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Vertical Transmission

Hatch Stokes

President ASM

In recent years there has been a growing concern about the increasing shortfall in a trained workforce in pathology and related fields. This culminated last October with the release of the, so called, Legg report, entitled *The Australian Pathology Workforce Crisis* commissioned by the Department of Health and Aging. In the lead up to this report, and following its release, the Pathology Associations Committee (PAC), of which ASM is a member, has been working to revise and update the competency based standards for medical scientists. Longer serving members of the ASM may recall that a set of standards were first developed in 1993 and it is these standards that are currently being revisited in the light of government driven initiatives that may see the development of formal accreditation requirements for medical scientists. These potential changes of course will be highly relevant to many ASM members, particularly those who work in public and private pathology and diagnostic laboratories. As in the past, where relevant, we will be seeking input from all members so as to broadly represent the interests of the Society. Information on this issue can be found on the ASM website where the documents I referred to above can also be found. This information will be updated as it becomes available. I am particularly grateful to John Merlino, Silvano Palladino, Stephen Graves and Carol Ginns who have been working very hard to represent member's interests on this topic. As everyone is aware, we are entering the jubilee year of ASM so it is an exciting one that I am especially looking forward to. We have a number of special events planned and the National meeting will be bigger than ever. In late breaking news I can tell you that we will have a second Nobel Laureate at Perth. Harald zur Hausen from the German Cancer Research Centre will give the Bazeley Oration. This is in addition to the many other outstanding scientists attending, including Bonnie Bassler, Rita Colwell and of course our own Barry Marshall. As well a number of former Presidents from the early years of the Society have also agreed to attend. In addition to providing an opportunity to catch up with long standing colleagues, former Presidents will be actively participating in the meeting. Apart from the celebrations, 2009 will also see a number of service improvements to members. We have recently invested additional funds in the National Office that will improve the ASM web site. Also, you can look forward to improved communication generally via a major database upgrade that will provide for better on line member services including membership renewal. Look out for these changes as the year progresses.

The winners of each of our two new postgraduate travel awards have been determined. The inaugural Burnet/Hayes Travel award winner is Richard Harvey from the University of Adelaide. Richard will be presenting at the SGM meeting in May and will be spending time at a collaborator's lab in the UK. The second Millis/Colwell Travel Award has been won by Stephanie Bell from the University of Western Australia. Stephanie will be engaging in similar activities in the USA. These awards will be offered annually so I would encourage student members to look out for the call for applications in the next round later in the year. Each of these awards is worth up to $6,500 provided by your ASM plus free SGM or USA ASM meeting registration. We will shortly know who the USA and UK winners will be who will be coming to Perth.

Keryn Christiansen will finish her term as Past-President in July. As there was only one nomination for President-Elect there was no need for an election for this position. Therefore, on the recommendation of Council, I am pleased to advise that the incoming President-Elect is Professor John Turnidge from South Australia. Like Keryn, John has a long history of service to the ASM and has previously served on Council. Finally, Ailsa Hocking has announced that she will be stepping down as Chair of the *Microbiology Australia* Editorial Board. Ailsa has been in this position for many years and has been on the Board for even longer. Her efforts in helping the journal grow are greatly appreciated and on behalf of all ASM members I wish her well for the future.

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Water sustainability: future directions

Simon Toze

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Whether you're a believer or a sceptic about global warming and the influence of human activity on the climate, there is little argument about the current impact of drought and changing rainfall patterns on Australia. The Australian community is coming to grips with the fact that we need to be cleverer on how we use water. This has resulted in a significant increase in interest about water sustainability and has increased demands on governments at all levels to improve water usage and efficiency.

There are a number of methods being currently considered or used in different places in Australia to improve water efficiency and to generate 'new' water sources. One of these sources is the recycling of water that would normally have been discarded. Ten years ago there was only sporadic activity by a few utilities and researchers 'testing the water' on how to most effectively recycle water. There were some water recycling success stories, for example Managed Aquifer Recharge in South Australia and Western Australia, and third pipe systems in NSW. Overall, however, there was a wide level of scepticism and reluctance from state regulators along with public ignorance and misperception. Water recycling was considered too difficult, too expensive and not worth the health and political risk.

Recent changes to the Australian climate have had a significant impact on these opinions. In the last 10 years there has been a large increase in water recycling in most Australian states. Surveys have also shown that the Australian community now generally strongly supports the recycling of water, frequently placing it at the top of priorities they believe water utilities should be focusing on (for example see the results of a recent Western Australian community engagement program [1]). Developers have also realised that there can be commercial gain by setting up water recycling in new developments. Often water recycling applications suggested by developers push the boundaries of what is known about treatment capability as well as potential health and ecological risks.

At the same time, new Australian national water recycling guidelines has been produced. These guidelines use a risk management approach, incorporating techniques such as multi-barrier systems, hazard analysis and critical control point (HACCP), quantitative microbial risk assessment (QMRA) and assessment of environmental impacts. The impact and role of microorganisms is a central theme in these guidelines.

Despite the improvements in the guidelines, the greater enthusiasm, and increases in water recycling, there are a number of issues where greater understanding is needed. These include assessments of health risk, water treatment efficiency, management and operational issues, and ecological impacts. A failure to understand these issues could result in operational problems, outbreaks of disease in the community, or damaged environments. Alone, this shows that research remains an important part of any water recycling scheme and for water recycling in general. In addition, however, the public have shown that they have the greatest trust in the advice and activity of researchers [2]. Current microbiological research on water recycling includes detection of pathogens and their removal, the biodegradation of organic chemicals and nutrients, and improved water treatment – particularly the potential for using environments as part of active treatment systems, quantitative risk management, and ecological impacts.

Internationally, Australia is at the forefront of water recycling. This edition of *Microbiology Australia* has papers from a number of researchers who are well-known for their active research on various aspect of microbiology associated with water recycling. It is through their efforts that we can continue to increase the amount and acceptance of recycled water in Australia and assist to solve one of the major issues facing the country in the 21st century. I would like to thank the authors for the time and effort they have taken to write their papers and I hope it provides you with an update of this rapidly growing area.

References

1. Water Corporation (2008) Water Forever Reflections Paper http://www.watercorporation.com.au/P/publications_diversity.cfm
2. Po, M. *et al.* (2005) Predicting Community Behaviour in Relation to Wastewater Reuse: What Drives Decisions to Accept or Reject? Water for a Healthy Country National Research Flagship. CSIRO Land and Water, Perth, Western Australia, 128pp.

Simon Toze is a principal research scientist at CSIRO. His research interests are managed aquifer recharge, indirect potable re-use of water and the fate and behaviour of microbial pathogens in aquatic environments.

Pathogen survival in recycled water

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Water shortages affect more than 2 billion people worldwide in over 40 countries, with 1.1 billion people living without sufficient drinking water [1]. Captured stormwater and treated wastewater can be used for supplementing non-potable water supplies. However presence of enteric pathogens in the reclaimed water can lead to potential health hazards [2].

Pathogens can be actively removed during residence in environmental systems such as rivers, reservoirs. Their survival is influenced by a range of factors in the receiving environment. Enteric viruses, followed by protozoan pathogens, are of concern in recycled water due to their relatively low infectious dose and resistance to disinfection processes. A better understanding of the major factors influencing the decay of pathogens in the environment will greatly assist risk-based regulatory frameworks that are required to minimise health risks from the use of recycled water.

The intended use of reclaimed water influences the decision the level of treatment required. The final pathogen type and numbers in the reclaimed water are determined by the type of water reclamation process used [3]. Secondary wastewater treatment processes such as coagulation, flocculation and sedimentation generally result in 2-5 log reduction in pathogen numbers [4]. However, infectious viruses and protozoan pathogens can survive secondary treatment and in some cases are present in the tertiary treated effluent [2, 5]. Advanced treatment technologies such as reverse osmosis and advanced oxidation appear as viable options, in particular for indirect potable re-use application. The combination of these treatment methods can result in more than 6 log reduction in pathogen numbers but is expensive to run [4].

It is generally accepted that pathogens lose viability in water and other environments over time [6-8]. A range of factors has been implicated in the inactivation of pathogens in reclaimed water including temperature [9], dissolved oxygen [10], organic carbon concentration [6], pathogen types [7] and autochthonous microorganisms [6]. This article presents data from three different water recycling projects where the effectiveness of natural processes to reduce pathogen numbers were studied.

The scenarios

Scenario 1: irrigation of sporting ovals with recycled water

The first scenario was a recycling scheme to provide water for irrigation of sporting ovals. The question to be answered was if there was a chance of bacterial pathogens persisting on the grass, thus posing an increased risk of skin infection from athletes using the ovals. It was also thought that knowledge of the degree of persistence of pathogens on the oval surface could assist in improving the management of the water recycling scheme.

The recycling system used secondary treated effluent from a local sewage treatment plant in Perth which had undergone rapid sand filtration and chlorination [7]. The ovals were irrigated late at night to minimise the potential of human contact and allow a minimal drying time prior to potential use of the ovals. To determine the actual decay times of selected bacterial pathogens on the ovals, survival experiments were undertaken, one during summer and one in winter. The experiments were done by irrigating selected areas of the grass around the sporting complex with treated effluent seeded with pathogens [7]. The sites varied by the amount of sunlight and shade. Grass surface and thatch samples were collected hourly and processed to determine the number of seeded pathogens remaining.

Scenario 2: managed aquifer recharge (MAR) of treated effluent

In the second scenario, the treated effluent was used for a managed aquifer recharge (MAR) scheme where treated water was recharged to the underlying aquifer via infiltration galleries [11]. The recharged water was recovered 50m down gradient for use

for green space irrigation. As with the first scenario, the potential presence of pathogens could pose a health risk so the ability of the aquifer to remove pathogens was assessed to enable a risk assessment to be undertaken. The survival experiment was done in a monitoring well located at 7m down gradient from the infiltration galleries. This well was drilled to a depth of 10.60m and was shown, through chemical analysis of the groundwater, that it was in the plume of the recharged water, thus ideally located for the pathogen inactivation study.

The inactivation experiments were done using nylon diffusion chambers (7-14mL capacity) fitted with 100K MWCO filters on both ends (Figure 1). The pathogens were placed inside the chambers, with groundwater taken from the MAR site. The diffusion chambers were designed to allow groundwater to pass through the chambers but to prevent the movement of the pathogens into the aquifer. A number of bacterial, viral and protozoan pathogens and indicator microorganisms were used in the diffusion chambers. Selected chambers were collected at predetermined times and the number of detectable microorganisms in the chambers were determined.

