vancomycin-resistant enterococci (VRE). In general, these have caused low-grade infections and are widespread in the community²³. A different situation is found in the USA, where enterococci are the third commonest cause of nosocomial infections, which are often serious, and bacteraemias have a mortality of 42–68%. Resistance there is mainly related to hospital use of vancomycin, particularly in patients undergoing peritoneal or haemo-dialysis. A gene, *esp*, seems to be associated with virulence in some studies.

As vancomycin has been a mainstay of therapy for MRSA, the appearance of vancomycin-resistant staphylococci is to be dreaded. In 1992, the *vanA* resistance gene was transferred from VRE to staphylococci. Between 2002 and 2006, only seven isolates of vancomycin-resistant staphylococci were reported in the USA²⁴. All were in patients with prior enterococcal and MRSA infections. No person to person transmission occurred, but the final outcome remains to be seen.

SARS

The *Severe Acute Respiratory Syndrome* emerged from Hong Kong in 2002-3. A coronavirus emerging from animals, it showed (as is often the case) significant pathogenicity for its new host, and like the noroviruses, a great propensity for aerosol spread, including up and down drains in tall buildings. WHO has records of 8,096 infections,

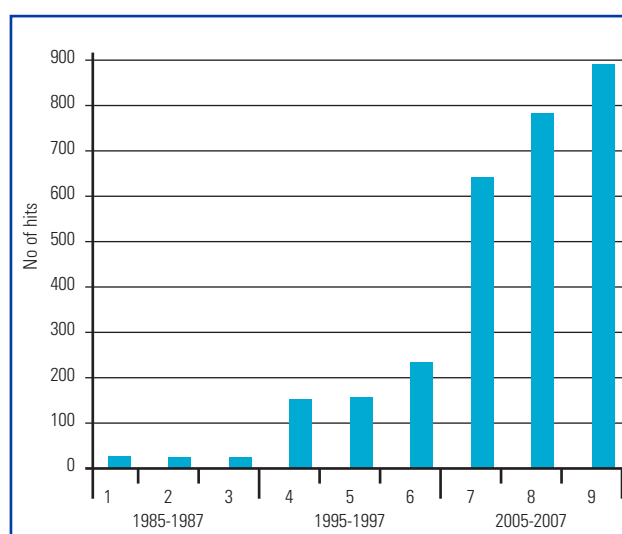


Figure 4. Result of entering MRSA into Pub Med: 1-3 = 1985-7; 4-6 = 1995-7; 7-9 = 2005-7

many related to hospitals, and 20% were in healthcare workers. Extremely strict infection control measures seem to have halted this epidemic, and it remains to be seen whether it will reappear.

Conclusion

This has been a personal view of the last 30 years and is in no way intended to be comprehensive. As a retired infection control professional, I am still somewhat schizophrenic – pleased that I was right in trying to warn others of the problem – but sad that it has taken the current situation to show that I was correct.

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The emergence of epidemic *Clostridium difficile* clonal lineages

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Abstract

The continued rise of *Clostridium difficile* infection worldwide has been accompanied by the rapid emergence and transcontinental spread of a highly virulent clone, designated PCR-ribotype 027. The 027 strains have risen from obscurity to become the most frequently isolated *C. difficile* strain type. Concomitantly, patients infected with this strain often experience more severe diarrhoea, more recurrent episodes and higher mortality. The rapid pandemic spread of the 027 clone provides a valuable opportunity to study the very recent emergence of a bacterial pathogen – a rare chance to monitor the evolution of bacterial virulence.

Clostridium difficile - the organism and the disease

Clostridium difficile is a Gram-positive, spore-forming, anaerobic bacillus that often resides in the gut of mammals and is the causative agent of *C. difficile* infection (CDI)^{1,2}. The hospital environment and patients undergoing antibiotic treatment provide a discrete ecosystem where *C. difficile* persists and where virulent clones thrive. Consequently, *C. difficile* is the most frequent cause of nosocomial diarrhoea worldwide^{1,2}. CDI includes a wide range of symptoms, from mild diarrhoea to life threatening pseudo-membranous colitis (PMC), and characteristically occurs after treatment with broad-spectrum antibiotics. Antibiotic therapies can disrupt the normal gut microflora and reduce the resistance of the gut to colonisation by pathogens. Following disruption of the protective gut microbiota, it is proposed that ingested or indigenous *C. difficile* spores germinate, colonise the gastrointestinal tract and produce toxins. Why *C. difficile* flourishes under these conditions is unknown. Colonisation and toxin production result in an acute inflammatory response, and severe damage to the intestinal epithelium ensues. Treatment of CDI is far from straightforward because broad-spectrum antibiotics exacerbate the disease, leaving only a few options, such as the administration of either metronidazole or vancomycin². The difficulty of averting CDI in hospitals is exacerbated by the necessity for patients to receive antibiotics for the complications for which they were hospitalised.

CDI is a major health care problem that can result in patient isolation, ward closures and, in extreme cases, hospital closure. In the UK, CDI is a notifiable disease with more than 55,000 hospital cases reported in the elderly alone in 2006, and 6,500 deaths attributable to CDI (UK Office for National Statistics). This shocking statistic effectively means that a person dies of CDI every hour in UK hospitals. The population at risk for CDI includes not only patients on antimicrobial and other therapies that can alter the balance of the gut microbiota (e.g. antacid/proton pump inhibitors and non-steroidal

anti-inflammatory drugs), but also the immunocompromised. Another feature of CDI is the high relapse rate (up to 55% of patients) due to re-infection or reactivation of infection^{1,2}. A further complication that will impact on the hospital epidemiology of *C. difficile* is the emergence of community-acquired CDI² that will provide a source of patients and visitors that can contribute to *C. difficile* infection and the spread of CDI. Given the continued use of broad-spectrum antibiotics, other drugs and the rising numbers of immunocompromised and elderly patients, the problems associated with CDI are unlikely to recede.

Clostridium difficile is known to produce several virulence factors, including two related UDP glycosylating toxins, named toxin A and toxin B. Additionally, 6% of *C. difficile* isolates produce an actin-specific ADP-ribosylating toxin³. In recent years, increasing numbers of pathogenic strains that cause CDI have been isolated, which lack *C. difficile* toxin (CDT) and have truncated versions of toxin A and/or B^{4,5}. Clearly, the production of these toxins alone cannot fully explain *C. difficile* pathogenesis. Other, as yet unidentified factors must be important in the transmissibility, survival and virulence of *C. difficile*, and in particular the emergence of epidemic clones.