Scenario 3: impact of wetlands to treat urban stormwater

In the third scenario, stormwater was collected and treated in a wetland prior to injection into an aquifer (300m) under an aquifer storage transfer recovery scheme in Salisbury, South Australia. The aim of the project was to determine if urban stormwater could be captured and transformed into potable water using the treatment capacity of the wetland and aquifer. A major factor influencing this conversion to potable water was the ability of the wetland and aquifer to remove any microbial pathogens that might have been present; a survival experiment was undertaken in the wetland during the winter season when most stormwater was available for capture. A pathogen decay experiment was undertaken using diffusion chambers similar to those described above and again tested using a range of bacterial, viral and protozoan pathogens and indicator organisms.

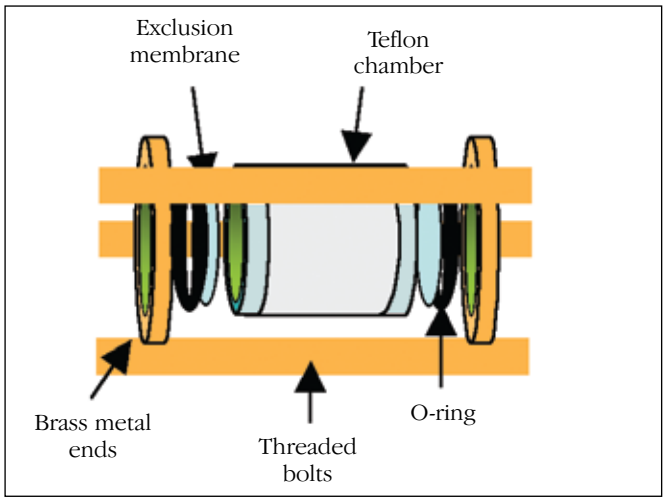

Figure 1. Schematic design of diffusion chamber.

Observations on pathogen decay

Our research has shown that pathogen inactivation occurred due to environmental processes in all three of the scenarios tested. The results indicate that different factors are most likely responsible for causing this decay in the different re-use schemes. Decay of the pathogens was faster on the grass surface irrigated with effluent compared with groundwater and wetland (Figure 2 and Table 1).

The pathogen inactivation on grass surfaces showed that inactivation was faster under sunlight during the summer compared with winter with 1 $\log_{10}$ reduction (T_{90}) varying from 3-12 hours (Table 1). Rapid inactivation of the bacteria on the grass surface irrigated with treated effluent was expected during the summer due to high ambient temperature and intensity of sun light. Slower inactivation during winter was possibly due to the combination of low temperature, high moisture and low solar radiation intensity compared with summer.

Inactivation of pathogens on the grass surface irrigated with effluent is primarily influenced by moisture content, sunlight and temperature, whereas in groundwater and wetland water chemistry, activity of indigenous microorganisms and temperature were found to have a dominant role. Unlike the impact of sunlight on grass surfaces, sunlight had no influence on pathogen survival in the groundwater and the wetlands (the wetlands are covered with shade cloth to deter birds). Thus other factors have a

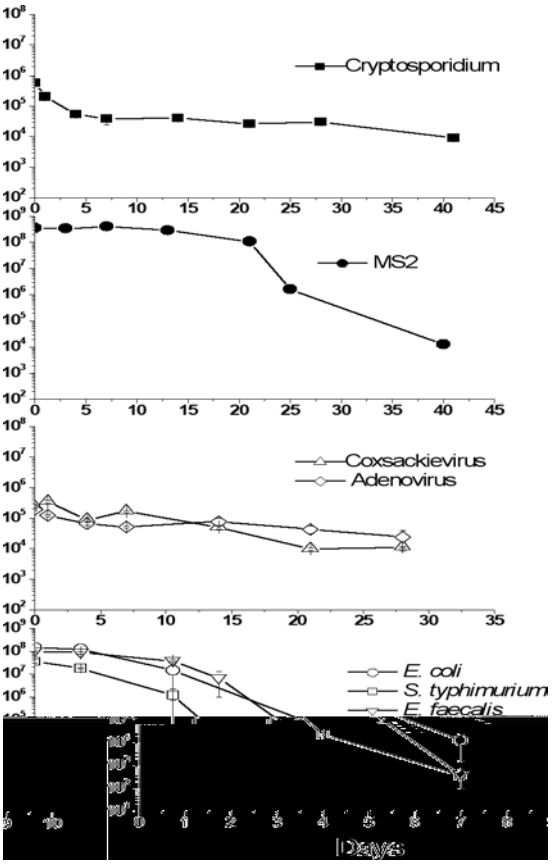

Figure 2. Inactivation of pathogens and indicator microorganism in groundwater during MAR with secondary treated effluent.

Table 1. One $\log_{10}$ (T_{90}) inactivation time of enteric microorganisms under three different water recycling processes.

Microorganism	Effluent on grass surface (hours)		Secondary effluent (days)	Stormwater (days)
	Summer	*Winter*	*Groundwater*	*Wetland*
E. coli	3.3	8.4	1	5
S. typhimurium	2.5	6.6	1	5
S. aureus	3.7	11.7	–	–
E. faecalis	4.2	7.7	1	6
MS2	14.3	12.5	14	–
Adenovirus	–	–	28	33
Coxsackievirus	–	–	18	–
Cryptosporidium	–	–	31	80

greater influence. The observed pathogen inactivation times were faster in the groundwater than in the surface wetlands. These differences are most likely due to the higher temperature of groundwater (22°C) than wetland (9°C). It has been established that autochthonous microorganisms contribute significantly to pathogen decay in aquatic systems such as aquifers [6]. It is also known that temperature has a secondary influence on the activity of these autochthonous microbes, thus explaining the differences between the inactivation rates observed in the groundwater and wetland areas [6, 12].

Pathogen type has also been noted to be important, with bacteria much more efficiently removed compared to the viruses and protozoa. In the schemes presented here, Cryptosporidium oocysts were the most resistant to decay. Thus it is important to also have a good understanding of the likelihood of different pathogen types to be present in any water source being considered for recycling.

Concluding remarks

In general, our studies have confirmed that environmental systems can be used to assist in the treatment of recycled water to achieve the removal of microbial pathogens. A range of different environmental factors can have an influence on the decay of microbial pathogens. The type of environmental factor that has the greatest influence depends on the environment where the recycled water is used or stored and local ambient conditions, in particular seasonal differences.

The type of pathogen that can be present in the recycled water is also very important. Our studies have shown that the greatest risks associated with pathogens in recycled water are the enteric viruses protozoan due to their resistance to environmental pressures and their low infectious dose. Results obtained to date indicate that inactivation of Cryptosporidium oocysts appears to be primarily temperature-driven and at higher ambient temperatures they may not be expected to survive long.

Research is continuing to further elucidate the role of different environmental processes have on the decay of a range of microbial pathogens. The results to date, however, have shown that environmental processes can result in a significant reduction in pathogen numbers and should be considered as effective barriers under the multiple barrier approach for risk mitigation.

References

1. WHO/UNICEF (2000) *Global Water Supply and Sanitation Assessment 2000 Report.* Geneva.
2. Gennaccaro, A.L. *et al.* (2003) Infectious *Cryptosporidium parvum* oocysts in final reclaimed effluent. *Appl. Environ. Microbiol.* 69, 4983-4984.
3. Rose, J.B. *et al.* (1996) Removal of pathogenic and indicator microorganisms by a full-scale water reclamation facility. *Wat. Res.* 30, 2785-2797.
4. Toze, S. (2006) Water reuse and health risks-real vs. perceived. *Desalination* 187, 41-51.
5. Gantzer, C. *et al.* (1994) Poliovirus-1 adsorption onto and desorption from montmorillonite in seawater – survival of the adsorbed virus. *Environ. Technol.* 15, 271-278.
6. Gordon, C. and Toze, S. (2003) Influence of groundwater characteristics on the survival of enteric viruses. *Appl. Microbiol.* 95, 536-544.
7. Sidhu, J.P.S. *et al.* (2008) Survival of enteric microorganisms on grass surfaces irrigated with treated effluent. *J. Wat. Heal.* 6, 255-262.
8. Yates, M.V. *et al.* (1985) Virus persistence in groundwater. *Appl. Environ. Microbiol.* 49, 778-781.
9. Allwood, P.B. *et al.* (2003) Survival of F-specific RNA coliphage, feline calicivirus and *Escherichia coli* in water: a comparative study. *Appl. Environ. Microbiol.* 69, 5707-5710.
10. Jansons, J. *et al.* (1989) Survival of viruses in groundwater. *Water. Res.* 23, 301-306.
11. Bekele, E, *et al.* (2005) Improvements in wastewater quality from soil and aquifer passage using infiltration galleries: case study in Western Australia. In: Recharge systems for protecting and enhancing groundwater resources. Proceedings of the 5th International Symposium on Managed Aquifer Recharge, Berlin, June 2005.
12. Quinonez-Diaz, M.J. *et al.* (2001) Removal of pathogenic and indicator microorganisms by a constructed wetland receiving untreated domestic wastewater. *J. Environ. Sci. Health*. A36, 1311-1320.

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Simon Toze – Please see details on page 4.

Pathogens and indicators in wastewater matrices

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As climate change and increasing population sizes continue to place stress on water resources, communities are increasingly looking to recycled water as a supplementary water source, whether for drinking water, domestic irrigation, industrial or agricultural use. Protecting public health by ensuring the safety of water supplies is a key concern for the water industry and health authorities.

Guidelines for the safe use of recycled water require monitoring for the removal of key enteric pathogens but these are reliant on traditional indicators such as *Escherichia coli (E. coli)*, coliforms and faecal coliforms to demonstrate the microbiological quality of the water. However, as with potable water, it is impractical and uneconomical to screen recycled water for every possible enteric pathogen. To reduce the costs of monitoring wastewater, a preferred option would be to use an indicator organism that correlated with the presence of a pathogen or class of pathogens [1] (also termed an index organism). However, finding such an organism is unlikely since it would require an exclusive association between the pathogen and indicator organism. Indicators such as *E. coli,* while valuable in the context of ensuring the safety of potable water supplies, are of less value in domestic wastewater applications because this matrix is faecally contaminated by default, so faecal indicators will always be present while pathogens may be absent.