***Clostridium difficile* – a continually evolving pathogen – and the emergence of 027 strains**

Alarming, in the past five years, a new group of highly virulent *C. difficile* strains has emerged to cause outbreaks of increased disease severity in North America and Europe. Several studies have revealed that patients infected with these PCR-ribotype 027 strains have more severe diarrhoea, higher mortality and more recurrences^{6,7}. Prior to 2003, only a handful of these strains were isolated in the UK, whereas currently most UK isolates are now PCR-ribotype 027. This is also mirrored in Canada, where 027 strains were undetected in 2000, but reached 75.2% of all PCR-ribotyped strains by 2003⁸. Indeed, the rise of 027 in Europe has warranted the formation of a European-wide surveillance group⁹. At the last count, 027 strains had been reported in 16 European countries⁹.

The earliest retrospectively recorded 027 isolate was CD196, in 1985, which is a non-epidemic strain isolated from a patient with CDI in a Paris hospital³. Probably the next recorded 027 isolate was a non-epidemic strain designated BI-1, which was from a patient with CDI in a Minneapolis hospital in 1988¹⁰. Since 1988, further BI designated strains (all PCR-ribotype 027) have been isolated and characterised by Gerding and colleagues, representing a useful time-line of the evolution of 027 strains¹⁰. They have recently shown that later epidemic strains, such as BI-6, are particularly virulent in the hamster model of CDI, consistently causing death within 48 hours of administration¹⁰.

The sharp worldwide increase in reported incidence of CDI in the last decade has been the driving force for the development of a variety of molecular typing approaches to study the epidemiology of *C. difficile*. These include pulse-field gel electrophoresis (pulsotypes), restriction endonuclease analysis, toxinotyping (based on sequence data of toxins A and B), multi-locus sequence typing (MLST) and PCR-ribotyping¹¹. The most widely accepted method is PCR-ribotyping, where more than 100 easily distinguishable groups have been identified, based on mutations in the relatively stable rRNA sequence¹². This method has been used to define the so called 027 strain lineage, which was the 27th strain type to be identified with a unique rRNA profile (note, 0 in 027 is a zero and not a letter O, and

should not be confused with O-antigen typing systems). Generally, these typing methods are congruent, as exemplified by the fact that 027 is invariably NAP1 (North American pulsotype 1), BI (by restriction endonuclease analysis), toxinotype III and all have the same MLST sequence type. More recently, multi-locus variable number of tandem repeat analysis (MVLA) has been used to sub-type clones of 027, but this has limited discriminatory potential¹³.

What is so unique about the 027 strains?

The reason(s) why the 027 strains have burst onto the scene remain to be fully determined, and it is likely to be due to multiple factors that have evolved to increase the survival, transmissibility and virulence of the clonal lineage. The pathogenicity locus (PaLoc) of *C. difficile* includes the toxin A and B genes, along with their regulatory components, including *tcdR* (a sigma factor), and *tcdC* (a negative regulator which destabilises the TcdR-holoenzyme to prevent transcription of the PaLoc)¹⁴. For some time, toxin production has been the main focus of study when addressing virulence of *C. difficile*. It has been reported that some 027 strains can produce more toxin *in vitro*¹⁵, which was initially attributed to deletions in the negative regulator *tcdC*. However, the 18 base pair (bp) in-frame deletion was found to have no effect on toxin production¹⁶. Two further deletions have been identified within *tcdC*, a 39 and a single bp deletion. The single bp deletion results in the formation of a stop codon and truncation of the protein, potentially leading to increased toxin production. However, various deletions have been identified in *tcdC* in non-epidemic PCR-ribotypes as well¹⁶, suggesting the increased virulence cannot solely be attributed to these deletions. More recently, sequence analysis of the toxin B gene has shown the 3' end, which encodes the binding domain, to be variable compared to most other *C. difficile* strains. This suggests the possibility that toxin B from 027 strains may have a more distinct binding capacity than the less virulent counterparts¹⁷. All strains encountered to date appear to have an intact actin-specific ADP-ribosylating toxin which may play a contributory role in virulence. Additionally, the use of fluoroquinolone antibiotics in hospitals has been shown to be a significant risk factor for the emergence of 027 strains and the appearance of more severe CDI¹⁸.

Apart from classical virulence determinants, such as toxin production and antibiotic resistance, other factors, such as increased colonisation of the gut mediated via enhanced germination of spores, increased resistance to bile salts or increased transmissibility manifested through sporulation, might explain the emergence of epidemic 027 strains. A recent report comparing three "historical", Swedish, non-epidemic 027 strains with an epidemic strain concluded that the epidemic strain sporulated more readily than its three non-epidemic counterparts¹⁹. Another report has found that epidemic 027 strains bind more readily to human intestinal epithelial cells than other strains²⁰.

However, other reports suggest that infection with 027 strains does not cause more severe disease²¹. It could be that the 027 lineage is in the process of "burning itself out" and is evolving to have a less dramatic effect on its human host. Methods that can rapidly distinguish 027 strains are desperately required to pinpoint host and environmental factors that might influence the development of CDI. The lack of understanding of the population biology of *C. difficile* means that we do not know where epidemic clones, such as 027, have emerged from and how they are continuing to evolve.

Beyond 027 and other epidemic clones

Although widespread, 027 strains do not account for all cases of *C. difficile* and other PCR-ribotype clones that are emerging. The rapid spread of the PCR-ribotype 027 strain has distracted attention from other virulent strains of *C. difficile*. For example, toxin A-B⁺ strains have emerged in the past decade in parts of Asia and Europe^{4,5}. The A-B⁺ strains, which are invariably PCR-ribotype 017, are another example of an epidemic *C. difficile* clonal lineage. As toxin A assays are sometimes used to identify *C. difficile* in stool samples, toxin A-B⁺ strains are likely to be under-represented in infection statistics. A report from Korea showed that 50.9% of 106 isolates, most of which caused severe PMC in patients, were A-B⁺. A recent report from Ireland showed that 95% of 85 isolates were A-B⁺ (ribotype 017) and were invariably fluoroquinolone-resistant⁵. Other recently emerging strains include PCR-ribotypes 053, 078 and 106²², of which 078 is particularly interesting, as it has been found in both animals and humans, forming the major PCR-ribotype in both calves and pigs²². A foodborne link to the acquisition and transmission of *C. difficile* strains seems likely. Type 078 isolates contained genes for toxin A, toxin B, binary toxin, and the 39 bp deletion in toxin regulator gene (*tcdC*), as well as a point mutation at position 184, resulting in a stop codon. In Holland, from February 2005 through February 2008, the incidence of type 078 among isolates obtained from 1,687 patients increased from 3% to 13%. Compared with patients infected with type 027, patients infected with 078 strains were younger, and more frequently had community-associated disease²³.

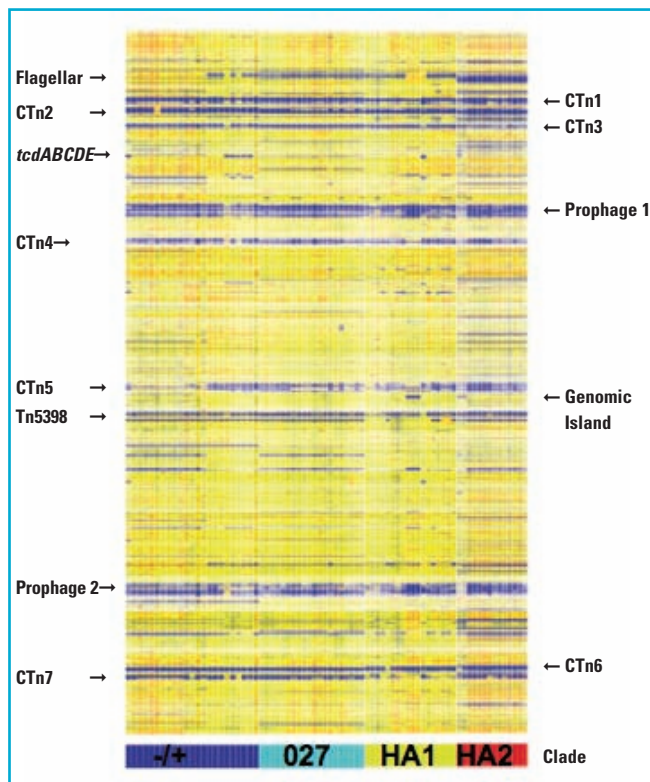


Figure 1. Whole-genome comparison of 75 *C. difficile* strains in relation to the sequenced strain 630 using microarray analysis. A horizontal line represents the presence (yellow lines) or absence/high divergence (blue lines) of each gene from CD0001 (top) to CD3679 (bottom). Selected variable genomic islands (blue bars) are highlighted and are indicated at the sides. Strains are grouped into four clades and are shown as A-B⁺, 027, human and animal 1 (HA1) and human and animal 2 (HA2).

Comparative genomics of *C. difficile*

Given the medical and economic importance of CDI, and the difficulties in studying the genetics of the organism, a representative *C. difficile* strain was fully sequenced and published in 2006²⁴. The strain chosen, 630 (PCR-ribotype 012), was a multi-drug-resistant isolate from a patient with pseudomembranous colitis at a hospital in Zurich. The full sequence revealed a 4.29 Mb chromosome with 3,679 predicted coding sequences. A large proportion (11%) of the genome consists of mobile genetic elements which are putatively responsible for the acquisition by *C. difficile* of an extensive array of genes involved in antimicrobial resistance, virulence, host interaction and the production of surface structures²⁴.

However, strain 630 is not representative of *C. difficile* species. In order to compare the genome content of different strains, hybridisation of genomic DNA from sample strains to a microarray that includes all predicted coding sequences from the sequenced strain can be undertaken. In order to answer specific questions relating to gene differences and phylogeny, comparative phylogenomics (whole genome comparisons of bacteria using DNA microarrays combined with Bayesian-based algorithms to model the phylogeny) has been developed and applied to *C. difficile*^{25,26}. Comparative phylogenomic studies revealed four distinct clades from 75 strains sampled (Figure 1). These included a 027 specific clade, an A-B⁺ clade and two further clades that contained a mixture of animal and human isolates (HA1 and HA2)²⁶. The 027 and A-B⁺ strains were from diverse geographical origins, confirming the transcontinental spread of both these clonal lineages. Further data analysis revealed micro-heterogeneity and micro-evolution among the 027 strains, with CD196 and BI as progenitor strains²⁶.

No doubt these sequencing and DNA microarray studies will be the first of many used to discover the genome content and diversity of the species. For example, strain QCD32q58, that ravaged hospitals in the Quebec area, Canada, and strain R20291, that caused a severe CDI outbreak at the Stoke Mandeville hospital in the UK, are two examples of 027 strains currently being sequenced and analysed. Comparison of these genomes to the so called "historic non-epidemic" 027 strains may be particularly revealing in terms of pinpointing the subtle genetic differences that have enabled the modern 027 counterparts to be so problematic. Initial sequence data shows several regions of genetic difference between the 027 strains and between other PCR-ribotypes, confirming that *C. difficile* is continuing to evolve.

Conclusions and future perspective

In evolutionary terms, *C. difficile* is clearly a pathogen on the move. Even the newly emerged clonal lineage, 027, is evolving with the recent emergence of fluoroquinolone and clindamycin-resistance^{8,20,27}. MLST is generally accepted as the gold standard for studying the population structure and evolution of bacterial pathogens, but is unsuitable for distinguishing strains from clonal lineages such as the 027. Some success in distinguishing 027 strains has been possible through using MLVA, but this has limited discriminatory points for effective analysis. The complete genome sequence of any bacterium is the definitive standard to fully distinguish between strains, and with the advent of next generation sequencing and reduced sequencing costs, this may become a reality. Alternatively, next generation sequencing, which has been developed extensively for determining Single Nucleotide Polymorphisms (SNPs) in human

Unanswered questions relating to emergent clones and CDI

- Is rapid sporulation and/or germination important in the survival and spread of epidemic 027 strains?
- Do 027 strains have different toxin B binding properties that alter their receptor specificity and avidity?
- Are there common determinants shared by 027, A⁺B⁺ and 078 strains that might explain their epidemic nature in terms of survival, transmissibility and virulence?
- What is the evolutionary origin of 027, A⁺B⁺ and 078 strains and will they be superseded by other evolving clonal lineages?
- Could 027, A⁺B⁺ and 078 strains evolve to become more virulent?

genetic studies, may have wider applications in bacterial genetic studies. Such SNP analysis has recently been applied to provide the detailed epidemiological distinction and micro-evolution of the clonal bacterial pathogen *Salmonella* Typhi²⁸. This can be scaled up for 96-well microtitre plate analysis using Illumina Golden Gateway SNP CHIP technology. This could be usefully applied to study fine genetic differences between 027, A⁺B⁺ and 078 strains, to define their evolutionary origin and to monitor how they will evolve in the future. These concerted efforts will provide the basis for rational strategies to reduce the burden of CDI and more broadly for research in the pathogenesis and emerging infectious diseases. Despite their diverse geographical origins, from Asia to the USA, the A⁺B⁺ and 027 strains are clonal according to MLST and other analyses. The rapid transcontinental emergence of two hypervirulent *C. difficile* lineages provides a unique opportunity to study the very recent emergence of a pathogen. At the same time, there is a public health imperative to continue to monitor for the emergence of further epidemic *C. difficile* clones.