The ideal indicator

The ideal indicator mimics the behaviour and characteristics of a pathogen but is itself easier and faster to isolate, culture or identify, is non-pathogenic and is a cheaper alternative to direct detection of the pathogen. The indicator should be present when the human pathogen is present and absent when the pathogen is absent, have similar environmental requirements, pattern of die off and susceptibility to disinfection. The presence or absence of the indicator makes its selection difficult as pathogens are not part of the normal microbial flora of the human system; they are only present in and excreted by infected individuals, with infection often being seasonal and related to prevalence within the community.

The continued operation of wastewater treatment plants requires constant monitoring of key parameters. The behaviour of different types of pathogens becomes problematic when selecting an indicator because it is unlikely that a single indicator will be representative of all pathogenic bacteria, viruses, protozoans and helminths, requiring indicator selection to be tailored for the pathogens and treatment process of interest. Traditionally, indicators have been used to suggest the presence of pathogens [2], although there is no direct correlation between numbers of any particular indicator and enteric pathogens [3].

Alternative indicators

Rather than selecting an indicator that is ideal for an individual pathogen, the opportunity exists to select an indicator that is representative of the process efficacy [1]. Process indicators are an organism or group of organisms that demonstrate the efficiency of a process, such as total heterotrophic bacteria or total coliforms for chlorine disinfection. Model or index organisms are a group or species that behave in a similar manner to the pathogen of interest. This relies on the model organism such as *E. coli* having similar survival in the environment in response to disinfectants such as *Salmonella.* The presence of the model organism in a treatment plant can provide an index for the presence of the specific pathogen [1].

Enteric pathogens in wastewater

As mentioned, enteric pathogen presence in wastewater is dependent on the level of community infection. The types and numbers of pathogens that enter wastewater are likely to

be seasonal and, as such, not all pathogens will be detectable throughout the year. Seasonality is generalised and can vary depending on climatic conditions. This inconsistency and variability makes detection difficult and direct detection of pathogens from any water source tends to be time-consuming and expensive. As such, research to find suitable indicator microorganisms has been attempted by numerous groups around the world.

Bacterial pathogens and indicators

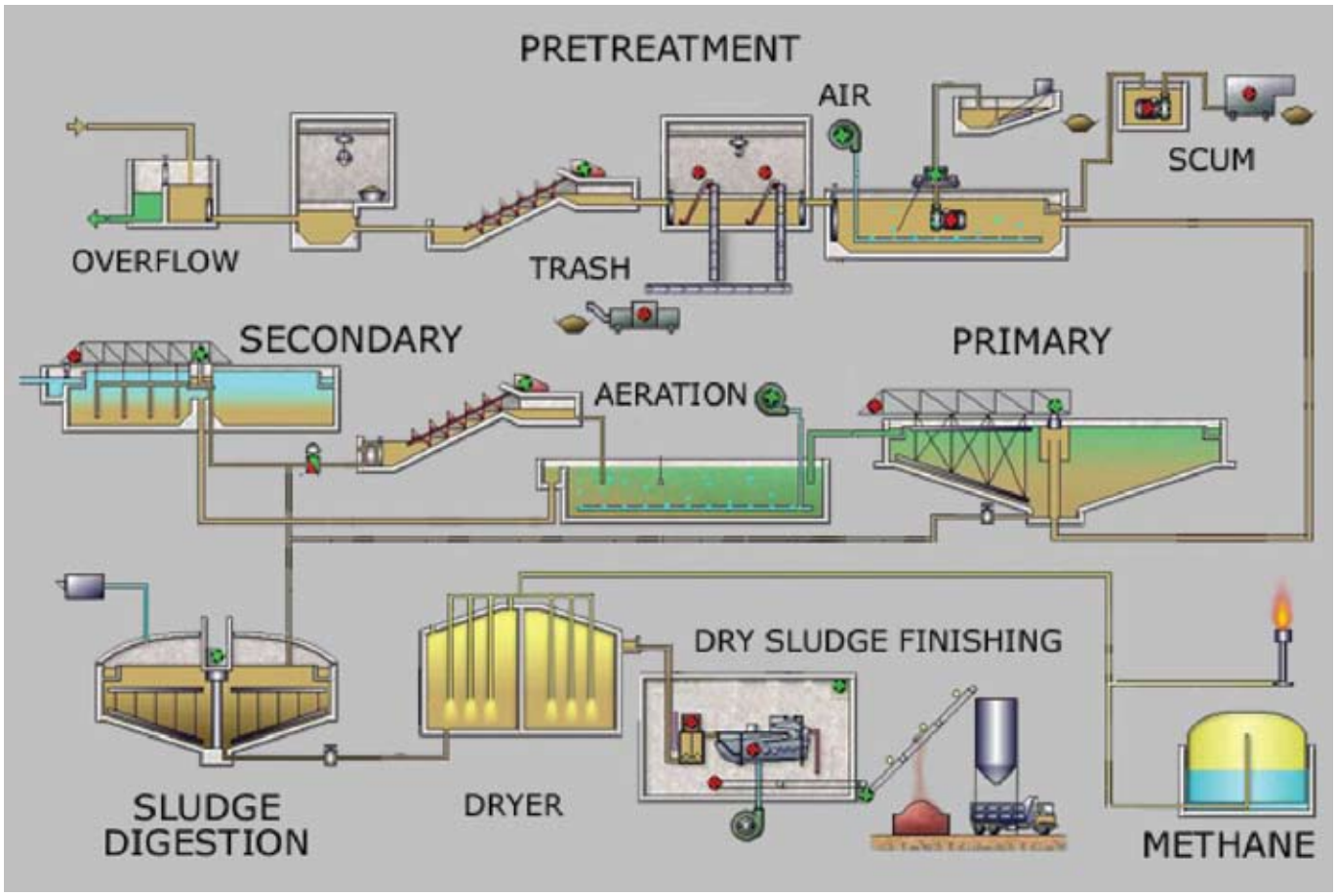
Bacterial pathogens are a major cause of gastroenteritis worldwide, with the leading cause of food-borne diseases from *Campylobacter, Salmonella* and *Shigella* [4]. The established methods for the detection of bacterial pathogens in wastewater is based on culture using artificial media, incorporation of selective agents or treatment to reduce background contaminants. Often, additional tests are required for confirmation of identity. The culture-based methods determine whether the cell is able to grow (in artificial conditions) but do not determine whether infection in a host is possible.

Campylobacter, Salmonella and *Shigella* are highly susceptible to standard disinfection processes, being more sensitive than *E. coli* to chlorine [5]. Therefore, this renders them less of an issue for the water industry provided treatment conditions are optimal [5]. In developed countries, the potential for issues only arises during system failure or upset, which can be due to heavy rains, breakdown or inappropriate monitoring.

Bacterial indicators include coliforms, enterococci (similar removal rates to coliforms [6]), *Bifidobacteria* and *Bacteroides fragilis.* All are non-pathogenic and present in high numbers in the human gut and faeces, but very little is known of the behaviour of *Bifidobacteria* and *B. fragilis* in wastewater treatment processes.

E. coli is considered to be an important bacterium to the water industry, both as a cause of water-borne outbreaks by *E. coli* 0157:H7 and as an indicator organism for the detection of faecal contamination. *E. coli* 0157:H7 has been reported in 31 outbreaks in the US between 1982-2002, accounting for 9% of all outbreaks by this pathogen [7]. Most facilities use faecal coliforms or total coliforms as an indicator, but neither group of organisms correlate with pathogenic bacteria removals (except *Salmonella)* [8].

Other bacteria of interest to the water industry include *Aeromonas hydrophila* (now listed on the United States Environmental Protection Agency Candidate Contaminant List (USEPA CCL)), *Klebsiella* species, *Pseudomonas aeruginosa* (potential for



Wastewater treatment plant, reprinted with permission from Josefpm, *Wikipedia.*

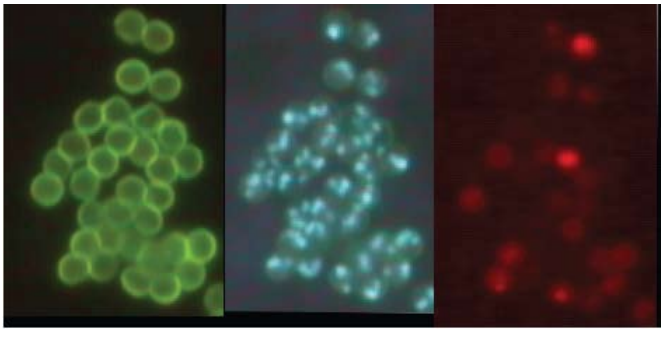
mucoid strains to be resistant to oxidant-based disinfection [9], *Mycobacterium* spp (listed on the USEPA CCL as an emerging pathogen and highly resistant to disinfection [10], and *Vibrio* species (although only in developing countries).

Clostridium perfringens offers a greater challenge because the spores are robust, survive longer, and are more heat- and chlorine-resistant than other bacteria in wastewater [11]. Harwood *et al.* [6] tested for *C. perfringens* at each point in the wastewater treatment process and determined that it was present in 93% of influent samples, 86% of biological treatment samples, 79% of filter effluent samples and 61% of disinfected effluent samples.

Protozoan pathogens and indicators

Protozoan parasites are numerous in wastewater, including *Cryptosporidium, Giardia, Entamoeba* and Microsporidia, which are of particular interest to the water industry. Methods are expensive and time-consuming, involving concentration from large volumes, purification using immunomagnetic separation and labelling with fluorescent antibody for enumeration under fluorescence microscopy. *Cryptosporidium* is highly resistant to chlorine-based disinfectants, has been implicated in a number of gastroenteritis outbreaks around the world, most notably Milwaukee, USA (1993) [12] and therefore has become highly important to the water industry. *Giardia,* although present at higher numbers than *Cryptosporidium,* has greater susceptibility to disinfection with chlorine and is therefore less problematic under effective operating parameters at wastewater treatment plants. Microsporidia is listed on the USEPA CCL as an emerging pathogen that can cause opportunistic infections. Although limited reports on water-borne outbreaks exist [13], Microsporidia has been detected in wastewater effluents [14]. Sensitivity to chlorine has been disputed, although John *et al.* [15] claim it is as sensitive to chlorine as *Giardia.*

Possible indicators for protozoa suggested in the literature include aerobic spores, anaerobic spores and particle profiling (particle size distribution). Spores have a greater resistance to chlorine than vegetative cells and as such can more reliably represent the disinfection of protozoa. Anaerobic spores such as sulphite reducing clostridia have been suggested as a surrogate for *Cryptosporidium* in wastewater, although numbers do not correlate with the pathogen [6]. Particle profiling has been developed as a useful tool for microbial detection in untreated raw wastewater where direct agricultural use is in place [16], with correlation between particle removal and the removal of faecal coliforms and *Salmonella* spp observed. This has so far been untested for protozoa and further data are required to validate the reported correlations.