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Infection control in developing countries

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Introduction

Developing countries are normally defined as those lacking the level of nationwide industrialisation, infrastructure and technological advances normally found in Western Europe and North America. Many of the countries in Africa, Asia, Central & South America, Oceania and the Middle East fall in this 'developing' category and often face additional challenges in terms of lower levels of literacy and standards of living. Nevertheless, within this broad group, there are various sub-categories, each having different characteristics as well as economic strengths. Indeed, some are relatively wealthy, oil exporting nations or newly industrialising world economies; a considerable number are middle income countries. At the end of the development scale lie around fifty very poor nations with predominantly agricultural economies, which tend to be heavily dependent on external aid.

From a medical perspective, many developing countries are often characterised by significant health and hygiene issues. Indeed, it has been estimated that more than one billion inhabitants in these countries do not have access to safe water and even fewer to basic sanitation¹. Around 1.5 million children in the developing world die per year; diarrhoea is responsible for more than 80% of these deaths². One of the reasons for this state of affairs is the low expenditure and budgetary allocation within the poorer countries of the world towards health. Indeed, the proportion of annual expenditure for health related initiatives in many developing countries is often less than 5% of Gross Domestic Product (GDP), sometimes less than 0.1%³.

Healthcare-associated infections in developing countries

Unlike more affluent countries, infectious diseases continue to pose a heavy burden of morbidity as well as mortality in developing nations⁴. Among the more important disease entities are a wide range of respiratory diseases, including tuberculosis, various gastrointestinal infections, AIDS and HIV, plus a spate of parasitic infestations of which malaria is the most significant. However, this situation is not limited to ambulatory settings and is equally relevant within healthcare institutions. Deficient infrastructures, rudimentary equipment and a poor quality of care contribute towards incidences of nosocomial infections which have been estimated to be between 2-6 times higher than those in developed nations⁵. In many instances, such figures are often 'guesstimates' because surveillance systems are often either non-existent or else unreliable. However, the limited studies on prevalence of healthcare-associated infections in some developing countries in the world suggest that up to 40% of these are probably preventable⁵. This situation appears to be particularly severe within intensive care settings, where up to 60 to 90 infections per 1000 care-days have been reported; mortality rates

from more severe infections, such as blood stream and lower respiratory infections, approach 25% in adults and more than 50% in neonates⁶.

The challenges of infection in healthcare facilities within developing nations is also of a wider spectrum than that normally found in equivalent hospitals in the western world. Numerous publications have highlighted the frequency by which community infections, such as cholera, measles and enteric pathogens, also spread nosocomially within such institutions^{7,8,9}. In many instances, outbreaks are traceable to an index case who would have been inappropriately managed in a background of overcrowding and limited hospital hygiene. Similar cases of transmission have also been reported in the case of respiratory infections. Tuberculosis transmission in healthcare facilities is a major occurrence in many African countries as well as parts of Asia and Latin America¹⁰. In many instances, this disease is strongly related to the rise of HIV within these same geographical regions and is not uncommonly complicated by increasing prevalence of multi-drug-resistant mycobacteria. Blood-borne infections are not restricted to HIV, alone. Hepatitis B remains a major nosocomial pathogen in many hospitals within the developing world¹¹. More dramatic and life threatening have been outbreaks of viral haemorrhagic fevers in institutions within several countries in the African continent¹². Hospitals are also liable to healthcare-associated infection caused by more conventional pathogens which, just like in their western counterparts, can carry the additional burden of antimicrobial resistance⁴. Unfortunately, data on the prevalence of resistance in nosocomial pathogens is poorly documented in the developing world. However, recent publications suggest that this may be even more common than in developed countries. Recent publications from the Mediterranean region have highlighted proportions of meticillin resistant *Staphylococcus aureus* to exceed 50% in several countries in the Middle East, with resistance to third generation cephalosporins in *E. coli* exceeding 70% in some participating hospitals¹³. There may be diverse and often complex backgrounds to this epidemiological situation.

Factors facilitating transmission and management of nosocomial infections

The infrastructure of healthcare facilities in some of the poorer nations often lacks basic requirements for the prevention of transmission of infectious diseases. Inadequate or unsafe water supply, together with lack of resources or equipment for effective environmental cleaning, is often compounded by significant overcrowding due to inadequate beds to cope with demand¹⁴. There is often lack of strategic direction as well as effective planning for healthcare delivery at both national as well as local levels. A functional sterilisation department is by no means standard in every hospital, even in the larger urban institutions. Other areas of concern include poor awareness or knowledge about communicable disease transmission among healthcare workers and lack of commitment within senior management¹⁵. This is particularly relevant in developing countries, where nurses, doctors and patients are often unaware of the importance of infection control and its relevance to safe healthcare¹⁶. Medical practitioners may have a tendency to be



Figure 1. Hospital conditions in developing country

heavily committed towards individual patients and disinclined to think of them in groups, a concept which is the antithesis of basic infection prevention and control¹⁷. They are often unaware of risks of nosocomial infections, attributing such possible developments to be natural or inevitable¹⁸. On the other hand, nurses have more intimate contact with patients and are trained to take care of patients in groups. Although this increases the potential to serve as sources of cross-transmission, nurses are likely to be more positive towards infection control policies. However, this is hindered by the comparatively low status offered to nurses in the developing world and also complicated by a gender bias in environments where emancipation of women has been slow. Attitudes of senior medical staff may further compound the problem through personality clashes, resistance to change or improvement as well as reluctance to working in tandem with other health professionals. Non-existent litigation further accentuates lack of accountability at various levels. Furthermore, many patients have limited expectations, already regarding themselves fortunate to have any sort of institutional care and, as a result, accepting a significant degree of morbidity as part of their hospital stay. It must be emphasised that even in the poorer countries, this set of circumstances is by no means universal. It is not uncommon that, even where most of the hospitals in a country lack all these basic requirements, individual institutions (often either private or NGO managed) would be in a position to offer healthcare as well as infection control standards of the highest quality. However, it would only be a small minority of patients, often coming from a more affluent background, that would be able to benefit from them.