***Cryptosporidium* and *Giardia* observed under fluorescence microscopy and DIC.**

Viral pathogens and indicators

Viral pathogens are a major cause of gastroenteritis worldwide. The established methods for the detection of viral pathogens in wastewater are based on concentration of virus particles followed by cell culture for culturable viruses and polymerase chain reaction (PCR) or reverse transcription PCR (RT-PCR) for non-culturable viruses. Culture-based methods are able to determine infection within an animal host cell, while the molecular methods such as PCR and RT-PCR are only able to determine virus presence (and potentially numbers) or absence, not infectivity.

Culturable viruses important in wastewater include many of the enteroviruses, a limited range of the adenoviruses and reoviruses (rotavirus). Non-culturable viruses include norovirus, rotavirus, human calicivirus, Hepatitis A virus, Hepatitis E virus and polyomavirus. Human infective viruses are unable to replicate in the environment as they require a suitable host. Viruses such as polyomavirus and reovirus can cause asymptomatic infection in childhood, with a high level of seroconversion in the community, but are not generally considered pathogens. Viruses in general are highly sensitive to disinfection with chlorine and as such are treatable within the wastewater treatment process.

A range of viral indicators, including bacteriophage, enteric virus genomes, poliovirus vaccine strain (now discontinued), polyomavirus and reovirus, have been suggested and tested through wastewater treatment processes. Bacteriophage offer the easiest option for enumeration as this is an agar plate based assay and is complete within 24 hours. However, Harwood *et al.*[6] found no correlation between coliphage and enteric virus removal by wastewater treatment processes (in particular filtration and disinfection). Alternatively, enteric virus genomes, although relying on recovery from effluents prior to PCR, offer a faster result because there is no culture step involved. Correlation between cultured virus and enteric virus genomes has not been demonstrated [17,18], and may potentially overestimate the health risk.

Helminth pathogens and indicators

Helminths have the highest prevalence in tropical and subtropical regions and areas with inadequate sanitation, usually in developing countries, but also occur in rural areas of the south-eastern United States [19]. Detection of helminth ova from wastewater involves either centrifugation or sedimentation, followed by flotation and examination by microscopy. Due to the size of helminth eggs, such as *Ascaris lumbricoides,* the majority are removed through sedimentation processes in wastewater treatment and thus become more problematic in biosolids [20]. Particle profiling has been reported as a useful indicator for the removal of helminths from wastewater, with a high correlation of R^2=0.98 observed between numbers of helminth ova and the volume of particles of 20-80 microns [16].

Conclusion

The selection of individual microorganisms as indicators for the presence and removal of pathogens is a difficult task. Due to the seasonality of pathogens circulating in the community, the selection of an appropriate indicator that behaves in the same manner as the pathogen is hindered because it will not mimic the pathogen presence and absence within the wastewater stream. As an alternative, the indicators can be used as conservative markers of pathogen removal, treatment efficiency or indicative of pathogen behaviour using the process indicator, faecal indicator and model/index organisms guide set out by Ashbolt *et al.* [1]. The need to improve detection of pathogens or improved indicators is important to water recycling in conjunction with the risk management approach adopted in the Australian Guidelines for Water Recycling [21].

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References

1. Ashbolt, N.J. *et al.* (2001) Indicators of microbial water quality. In: *Water Quality: Guidelines, Standards and Health.* World Health Organization (Fewtrell, L. and Bartram, J., eds). IWA Publishing, London, UK.
2. Berg, G. (1978) The indicator system. In: *Indicators of Viruses in Water and Food* (G. Berg, ed), pp1-13, Ann Arbour Science Publishers, Ann Arbour, MI.
3. Grabow, W.O.K. (1996) Waterborne diseases: update on water quality assessment and control. *Water SA* 22, 193-202.
4. World Health Organisation (WHO) (2009) www.who.int/topics.
5. Westrell, T. *et al.* (2003) A theoretical approach to assess microbial risks due to failures in drinking water systems. *Int. J. Environ. Health Res.* 13, 181-197.
6. Harwood, V.J. *et al.* (2005) Validity of the indicator organism paradigm for pathogen reduction in reclaimed water and public health protection. *Appl. Environ. Microbiol.* 71, 3163-3170.
7. Rangel, J.M. *et al.* (2005) Epidemiology of *Escherichia coli* O157:H7 outbreaks, United States, 1982-2002. *Emerg. Infect. Dis.* 11, 603-609.
8. Koivunen, J. *et al.* (2003) Elimination of enteric bacteria in biological-chemical wastewater treatment and tertiary filtration units. *Water Res.* 37, 690-698.
9 Grobe, S. *et al.* (2001) Capability of mucoid *Pseudomonas aeruginosa* to survive in chlorinated water. *Int. J. Hyg. Environ. Health* 204, 139-142.
10. Falkinham, J.O., 3rd. (2002) Nontuberculous mycobacteria in the environment. *Clin. Chest Med.* 23, 529-551.
11. Venczel, L.V. *et al.* (2004) Inactivation of enteric microbes in water by electro-chemical oxidant from brine (NaCl) and free chlorine. *Water Sci. Technol.* 50, 141-146.
12. MacKenzie, W.R. *et al.* (2004) A massive outbreak of cryptosporidium infection transmitted through the public water supply. *N. Engl. J. Med.* 331, 161-167.
13. Cotte, L. *et al.* (1999) Waterborne outbreak of intestinal microsporidiosis in persons with and without human immunodeficiency virus infection. *J Infect. Dis.* 180, 2003-2008.
14. Sturbaum, G.D. *et al.* (1998) Detection of *Cyclospora cayetanensis* in wastewater. *Appl. Environ. Microbiol.* 64, 2284-2286.
15. John, D.E. *et al.* (2005) Chlorine and ozone disinfection of *Encephalitozoon intestinalis* spores. *Water Res.* 39, 2369-2375.
16. Chavez, A. *et al.* (2004). Particle size distribution as a useful tool for microbial detection. *Water Sci. Technol.* 50, 179-186.
17. Gantzer, C. *et al.* (1998). Detection of infectious enteroviruses, enterovirus genomes, somatic coliphages, and *Bacteroides fragilis* phages in treated wastewater. *Appl. Environ. Microbiol.* 64, 4307-4312.
18. Choi, S. and Jiang, S.C. (2005) Real-time PCR quantification of human adenoviruses in urban rivers indicates genome prevalence but low infectivity. *Appl. Environ. Microbiol.* 71, 7426-7433.
19. CDC: Centre for Disease Control, Division of Parasitic disease (2005) www.dpd.cdc.gov/DPDx/HTML/Microsporidiosis.htm
20. Nelson, K.L. (2003) Concentrations and inactivation of Ascaris eggs and pathogen indicator organisms in wastewater stabilization pond sludge. *Water Sci. Technol.* 48, 89-95.
21. NWQMS (Natural Resource Management Ministerial Council, Environment Protection and Heritage Council and Australian Health Ministers Conference (2008) Australian Guidelines for Water Recycling. Canberra, Australia.

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Pathogens in recycled water: are they measurable?

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In developed countries water managers are constantly under pressure to provide the clean and safe water. Traditionally, and for at least the past 100 years, the management of biological water quality has relied on the use of microbial indicator organisms to assess the potential risk of water-borne disease. However, over the past few years, there have been a number of critical reviews of guidelines and standards for managing risk in water storage, treatment and supply. International, national and state agencies have initiated these reviews and have all generally agreed that technology for alternative methods, in place of the use of indicator organisms for risk assessment of microbial water quality, has not advanced to point where there is an obvious replacement. However, even in the last 3 years, improvements in genetic techniques, such as real-time quantitative PCR and DNA microarrays are making advances that may allow us to consider alternatives to using indicator organisms in the foreseeable future. Here we present the issues and pros and cons associated with the use of indicator organisms compared to the use of molecular biology approaches for microbial risk management in recycled water. The current state of the legislation and guidelines is also discussed.

There is no doubt that Australia and other parts of the world are facing enormous challenges in providing fresh, clean water to a growing population, which is also complicated by changing weather patterns and environmental degradation. The water challenge faced by providers, managers and scientists is driving exploration into alternative sources for water other than dams and rivers. The National Water Initiative (NWI) released by the Council of Australian Governments (COAG) in June 2004 endorsed improving opportunities for water recycling. It is essential for the successful implementation of water recycling schemes to enable the industry to portray trust and confidence to the community that we have addressed, and can control, all health risks associated with recycled water. The ability to directly detect pathogens using the most accurate techniques will be critical for water managers and providers to confidently assess and manage risks of existing and new water sources.

How pathogens are monitored in water

In the most part, traditional and standard indicator organisms such as faecal coliforms and the more specific measurement of *Escherichia coli* have been the most acceptable risk management tool based on the available technology. However, we do know that many measurable pathogens such as *Cryptosporidium parvum* [1], *Legionella pneumophila* [2] and some viruses [3] are more resistant to conventional water treatment and can persist in the environment significantly longer than coliforms and *E. coli*. Many other studies have demonstrated very poor correlation with pathogenic organisms and indicators in water [3-7].

Guidelines for water recycling

Current water recycling guidelines and recommendations from the WHO, NHMRC and various state government agencies have based the microbial risk assessment on numbers of indicator organisms (total coliforms, faecal coliforms and *E. coli),* parasites such as helminth eggs, some protozoa such as Giardia/*Cryptosporidium* and MS2 phage for viruses [8-10]. However, the use of indicator organisms to assess public health risks can have serious limitations and have not always protected public health to the desirable level [3, 4, 11-15]. Examples of where indicator organisms have failed include the Milwaukee drinking water contamination incident where over 400,000 people were infected with Cryptosporidium when the indicator organism tests apparently demonstrated acceptable cell numbers [16].

The NHMRC's review of the use of coliforms [9], as indicator organisms, and the revised Australian Drinking Water Guidelines, have recognised the inadequacies of this culture-based method and are advocating the role of gene technology for the direct detection of pathogens. The NHMRC have acknowledged that the take-up of gene technology (such as DNA microarray and fluorescent *in situ* hybridisation) for enumerating organisms has been well adopted by the medical industry but not the water industry [9].

What are the alternatives?

Over the past 20 years, several alternatives to using indicator organisms to assess microbial water quality have been investigated. Alternative surrogates to the coliforms and *E. coli* such as bacteriophages, chemical indicators such as sterols and an array of molecular genetic techniques are all examples of attempts to improve the accuracy of microbial risk quantification. The several gene-based techniques that look promising for the water industry include the quantitative polymerase chain reaction (qPCR), multiplex PCR and microarray DNA technology. To make the quantum step to using these techniques for routine monitoring in a drinking water supply, they must prove to be specific, reliable and sensitive. The confidence in using molecular techniques routinely will also come when they can be reliably validated against the traditional culture-based methods and the quality control and accuracy issues are addressed.

The PCR for detection of pathogens

The PCR, only discovered in the mid 1980s [17, 18], has advanced dramatically in the past 20 years. So much so, that it is now routine in molecular biology laboratories. The invention of this method has revolutionised the study of microorganisms. Until recently, the results of the PCR were presence/absence, without any methods available to quantify the amount of starting material (i.e. number of cells). The real-time PCR or qPCR methods are now considered a fast and efficient tool to quantify target genes capable of identifying organisms from all types of samples containing microbes.

Multiplex PCR involves the molecular detection of several genes or DNA sequences in a single amplification reaction. In the context of risk assessment in the water industry, this allows the detection of multiple organisms simultaneously. Though multiplex PCR requires laboratory facilities and specialised equipment to perform the analysis, recent advances in the field of molecular biology have made the outlay for the necessary equipment affordable and the amplification reactions cost-effective compared to traditional detection methods. The main drawback of this method is that it only detects the presence or absence of the organisms to be detected and in itself gives no indication of cells numbers. Some groups have tried to overcome this to make multiplex PCR semi-quantitative by serially diluting the sample until a negative result is obtained. This can be time-consuming and can increase running costs due to the need for multiple reactions and the use of standards with known concentrations of the organism of interest.

As with multiplex PCR, real-time PCR can detect either one or several organisms of interest in a single reaction within a matter of hours, with the added advantage of being fully quantitative. This technique utilises the standard PCR methods for DNA amplification with the addition of a marker which fluoresces when bound to DNA. Analysis of the level of fluorescence allows the quantification of the amount of amplified DNA and, when compared to standards of known concentration, the number of organism in the original sample. Recent advances in the field allow probes with distinct fluorescence profiles to be designed for specific organisms, allowing the detection and enumeration of several organisms of interest in the same reaction. One of the main drawbacks of this technique is that it requires expensive specialised equipment and skilled operators to perform the analysis. Additionally, the fluorescent probes and markers necessary for the technique increase the running cost to perform real-time PCR markedly compared to multiplex PCR or culture-based analysis.

Genetic microarrays

DNA microarray, first developed at Affymetrix Inc [19], is one of the most exciting developments in microbiology that has potential to be exploited as a risk management tool for water quality assessment. This technique allows the simultaneous detection of potentially over 400,000 gene sequences in a single sample. In short, DNA probes specific for the gene or organism of interest are spotted on to a microarray chip. Recent improvements of the planar glass chip include the use of the silicon wafer or rounded beads [20]. The extracted DNA from the sample is then tagged with a fluorescent marker and hybridised with the chip containing the probes. The level of fluorescence of the microarray chip is then analysed to confirm the presence or absence of the DNA specific for organisms of interest within the sample. By measuring levels of fluorescence, this technique can be made quantifiable to provide indications of the concentration of the target DNA within the sample.

Currently, the major drawback of this technique is cost, as it requires expensive and specialised equipment and analysis of the data obtained through microarray requires highly skilled operators. Performing microarrays is also more time-consuming than PCR-based methods, as a preliminary amplification step must be performed to allow the detection of DNA that may be in low concentrations within the sample. Also, as with the other molecular techniques profiled here, microarrays require highly

purified DNA samples, free of the many contaminants which co-purify with DNA present in environmental sample. There are limitations with the use of DNA microarrays for the detection of pathogens in water; however, improvements in the sensitivity, quantification and possible direct hybridisation of this method could see it as a tool used in water labs for routine testing in the near future.

The future

Bringing together the potential automation of sampling, concentration, extraction, hybridisation, detection and reporting would be the most ideal tool for the monitoring of pathogens – Figure 1 conceptually demonstrates how this may occur. Only a couple of decades ago, this was not thought to have been possible. Theoretically, at least, as recognised by a number of authors, it is now thought to be possible when the limitations of sample preparation, validation and proof of concept can be overcome [20-22].

Viability and infectivity of a microbe detected in water is a factor that can determine the true extent of a public health risk. Molecular methods can potentially provide much more information about the physiological state of an organism, particularly microarrays, where functional genes can be monitored.

Direct detection of pathogens rather than indicators in water would allow more accurate risk assessment and management. The implications of both culture-dependent and molecular genetic techniques on risk assessment are summarised in Table 1. While genetic techniques do promise a bright future for water management, there are still some hurdles to overcome. Many issues with expense and need for skilled operators are being addressed. It is also now possible to carry out PCR in the field; affordable and hand-held PCR machines are available [23]. However, issues with DNA and extraction and inhibitors of the PCR as with microarrays still need to be addressed before the technology can be considered as a replacement for culture-dependent methods.

Conclusion

Do we need to digress from indicator organisms? In the best interest of public health and the use of recycled water for drinking purposes, it is essential that we fast-track the improvements required to determine pathogen numbers, viability and infectivity using molecular techniques. The immediate future is exciting for water testing laboratories given this ability to ensure public health safety with so much more confidence. It would be

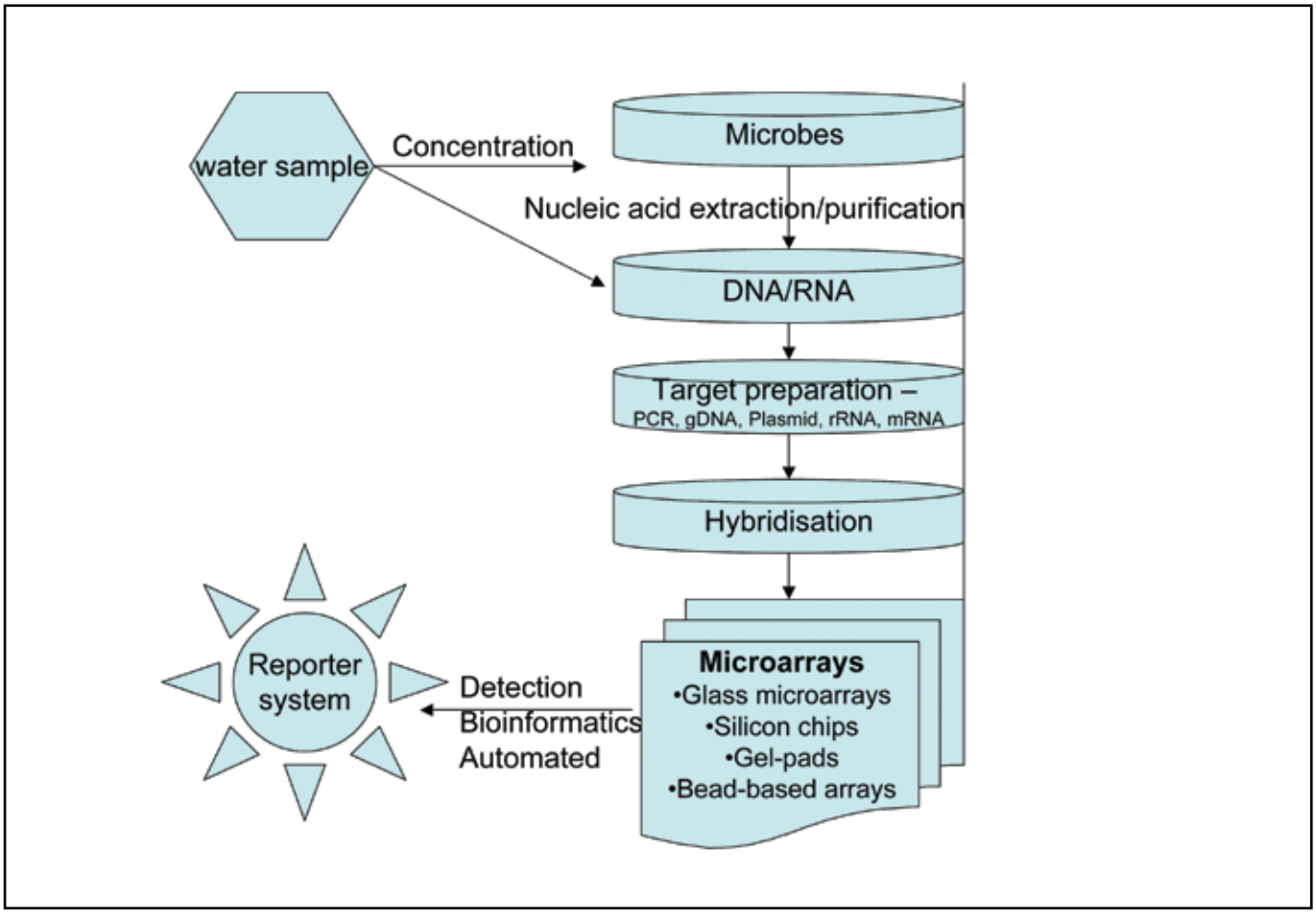

Figure 1. Idealistic diagram of how future pathogen monitoring in water may be carried out.

Table 1. Comparison of conventional culture-dependent techniques with genetic techniques with implication for risk management (Stratton *et al.* 2006).

Culture-dependent risk management implications
Can take up to 5-10 days for results – delayed response increases exposure
Implied results – real risk over- or under-estimated; only lends itself to qualitative risks assessment (absence or presence of risk, not extent of risk)
Only grows viable organisms – risk is underestimated
Some pathogens more resistant – risk is selectively underestimated
Accuracy and repeatability – questionable risk quantification
Molecular genetics
Results within 24 hours – decreased potential for exposure
More specific results – risk quantification possible
Under estimates (false negatives) – risk underestimated
Accuracy and repeatability; easier to include controls – risk quantification possible
Specificity – can detect the actual pathogen, virulence factors or toxin genes that culture techniques either cannot or would take lengthy experiments to do so

irresponsible for the water industry not to continue to actively develop more specific and reliable technologies for measuring pathogens in water.