The risks of infection in hospitals within the developing world are not only restricted to the patients who receive care within them. Occupational health is an equally low priority in many of these facilities and, as a result, it is not uncommon for healthcare workers to also be exposed to, and become infected by, pathogens causing healthcare-associated infections, including viral hepatitis, HIV and tuberculosis.

In such limited resource environments, and in situations where medical practice is biased towards intervention rather than prevention, it is not surprising that basic infection control programmes are often lacking, particularly in smaller hospitals in rural areas¹⁹. Even within larger urban facilities, infection control teams, composed of both an infection control nurse as well as a doctor, who have been trained and have managerial backup, are very

much in the minority. They are often restricted to academic institutions or heavily funded government or private tertiary care units. Even where present, these teams tend to encounter numerous logistical obstacles, including lack of administrative, clerical and IT support. Infection control output therefore tends to be significantly variable; policies and procedures are either absent or lack consultation, evidence base or suitable addressing of local needs.

Healthcare professionals also face significant challenges in the diagnosis and treatment of infectious disease⁴. Diagnostic facilities are often lacking. Laboratories may be absent or limited as a result of inadequate resources of both a material as well as human resource nature. Trained laboratory scientists are very much in the minority and the implementation of quality control programs to ensure validity of the laboratory's output is not viewed as crucial. This situation is worsened by possible lack of confidence in the laboratory from clinicians who would prefer to undertake treatment, based only on clinical judgement or recommendations from other countries rather than local epidemiology. One reason for this is the lack of feedback of local resistance data²⁰. This risks inappropriate treatment which would not properly cover local resistance prevalence patterns. Another major factor hindering the treatment of infectious disease is the presence of poor quality (or even counterfeit) antimicrobials, with little or no active ingredient within the formulation²¹.

Addressing the challenge

It is therefore clear that, in order to improve the effectiveness of infection control in many developing countries, a multifactorial set of initiatives needs to be undertaken that are both feasible as well as achievable in this background of economical and social deficits¹⁵. It is essential that infection control teams increase their presence within hospitals in these regions. These key personnel must be provided with the necessary training as well as administrative support and facilities in order to deliver the required services. Such teams would be able to identify the major challenges and assess relevant risks through tailored surveillance programmes. Surveillance constitutes a challenge in such environments, since it is often time consuming and resource dependent²². In addition, it requires a reasonable level of laboratory support. Nevertheless, it is possible, using simplified definitions of healthcare associated infections, as suggested by the World Health Organisation, to achieve a surveillance programme even with very limited resources²³. Such initiatives need to concentrate on the more serious infections and document their impact in the respective facility. Trained infection control personnel would also be appropriate drivers to eliminate wasteful practices which siphon resources away from truly effective practices. Dogmas include routine use of disinfectants for environmental cleaning, use of unnecessary personal protective equipment such as overshoes, excessive waste management procedures which treat all waste generated in the hospital as infectious. Infection control teams will be able to spearhead cost-effective interventions based on training of healthcare workers to comply with relevant infection control measures related to standard precautions, isolation together with occupational health and safety. It is possible to achieve significant reduction in the prevalence of healthcare-associated infections through low cost measures; interventions aimed at preventing cross transmission of infection are particularly effective.

There is no doubt that one of the most cost effective interventions in limited resource environments is improved compliance with hand hygiene. Indeed, the World Health Organisation has designated improvement of hand hygiene within healthcare facilities worldwide as a priority and chose this topic for its first Global Patient Safety Challenge under the banner, 'Clean Care is Safer Care'⁶. A comprehensive set of tools has been tested worldwide in pilot hospitals, the majority of which were in developing countries. The emphasis of this initiative focuses on the availability and utilisation of alcohol hand rub for patient contact situations where hands are physically clean. This is made possible through local manufacture of inexpensive, good quality products according to a validated formula. A multimodal strategy requires these alcohol hand rub containers to be available at point of care and for the staff of the hospital to receive adequate training and education in their use. Hand hygiene practices are monitored and feedback on performance regularly provided to the users. Reminders in the workplace sensitise awareness and belief among healthcare workers in general.

It is equally clear that in order to achieve effective improvement in infection prevention and control, healthcare facilities in many developing countries need support and assistance. Experience suggests that one of the most effective and rapid methods for improving infection prevention and control within a country or region is to form an organisation in which interested members interact regularly to review practices and share information²¹. This maximises group skills and reduces the sometimes steep learning curve, since formal preparation in this field often lags behind the need. In addition, the organisation provides credibility for changing practices and can develop practice guidelines and official regulations in the field.

Conclusion

Infection prevention and control in healthcare facilities within the developing world continues to offer numerous challenges as a result of reduced resources related to socio-economics, infrastructure and human resources. However, it is possible to achieve substantial progress even within such challenging circumstances through a programme led by trained and empowered infection control professionals. Such initiatives need to concentrate on low cost, high impact interventions and emphasise training, backed by interaction and networking with colleagues and societies within the country itself and beyond.

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Oxoid supports the International Federation of Infection Control



Oxoid is proud to support the work of the International Federation of Infection Control (IFIC). This charity assists the establishment of infection control infrastructures and knowledge-base, particularly within low resource countries. It also provides a communication network of support for its member societies and individuals, as well as promotes high quality educational opportunities. These include publications, such as the *International Journal of Infection Control* (which is freely accessible at www.ijic.info) together with teaching materials, training programs, conferences and workshops at no or low cost. The IFIC web site (www.thelific.org) provides a forum where infection control professionals can obtain information, download resources and network with IFIC members and corporate sponsors.

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Biofilms in Water Systems – origins and treatment

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Introduction

Micro-organisms can attach at air-liquid, liquid-liquid and solid-liquid interfaces in aqueous environments¹, maturing into heterogeneous but highly ordered communities of biofilms that exhibit stacks or fronds of aggregated microcolonies surrounded by a primitive vascular system of water channels to conduct nutrients and products in a mosaic structure (Figure 1)². These biofilms are frequently predated at the bottom and higher up by eukaryotic grazers, such as amoebae and ciliates, as a food source (Figure 2)³. Consequently, biofilms are ubiquitous in both the natural and built environments, be it on the dentition in animals, on phylloplanes, on rocks and sediments or as algal blooms in lakes and marine environments, or water holding tanks and pipe supplies. Many of these environments might be considered physically and chemically hostile due to exhibiting a wide range of temperatures, oxygen concentration, pH, solar irradiation intensity and desiccation. Such environments may present nutrient deprivation or exposure to toxic molecules, such as metal ions, industrial organic pollutants and antimicrobial agents. For example, biofilms continue to thrive near deep sea smoker vents, where temperatures can exceed 140°C and toxic concentrations of hydrogen sulphide are expelled⁴. As such, biofilm formation can be considered as an important adaptive response to extreme environments, and we should not be surprised that biofilm communities do well in hot water calorifiers and hot water piped supplies in hospitals and other institutional buildings where water temperatures rarely exceed 60°C⁵.