The development of more rapid and reliable technologies can aid in alerting to disease potential. Accuracy in determining the actual presence of pathogens, as against elevated numbers of indicator organisms, will avoid false alerts (and the subsequent loss of faith in service providers). The converse of more rapid and accurate identification of the presence of pathogens has the obvious benefits of reducing exposure and avoiding situations such as those experienced at Milwaukee. Ultimately, the ability to quantify the number of pathogens present will permit validation of whether infectious doses are present in an incident, and therefore diversion of product or implementation of remedial measures in line with HACCP procedures can be immediately implemented.

References

1. Chauret, C.P. *et al.* (2001) Chlorine dioxide inactivation of Cryptosporidium parvum oocysts and bacterial spore indicators. *Appl. Environ. Microbiol.* 67, 2993-3001.
2. Kim, M. *et al.* (2008) Source tracking of microbial intrusion in water systems using artificial neural networks. *Water Res.* 42, 1308-1314.
3. Baggi, F. *et al.* (2001) Persistence of viral pathogens and bacteriophages during sewage treatment: lack of correlation with indicator bacteria. *Res. Microbiol.* 152, 743-751.
4. Allwood, P.B. *et al.* (2003) Survival of F-specific RNA coliphage, feline calicivirus, and *Escherichia coli* in water: a comparative study. *Appl. Environ. Microbiol.* 69, 5707-5710.
5. Baker, K.H. and Herson, D.S. (1999) Detection and occurrence of indicator organisms and pathogens. *Water Environ. Res.* 71, 530-551.
6. Fernandez, M.C. *et al.* (2000) *Aeromonas hydrophila* and its relation with drinking water indicators of microbiological quality in Argentine. *Genetica* 108, 35-40.
7. Nelson, K.L. (2003) Concentrations and inactivation of Ascaris eggs and pathogen indicator organisms in wastewater stabilization pond sludge. *Water Sci. Technol.* 48, 89-95.
8. Natural Resource Management Ministerial Council *et al.* (2007) *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 2).* Augmentation of Drinking Water Supplies. Draft for Public Comment.
9. NHMRC (2003) Review of Coliforms As Microbial Indicators of Drinking Water Quality. (Stevens, M. *et al.,* eds).
10. WHO (1996) Guidelines for drinking water quality, 2nd edition, volume 2. In: *Health Criteria and Other Supporting Information*.
11. Efstratiou, M.A. *et al.* (1998) Correlation of bacterial indicator organisms with *Salmonella* spp., *Staphylococcus aureus* and *Candida albicans* in sea water. *Lett. App. Microbiol.* 26, 342-346.
12. Fattouh, F.A. *et al.* (2004) Recovery of somatic coliphages in wastewater and seawater samples in relation to bacterial indicator organisms and water hydrochemical parameters in Kaiet Bay station, Alexandria. J. *Water Supp. Res. Technology-Aqua* 53, 183-192.
13. Horman, A. *et al.* (2004) *Campylobacter* spp., *Giardia* spp., Cryptosporidium noroviruses and indicator organisms in surface water in southwestern Finland, 2000-2001. *Appl. Environ. Microbiol.* 70, 87-95.
14. Jin, G. *et al.* (2004) Comparison of *E. coli,* enterococci, and fecal coliform as indicators for brackish water quality assessment. *Water Environ. Res.* 76, 245-255.
15. Tallon, P. *et al.* (2005) Microbial indicators of faecal contamination in water: A current perspective. *Water Air Soil Poll.* 166, 139-166.
16. MacKenzie, W.R. *et al.* (1994) A massive outbreak in Milwaukee of Cryptosporidium infection transmitted through the public water supply. *New Eng. J. Med.* 331, 1035-1041.
17. Lawyer, F.C. *et al.* (1989) Isolation, characterization, and expression in *Escherichia coli* of the DNA polymerase gene from *Thermus aquaticus. J. Biolog. Chem.* 264, 6427-6437.
18. Saiki, R.K. *et al.* (1985) Enzymatic amplification of β-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia. *Sci. Total Environ.* 230, 1350-1354.
19. Lipshutz, R.J. *et al.* (1999) High density synthetic oligonucleotide arrays. *Nature Genet.* 21, 20-24.
20. Call, D.R. (2005) Challenges and opportunities for pathogen detection using DNA microarrays. *Crit. Rev. Microbiol.* 31, 91-99.
21. Bosch, A., *et al.* (2008) New tools for the study and direct surveillance of viral pathogens in water. *Curr. Opin. Biotechnol.* 19, 295-301.
22. Lemarchand, K. *et al.* (2004) Molecular biology and DNA microarray technology for microbial quality monitoring of water. *Crit. Rev. Microbiol.* 30, 145-172.
23. Lebuhn, M. *et al.* (2004) Evaluating real-time PCR for the quantification of distinct pathogens and indicator organisms in environmental samples. *Water Sci. Technol.* 50, 263-270.

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The impact of biofilms on water quality in long pipelines

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Limited water availability and increased water demand necessitates the use of long pipelines to distribute potable and non-potable water for human consumption or other purposes. The effects of microbial growth and activity on the quality of distributed water have been studied for many years, although in recent years much of this focus has shifted to understanding the effects of biofilms, rather than planktonic microorganisms, on water quality.

Recently, it was estimated that 95% of all biomass in water distribution systems is in the form of pipe-wall biofilms, with only 5% of all biomass in the bulk water phase [1]. Under favourable conditions, biofilms can impact water quality by increasing disinfectant demand, creating taste and odour problems, harbouring opportunistic pathogens and contributing to the potential for discoloured water events. More research is required to inform the development of guidelines for the management of biofilms in long pipelines to ensure the delivery of safe drinking water and to minimise impacts on water quality.

The *Australian Drinking Water Guidelines* (ADWG) [2] provide a framework for the management of the catchment, treatment and distribution systems for provision of safe drinking water. The ADWG clearly state that "... the greatest risks to consumers of drinking water are pathogenic microorganisms..."; however, the ADWG does not currently contain any specific information that refers to or provides guidelines for the management of biofilms in water distribution systems. Current monitoring regimes and guideline values are based on the analysis of bulk water samples. Given the relative abundance of sessile microorganisms (biofilms) over planktonic microorganisms (bulk water), this approach at best provides a significant underestimate of the microbiological status of the water distribution system. Recent Australian and international research is shedding new light on the roles and impacts of biofilms, and how they can be managed in order to limit their impact.

Biofilms grow when they are provided with adequate sources of energy, carbon, nutrients, and favourable temperatures. Most water sources contain natural organic matter (NOM). Organic compounds within the NOM provide a suitable source of energy and carbon for growth by heterotrophic microorganisms. Assimilable organic carbon (AOC) has been defined as the fraction of dissolved organic carbon (DOC) which microorganisms use to produce new biomass. Specific tests are used to quantify the AOC content in distributed water in order to help determine the 'biostability' of the water [3, 4], although their use in Australia is limited to research applications.

In some cases, inorganic compounds can act as an important energy source for biofilm growth. In parts of Australia, the less reactive chloramine is used instead of chlorine to maintain disinfection in long pipelines. To achieve chloramination, ammonia is combined with chlorine to produce monochloramine. Ammonia serves as the energy source for growth of ammonia oxidising bacteria (AOB) which oxidise ammonia to nitrite [5]. Loss of ammonia due to nitrification prevents the formation of monochloramine which leads to poor or no disinfection. Nitrifying biofilms have the ability to fix carbon dioxide and therefore act as 'primary producers' by generating organic carbon in the form of new biomass that can then serve as an energy source of heterotrophic microorganisms.

Parts of the Goldfields and agricultural water supply system in the wheat belt of Western Australia are impacted by nitrifying biofilms which prevent the formation of monochloramine [unpublished data]. This system is characterised by long above-ground pipelines with relatively long detention times. In the warmer months, water temperatures regularly exceed 30°C and often exceed 40°C. These elevated temperatures provide ideal conditions for the biofilm growth and correlate well with

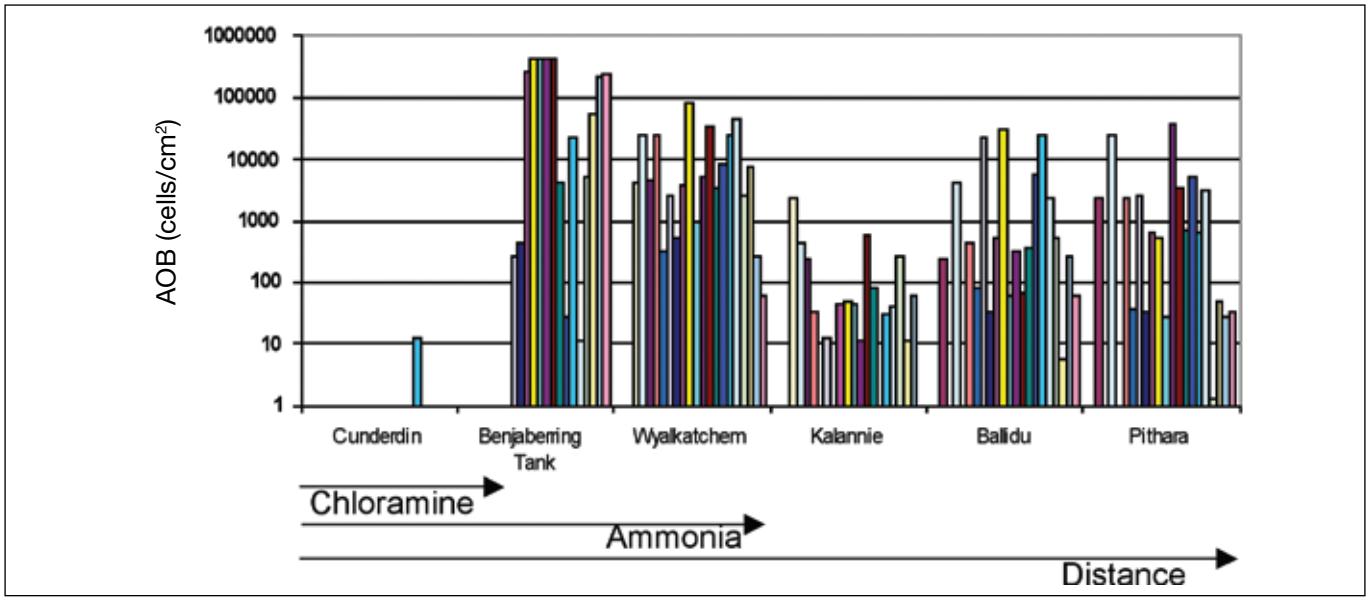

Figure 1. Numbers of ammonia oxidising bacteria (AOB) per cm² at different locations within parts of a chloraminated distribution system in Western Australia. Individual columns represent monthly or bimonthly samples collected from each site between early 2006 and mid 2008. Arrows indicate penetration of chloramine and ammonia and increasing distance through the system.

increases in ammonia oxidising bacteria in pipewall biofilms and concomitant loss of free ammonia in solution (Figure 1). Recent studies show that novel strains of nitrifying bacteria comprise a significant part of pipewall biofilms in the most active nitrifying zones [Ginige *et al.* in preparation].