Environmental biofilms usually comprise complex consortia of micro-organisms and can contain a high species diversity of aerobic and anaerobic bacteria, archae, viruses, fungi, amoebae, protozoa and nematodes, unlike the more commonly encountered monospecies infections, *in vivo*. Of concern to modern society is the threat that micro-organisms pathogenic to animals and/or man can survive and even flourish in such ecosystems. This is related to the sharp rise in the world population, itself, causing bacterial, protozoal and viral

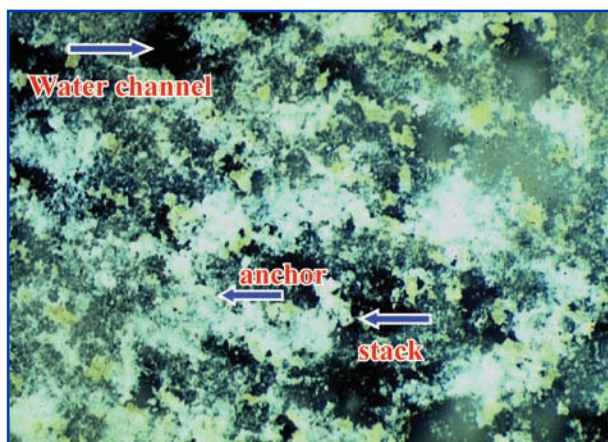


Figure 1. Heterogeneous biofilm showing stacks of microcolonies rising from the substratum and water channels

pathogen dissemination and amplification through person-person infective transmission, and the requirement of intensive agricultural practices to provide sufficient food exacerbating propagation and transmission of zoonotic pathogens. The need to dispose of increasing quantities of waste and wastewater from homes, industries and intensive animal rearing facilities has strained the resources of farmers and many sewage treatment utilities worldwide. Poor sewage and animal waste disposal, and intensive stocking of animals in fields near abstraction points built for supplying potable water, have increased the transmission of microbial pathogens back into the natural environment and to water supplying agricultural, industrial and domestic premises. Consequently, the normal water cycle has been perturbed by the introduction of toxic organic compounds and heavy metals from disposal of waste to land and leaching into watercourses. Eutrophication of rivers and lakes has occurred due to ingress of nitrogen and phosphorus resulting from excessive use of detergents, promoting biofilm formation and production of toxic by-products from cyanobacterial biofilm blooms, such as microcystin hepatotoxins and anatoxin neurotoxins. According to WHO statistics, developing countries no longer have a monopoly of waterborne diseases. Some 20 million people die each year of waterborne diseases, over one million of them in Europe⁶.

Potable water supplies to the built environment are not meant to be sterile

Against this challenging back drop of water pollution, water companies should be congratulated for the excellent job they do of abstracting water from non-pristine sources and treating it to high standards of potability. Their primary concern is to make potable water wholesome but not necessarily sterile. Filtration, clarification, ozonation and chlorination eliminate many waterborne pathogens at the treatment works, although common heterotrophic and autotrophic species, such as those belonging to *Pseudomonas*, *Sphingomonas* and *Nitrosomonas* genera, can survive⁷. In fact, the majority of microbial species present in the water and biofilms cannot be cultured, and we know little about their physiology and potential to cause harm. The treated water is then supplied through the distribution network to homes, institutional buildings and factories, frequently over many kilometres and taking many hours or days to reach the tap. This provides ample opportunity for the

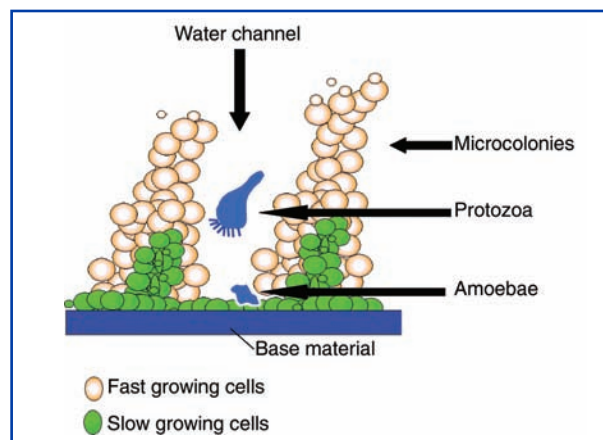


Figure 2. Open architecture structure of biofilm with fronds, water channels and grazing eukaryotic predators

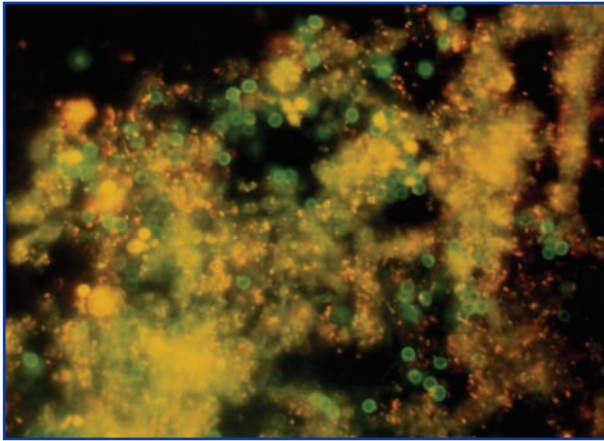


Figure 3. FITC-antibody-labelled *Cryptosporidium parvum* oocysts attached to potable water biofilm stacks stained with propidium iodide. The large green round cells are FITC-antibody labelled live oocysts of *C. parvum*, the large yellow/red cells are dead oocysts with compromised walls that have taken up propidium iodide which does not enter viable oocysts with intact walls.

heterotrophic and autotrophic species to attach to the mains pipe walls and form biofilms, despite the residual chlorination or chloramination which is applied, in many countries, to control microbial regrowth in the network. Biofilm formation can occur not only on the older cast iron pipes, associated with severe biocorrosion and deposit encrustation that may physically protect the biofilms, but also on the modern plastic pipe materials, such as polyethylene and cPVC, used in the distribution supply.