Conditions of elevated water temperatures and little or no disinfectant residual biofilm growth provide an abundant food source for protozoa and other bacterivorous microorganisms. *Naegleria fowleri,* the causative agent of primary amoebic meningoencephalitis (PAM), is a thermotolerant free-living amoeba (Figure 2) that is occasionally found (to date in at least three Australian states) in pipewall biofilms growing at temperatures >20°C. Although infections with *N. fowleri* are rare, they are usually fatal. No cases of PAM have been reported in Australia in recent decades, although several fatal cases have occurred in the USA this decade, some due to poorly disinfected drinking water.

Detection of *N. fowleri* is therefore an important part of water quality monitoring in potentially affected distribution systems. The current testing procedure employs a culture-based method to enrich and purify pure strains of thermotolerant *Naegleria* which are then typed using a PCR-based molecular test [6]. This testing procedure takes at least 2-3 days before a positive detection is confirmed due to the need to culture the *Naegleria* spp. A new PCR-based method has recently been developed that is capable of specifically detecting *N. fowleri* in bulk water or biofilm [Puzon *et al.* in preparation]. This method is at least as sensitive, and more rapid, than the current testing procedure as it does not rely on the need to culture *Naegleria* spp. This test has the ability to provide a quantitative estimate of *N. fowleri* cell numbers in less than 24 hours. The application of this new method will hopefully lead to improved monitoring and management of distribution systems to prevent colonisation by *N. fowleri.*

While they do not represent a health risk, water aesthetics issues such as taste and odour problems, or discoloured water events, are significant issues due to their ability to rapidly generate negative public perceptions. These issues often generate the greatest number of customer complaints made to water utilities. The formation of mature pipewall biofilms can lead to the generation of water aesthetics problems. A 'swampy odour' due to the formation of dimethyl trisulphide in distributed water was attributed to the actions of biofilm microorganisms [7]. Biofilm reactor experiments showed that dimethyl trisulphide production from potential organosulphur precursors like cysteine and methionine was insignificant in the absence of biofilms. Mature biofilms can metabolise organic compounds and are also able to oxidise and accumulate inorganic materials such as metals.

An example of this ability is shown in Figure 3, where an iron-rich upper layer was found to cover a biomass-rich lower layer. Iron and manganese oxidising bacteria in water distribution systems have been well studied [8, 9], although there is limited information on the rates of iron and manganese accumulation within mature biofilms and the factors that influence this, e.g. bulk water Fe and Mn concentrations, disinfectant residuals, biofilm age or thickness. Recent laboratory findings show that even with acceptably low concentrations of Fe and Mn (0.05 and 0.02mg/L respectively) in the bulk water, over time, stable biofilms have the ability to accumulate sufficient Fe and Mn to provide the potential for a discoloured water event [Ginige *et al.* submitted]. This finding suggests that prevention of biofilm formation is at

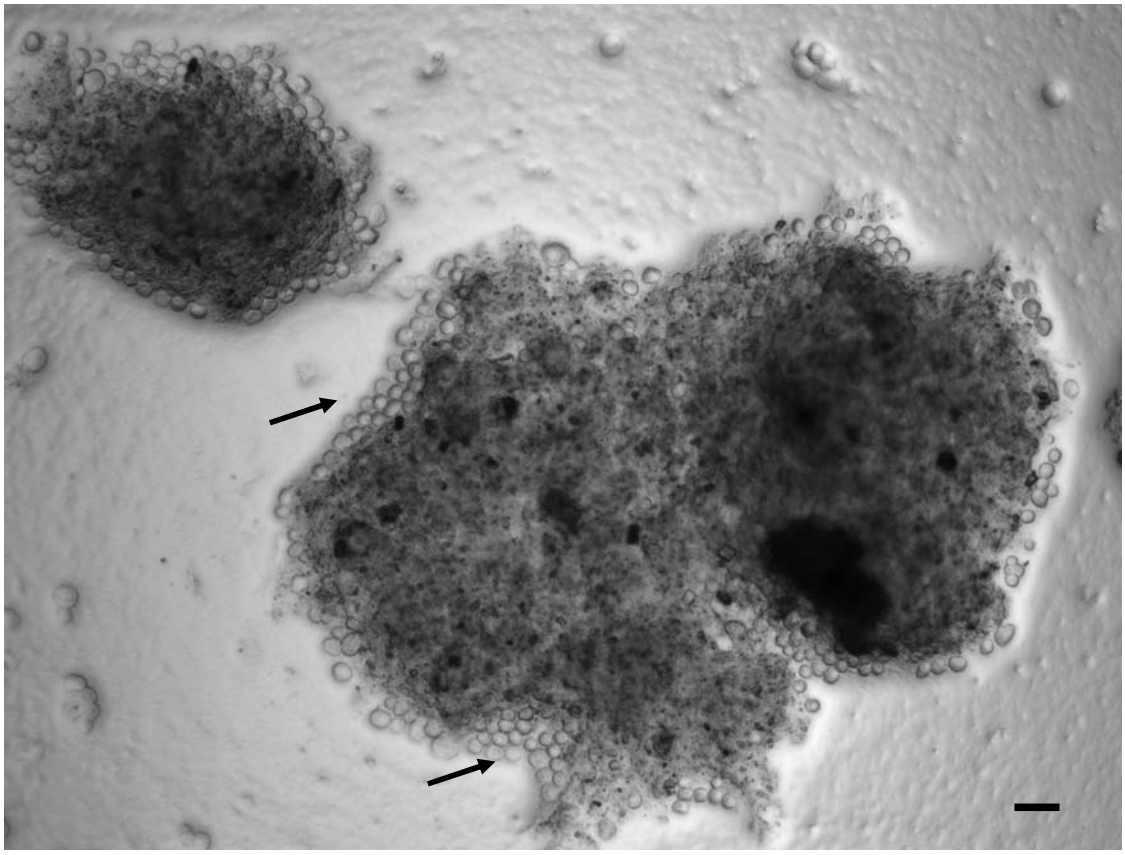

Figure 2. Photomicrograph of *N. fowleri* cysts enriched on a lawn of *E. coli* cells. Arrows indicate cysts. Scale bar=20μm.

Figure 3. Photograph of a mature biofilm with metal-rich upper layer above a biomass-rich lower layer on the pipewall surface within a distribution system (photo by Luke Zappia).

least as important as the effective treatment of water to achieve low concentrations of Fe and Mn.

Pipelines distributing lower quality non-disinfected water (e.g. stormwater, re-use water, untreated third pipe systems) likely provide more favourable conditions for biofilm formation. The level of management or control of biofilm formation within these systems will be dependent on the end-use of these waters and other considerations. Enhanced biofilm growth due to lower water quality will pose many of the same challenges as those outlined above; however, it has the potential to pose significant additional problems for treatment and distribution infrastructure through increased fouling of membrane-based filtration systems and also increased microbially induced corrosion.

Improved treatment of source water to remove AOC or other energy sources for microbial growth, combined with advanced disinfection strategies, represents the best way to prevent biofilm formation in drinking water distribution systems. For non-potable water distribution systems, more research is needed to better understand the likely impacts of biofilm growth on treatment and supply infrastructure, and to assess any potential hazards to human and environmental health.

Acknowledgements

The authors wish to acknowledge the Water Corporation of Western Australia for funding and collaborating on some of this research. The invaluable efforts of Jason Wylie and James Lancaster are also acknowledged.

References

1. Flemming, H.-C. *et al.* (2003) Contamination potential of biofilms in water distribution systems. *Wat. Sci. Tech. Water. Supp.* 47, 271-280.
2. NH&MRC and NMMERC (2004). Australian Drinking Water Guidelines 6.
3. Van der Kooij, D (1992) Assimilable organic carbon as an indicator of bacterial regrowth. *J. Am. Water Works Assoc.* 84, 57-65.
4. Van der Kooij, D *et al.* (1999) Maintaining quality without a disinfectant residual. *J. Am. Water Works Assoc.* 91, 55-64.
5. Regan, J.M. *et al.* (2002) Ammonia- and nitrite-oxidising bacterial communities in a pilot-scale chloraminated drinking water distribution system. *Appl. Environ. Microbiol.* 68, 73-81.
6. Robinson, B.S. *et al.* (2006) Rapid, sensitive, and discriminative identification of *Naegleria* spp. by real-time PCR and melting-curve analysis. *Appl. Environ. Microbiol.* 72, 5857-5863.
7. Franzmann, P.D. *et al.* (2001) The formation of malodorous dimethyl oliogosulphides in treated groundwater: the role of biofilms and potential precursors. *Water Res.* 35, 1730-1738.
8. Lechevallier, M.W. *et al.* (1987) Examination and characterization of distribution system biofilms. *Appl. Environ. Microbiol.* 53, 2714-2724.
9. Sly, L.I. *et al.* (1988) *Pedomicrobium manganicum* from drinking water distribution systems with manganese related dirty water problems. *Syst. Appl. Microbiol.* 11, 75-84.

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Quantitative microbial risk assessment (QMRA) for water re-use via aquifers

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Worldwide, there is an increasing interest in the recharge of aquifers as a method for augmenting urban water supplies. Managed aquifer recharge (MAR) can utilise a variety of non-traditional source waters including urban stormwater and reclaimed water from sewage effluent. However, these alternate water sources may contain a wide range of pathogenic hazards that pose risks to human health. Hence the safe use of recycling water via aquifers requires potential risks to be reduced to acceptable levels. This article outlines the approach recommended by the draft *Australian Guidelines for Water Recycling (AGWR) (Phase 2C Managed Aquifer Recharge)* [1] to quantify the aquifer treatment using a quantitative microbial risk assessment (QMRA) approach [2].

The first step in a QMRA is to define an acceptable level of risk; this is then used to set health-based targets for individual hazards. Assessing the disability-adjusted life years (DALYs), which accounts for the severity of each hazard, is the recommended approach advocated in AGWR [2], with an annualised risk of 10^{-6} DALYs per person not to be exceeded.