Biofilms act as a safe haven for pathogens

Rarely, filtration problems can occur at the treatment works, permitting ingress of chlorine-resistant species, such as *Cryptosporidium* oocysts, into the water network. These can survive prolonged residual chlorination, and also attach to the heterotrophic biofilms in the pipes (Figure 3)². More frequently, disturbances to the network can occur, including back-siphonage, cross connections, pitting corrosion, line breaks and/or repair of the distribution main, allowing ingress of contaminated water and increasing the chlorine demand, thereby leading to a loss of residual disinfectant further along the network.

Either as a result of survival at the treatment works or ingress into the network, it is clear that opportunistic pathogens such as *Legionella pneumophila*, the aetiological agent of Legionnaires' disease, can be found in the biofilms along with calcified deposits (Figure 4)⁸, as well as *Mycobacterium avium*, another important respiratory pathogen, and *Helicobacter pylori*, the aetiological agent of stomach ulcers and gastric cancer^{9,10}. Indeed, recent research using fluorescence *in situ* hybridisation (FISH) or PCR detection indicates that they may be present in high numbers, although whether they are viable with the potential to cause disease is the subject of much debate^{11,12,13}. For example, laboratory model studies have shown that *L. pneumophila* exposed to chlorine or monochloramine can become viable but non-cultivable (VBNC), thus going undetected using classical culture techniques, and they can be resuscitated when taken up by predatory amoebae existing as grazers in the biofilms^{14,15,16}. The problem presents when the pathogens actively or passively leave the biofilm or sections of the biofilm slough off the pipe walls due to changes in the shear force as the water velocity fluctuates during high or low demand. Some of these pathogens appear more resistant to chlorination than classical faecal pathogens and may be further protected within the sloughed biofilm bolus. Sloughed cells or biofilms then pose a potentially infective bolus

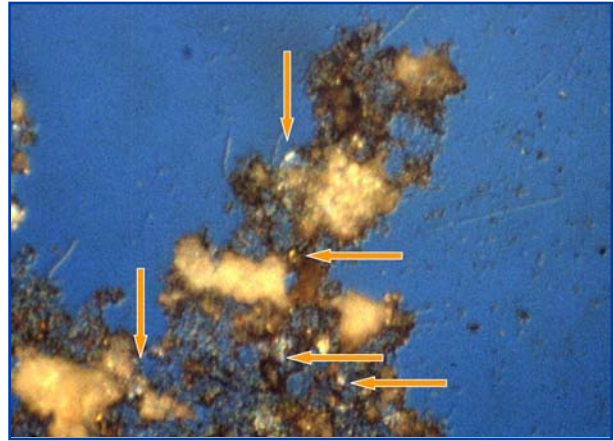


Figure 4. Episcopic differential interference contrast image of immunogold-labelled *L. pneumophila* in an autochthonous biofilm stack developed in hard potable water. The arrows mark microcolonies of cells. Large areas of lighter coloured material are deposited calcium carbonate scale.

that can recolonise further down the network and enter building plumbing systems. Delayed sloughing of biofilm associated pathogens may help explain why some waterborne outbreaks of disease persist for much longer than would be expected if pathogens such as *Cryptosporidium* spp. are transported directly through the mains network.

It is becoming increasingly difficult to rely on detection of even the classical faecal indicator of pollution, *Escherichia coli*, in drinking water supplies for assessment of water quality. In part, this is due to pathogens such as *C. parvum* being much more resistant to chlorine than *E. coli*, and water can not be assumed to have been disinfected if reliance is placed on cultivable *E. coli* detection. On the other hand, there are also limitations of using internationally approved culture and enzyme-based detection techniques for *E. coli* because of VBNC formation. Indeed, Juhna *et al.*¹⁷ were able to show, using an *in situ* cell elongation assay, that viable *E. coli* were still present in the mains distribution supplies of several European countries, at 0.001 to 0.1% of the total bacterial number in the samples, despite the inability to detect them using conventional culture recovery on agar media, or Colilert and Colisure chromogenic enzyme kits. Clearly, new internationally approved methodologies need to be agreed for detection of VBNC faecal indicators or pathogens.

Plumbing systems

From a public health perspective, it is important to keep cold water supplies cold and hot water supplies hot, e.g. national codes of practice recommend that hot water supplies should be maintained above 55°C, to prevent survival and growth of legionellae. Nevertheless, poor building design, with long runs of tortuous pipework and dead legs, can lead to significant decreases in the hot water temperature. This can be exacerbated if these pipes are poorly lagged and close to cold water pipes, hence resulting in heat transfer that lowers the temperature of the hot water supply and increases the temperature of the cold water supply. Under such conditions, biofilm can proliferate, and legionellae and mycobacteria can grow in a sheltered environment, frequently with static water flow and with the low oxygen concentration conducive to their physiology. Indeed, legionellae actually behave as micro-aerophiles, preferring biofilms with lowered redox potential zones, due to heterotrophic respiration lowering the oxygen and raising the carbon dioxide concentrations. *Helicobacter pylori* require elevated carbon dioxide concentrations for growth, but also need a pre-established, multi-species biofilm in order to be found in high numbers¹⁰.

It is rare that direct consumption of these contaminated waters leads to gastro-intestinal disease, perhaps with the exception of *H. pylori*, but the major health problem concerns aerosolisation of fine water droplets from splashing taps, shower heads, spa therapy pools, etc., when droplets of <5µm diameter can be inhaled and penetrate deep into the lung alveoli, avoiding the upper lung defences. In the alveoli, *L. pneumophila* can use its repertoire of defence mechanisms, evolved to defend against amoebal grazing in the environment, to overcome the lung macrophages and actually grow intracellularly. As such, man's transition from hunter-gatherer to living in a modern society, with the luxury of central heating, air conditioning and jacuzzis, has created a truly opportunistic pathogen which by chance, finds itself in the lung. Moreover, this respiratory challenge is exacerbated in patients with compromised lung function, such as middle-aged smokers.

Treatment strategies

Pathogen dissemination frequently occurs due to simultaneous failure of several barriers, such as reduced hot water temperatures, ineffective residual disinfection and poor maintenance of cold water holding tanks, leading to their contamination and subsequent contamination of the plumbing system. Several treatment strategies seem immediately obvious. Considering simple physical procedures: firstly, keep cold water supplies cold and hot water supplies hot by applying effective lagging materials and ensuring elementary engineering practices, such as avoiding cold water pipes being placed above hot water pipes, with the subsequent convection and radiation of heat. Secondly, keep hot water supplies above 55°C to prevent regrowth of heat tolerant pathogens, such as legionellae⁵. Some hot water systems operate closer to 50°C, but laboratory studies demonstrated that *L. pneumophila* will still colonise and persist in biofilms at this temperature, particularly on plastic pipe surfaces¹⁸. Maintaining the hot water temperature is particularly important when water consumption, and therefore flow, decreases at night. In one hospital study, it was shown that the temperature of hot water systems, maintained above 50°C during the day, decreased to 37°C or less during the night, and oxygen content of the water decreased from 6mg/l to almost zero; ideal conditions for the growth of legionellae⁵. Thirdly, in buildings that rely on cold water storage tanks for their water supply, these should be regularly cleaned and disinfected, and sealed to prevent ingress of dust or animal and bird faecal droppings that would increase the organic content of the water and introduce potential pathogens.

Considering chemical intervention, application of residual chlorination appears beneficial to control microbial regrowth, and shock dosing of high chlorine concentrations (50mg/l) into water systems suspected of causing Legionnaires' disease correlates with the abolition of outbreaks. However, we should be mindful that *L. pneumophila* can become VBNC in the presence of even several mg/l chlorine, and a plumbing system deemed free of legionellae may actually still be contaminated¹⁵. It is widely believed that monochloramine is a less reactive and therefore more persistent biocide than chlorine, making it ideal for penetrating biofilms and being a more effective biocide. However, it has again been shown that *L. pneumophila* will become VBNC in the presence of 10mg/l monochloramine¹⁶. Another alternative biocide used in plumbing systems is chlorine dioxide. At shock dose concentrations, this appears extremely effective at causing biofilms to shed from plumbing systems, as witnessed by the large amounts of deposits that can be flushed out. It also appears successful at reducing the numbers of legionellae present in the system¹⁹. However, it is not yet known if legionellae will survive in a VBNC form at shock dose or low dose concentrations.

Similarly, a wide range of biocides, such as bromine derivatives, are advocated to control legionellae in cooling water systems of institutional buildings²⁰. For example, 1-bromo-3-chloro-5,5-dimethylhydantoin was tested for efficacy against planktonic and biofilm bacteria on stainless steel and mild steel in a laboratory model to simulate a cooling tower water system. The consortium of bacteria growing in the chemostat model included legionellae, pseudomonads, methylobacteria and actinomycetes, and *Flavobacterium*, *Alcaligenes* and *Achromobacter* spp. At biocide concentrations of 1 or 2mg/l, bacteria in the planktonic phase were dramatically reduced in the culture, whereas only a 1 log drop in viable bacteria was detected in the biofilm. When the concentration of biocide was increased to 4 or 6mg/l, a 3 log reduction was observed in the number of viable bacteria recovered from the biofilm. This, again, illustrates how bacteria contained within a biofilm are more refractory to attack from antimicrobial agents. Significantly, legionellae appeared more susceptible to the biocide than other members of the microbial consortium and were not recovered from either biofilm or planktonic phases at biocide concentrations of 1mg/l.

In recent years, there has been a move from plumbing systems constructed of copper pipe to alternative pipe materials, such as stainless steel and even polybutylene in hot water systems, and polyethylene and cPVC in cold water systems. From a microbiological perspective, this might be considered unfortunate, since laboratory studies with potable water have indicated that biofilm formation and colonisation by *L. pneumophila* are reduced on copper, but increased on the plastics and, particularly, mild and stainless steels^{18, 21}. In the latter case, this may be due to the fact that *L. pneumophila* has a high requirement for iron²². It is unlikely that legionellae were present as VBNC on the copper because chemical signal analysis of unique fatty acids present in *L. pneumophila* showed that there were actually fewer cells (live or dead) in the biofilm²³. These laboratory studies were supported by a survey of hospital plumbing systems in Germany, where there was a clear correlation between the high prevalence of *L. pneumophila* in iron pipes, intermediate prevalence in plastic pipes and low prevalence in copper pipes²⁴. A study of hot water systems in Bologna also showed a negative correlation between the presence of copper and numbers of *L. pneumophila*²⁵. Similarly, copper surfaces have been shown to kill *H. pylori* and prevent it entering the VBNC coccoid state for environmental survival²⁶.

Copper has been recognised to exhibit antimicrobial properties since Antiquity, healing wounds and keeping water supplies wholesome, and we should not be surprised that it may be the ideal plumbing material. Similarly, silver also enjoys a long standing reputation as providing antimicrobial properties, and there has been interest into whether cold water tanks or the walls of cooling towers could be painted to help maintain water quality. This was investigated in one study, where biofilm colonisation and colonisation by *L. pneumophila* were investigated on surfaces coated with a silver-containing paint and exposed to potable water²⁷. Biofilm and legionellae numbers were indeed reduced, but the silver leached out of the paint after several weeks, and then the biofilms and legionellae numbers returned to their former levels.

Several companies have advocated the use of copper-silver ionisation systems as a way to control biofilms, legionellae and other pathogens in plumbing systems. Recommended levels are between 0.2 and 0.4mg/l for copper and between 0.02 and 0.04mg/l for silver, for

efficacy. However, these recommendations can vary, according to water quality and other parameters of the water system. Not only can these copper-silver concentrations reduce the need to use chlorine-based disinfectants, but also the companies suggest that hot water systems can then be safely operated at lower temperatures, reducing the risk of scalding to patients where washing facilities lack mixer taps, and reducing the energy costs to heat the water. A recent review has concluded that these ionisation systems appear very effective, particularly in soft water supplies²⁸. There has been concern that the silver and copper electrodes can become encrusted in hard water supplies, but the manufacturers use feedback sensors to regulate the ion concentrations released and compensate for water hardness, and also recommend regular cleaning of the electrodes.

Future considerations

It is clear that, if continued vigilance and maintenance of the plumbing and cooling systems are not adhered to, then biofilms and cultivable legionellae will return in high numbers. This may be due to recent ingress of the pathogen into the system or possibly resuscitation of existing VBNC bacteria. Whether such resuscitation occurs, and whether it is due to interaction with biofilm bacterial species or surviving amoebal species providing the appropriate growth environments or nutrients, remains to be investigated. Similarly, it remains to be seen, for the other biofilm-associated pathogens, such as mycobacteria and *H. pylori*, if VBNC is important for their survival, and whether new strategies can be developed to eradicate them.

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