DALYs are applied once pathogen numbers, dose-responses and exposures are determined; that is, after completion of a QMRA. This typically incorporates the following four steps:

- *Hazard identification.* AGWR [2] recommend using the reference pathogen hazards (Campylobacter, *Cryptosporidium parvum,* rotaviruses) to represent bacteria, protozoa and viruses respectively in the QMRA. These hazards are used both for stormwater and reclaimed sewage effluent. This step also includes consideration of the potential variability in pathogen numbers (typically assessment of the mean, median and 95th percentile numbers) as well as the treatment provided by the components of the MAR system.
- *Dose-response.* Establishes the relationship between the dose of the reference pathogen and the likelihood of illness.
- *Exposure assessment.* Identifies the population exposed to the hazard, and the pathway, quantity and duration of exposure. This step includes assessment of both the intended volume of the recycled water (e.g. ingestion of sprays from garden irrigation estimated at 0.1ml) and the frequency of the exposure (default 90 times / year for garden irrigation) [2].
- *Risk characterisation.* Calculates the DALYs to determine if the MAR scheme is of an acceptable risk.

The last step in risk assessment is to integrate information from hazard identification, hazard concentration considering any treatment barriers (including the aquifer), dose-response and exposure assessment, to determine the magnitude of risk. The magnitude of risk should be assessed on two levels – maximum risk (risk in the absence of any preventive measures such as treatment) and residual risk (risk that remains after consideration of existing preventive measures).

Determination of residual risk can be an iterative process, and will depend upon the residence time of the pathogen in the aquifer and its decay rate among other preventive measures. The draft *AGWR phase 2C Managed Aquifer Recharge* [1] advocate that the aquifer can be recognised as a treatment step much like conventional water treatment barriers, such as disinfection, for the deactivation of pathogens.

In cases where a simple exponential decay function can approximate the viable pathogens remaining, the numbers of pathogens in water recovered from a MAR scheme may be

described by $C_t = C_0\ 10^{-t/\tau}$ where C_0 is the initial number of pathogens in the recharge (n/L), t is the residence time (days) and τ is the time required for one-log10 removal. The required log reduction will depend upon the quality of the source water, the total log reduction of all treatment steps (not just the aquifer), the exposure scenario and any other water use controls in place (e.g. with holding periods for irrigation).

For example, the aquifer storage transfer recovery (ASTR) scheme in Salisbury, SA uses urban stormwater which is pre-treated in a wetland and then injected into a confined limestone aquifer prior to recovery from wells located 50m away. Wetland and aquifer residence time calculated by hydraulic modelling were an average of 10 and 270 days respectively. The combined treatment potential of the wetland and aquifer was estimated to be >6 log for removal of the reference pathogens, determined by insitu decay studies (to validate inactivation rates and residence times). The majority of this treatment (>4 log) was within the aquifer due to the longer residence time and warmer temperature than the wetland (~2 log for bacteria). The treatment potential of the ASTR scheme was determined to be satisfactory for use of the recovered water for drinking (residual risk $<10^{-6}$ DALYs). Recovered water and observation wells within the attenuation zone are being sampled as part of the verification monitoring program. Samples are analysed for microbial indicators and reference pathogens to assess the effectiveness of all barriers including aquifer treatment.

Multiple barriers (preventive measures), including source protection, exclusion of water from polluted sources, pre-treatment prior to recharge, aquifer treatment, post-treatment if necessary, and water use controls, together with following a risk management plan on how to manage and monitor these measures, will ensure that the residual risk is acceptable. By understanding each of the barriers an estimate of the required retention time in the aquifer can be made. These processes are described in the draft AGWR Phase 2C [1].

References

1. EPHC, NHMRC and NRMMC (2008) *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks, Phase 2C Managed Aquifer Recharge.* National Water Quality Management Strategy 21. Environment Protection and Heritage Council, National Health and Medical Research Council and Natural Resource Management Ministerial Council. Canberra, Australia. (Public consultation draft) http://www.ephc.gov.au/pdf/water/200805_WQ_GL_Draft_AGWR_MAR.pdf
2. NRMMC (Natural Resource Management Ministerial Council), EPHC (Environment Protection and Heritage Council) & AHMC (Australian Health Ministers' Conference) (2006). *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks. Phase 1. National Water Quality Management Strategy.* NRMMC/EPHC/AHMC, Canberra, Australia.

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Meeting the recycled water challenge for Sydney

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Sydney Water is seeking to maximise the delivery of recycled water meeting suitable standards for the intended use. The approach of health risk management through the 12 components of the national guidelines for water recycling is used in close consultation with the NSW Department of Health. Considerable effort is being put into demonstrating compliance with the guidelines when they are applied to specific recycling projects.

The recycling challenge

Sydney has very high quality surface water sources of drinking water. However, with a growing population, unpredictable rainfall, the impact of a prolonged drought and the potential impacts of climate change, these sources needed supplementing. The 2006 NSW Metropolitan Water Plan [1] sought to diversify the sources of water available in Sydney and at the same time to provide flows to maintain river health. The plan set an ambitious target for water recycling from sewage to save 70 billion litres ($70x10^9$ litres) of potable water by 2015 to be achieved through a diverse range of schemes covering domestic, industrial, irrigation and river replacement flows (Figure 1).

Implementing the Australian guidelines for recycled water

The *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1)* [2] (AGWR phase 1) were released in November 2006. Order of magnitude estimates of the efficacy of wastewater treatment processes required to meet health targets for a range of applications of recycled water were given, expressed as log-removal rates. Among others applications, the AGWR phase 1 covered large-scale treated sewage use for residential garden watering, car washing, toilet flushing, fire fighting and in industry. The *AGWR: Augmentation of Drinking Water Supplies* [3] (AGRW – ADWS) followed in May 2008. The latter expand on the principles and information provided in the phase 1 guidelines, including measured levels of pathogens in sewage and expected removal rates provided by a range of sewage treatment processes. The health-based targets for recycled water treatment guidelines have used quantitative microbial risk assessment (QMRA) to set targets for pathogen removal over the whole treatment train appropriate for the intended uses of the recycled water.

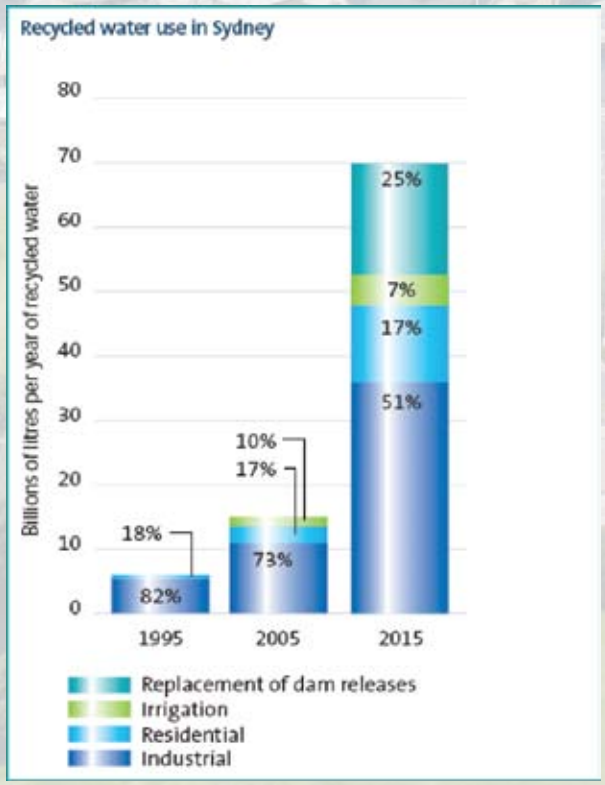

Figure 1. Sydney's recycled water targets for 2015 [1].

These Guidelines use the same 12 elements for managing water quality that are used in the *Australian Drinking Water Guidelines* (ADWG, 2004) and are based on international standard management systems. Some of the elements are:

- Management commitment to responsible use and management of recycled water.
- Supporting requirements that include research, validation of treatment barrier performance, documentation and reporting.
- Review, including verification monitoring and auditing.

Sydney Water is developing recycled water quality management plans to demonstrate that each recycling scheme is managed according to the guidelines. These are built on pre-existing ISO 9000 and ISO 14000 systems. Risk assessments are carried out for each scheme. Substantial contributors to risk are investigated. For example, pathogen presence and removal by treatment processes in place or proposed are validated, as is the extent of human exposure at the point of use. Sydney Water is also researching better understanding of the microbiological risks from recycled water.

Wollongong Stage 2 recycling implementation

Stage 2 of the recycled water scheme for the Wollongong sewage treatment plant (STP) will be the provision of recycled water to the Port Kembla coal terminal for dust suppression on the coal stockpiles. The coal terminal lies immediately south of the STP (Figure 2) and has historically used considerable amounts of potable water for this purpose (Figure 3). Secondary treated wastewater is supplied to the recycled water facility on the STP site (Figure 4).

Working with consultants from Water Futures, Sydney Water's Science and Technology staff undertook a QMRA using three model index pathogens – Campylobacter (bacterial index), rotavirus (viral index) and Cryptosporidium (protozoan index). Exposure was assessed through aerosol or ingestion for site workers and fire fighters.

A validation monitoring program using microbial surrogates *E. coli* (for bacteria such as *Campylobacter* spp.), MS-2 bacteriophage (for viruses including rotavirus) and *Clostridium perfringens* (for parasitic protozoa including *Cryptosporidium parvum*) was undertaken on primary, secondary and tertiary treatment processes at the Wollongong recycled water plant between December 2007 and April 2008 (n=17) (Table 1).

The QMRA undertaken for the (non-potable) use of recycled water at Wollongong Stage 2 did not identify any human health risks that exceeded the acceptable annual risk benchmark of 10^{-4} (1 infection per 10,000 persons *per annum).* A validation monitoring program demonstrated log reduction of pathogens

Figure 2. An aerial view of the Wollongong STP and the Port Kembla coal loading facility.

at the Wollongong Stage 2 recycled water plant exceeding those required in the 2006 *Australian Guidelines for Water Recycling* for industrial, municipal and fire fighting use, in most cases by many orders of magnitude. Values of 14.3 log reduction were achieved for bacteria (target 5.3), 9.3 log for viruses (target 6.5) and greater than 6.6 log reduction for protozoa (target 5.1), ensuring that recycled water was treated fit for its intended application in industry and irrigation as well as fire fighting.

The recycled water quality management plan for this scheme was endorsed by the NSW Health Department and approved prior to allow the data collection pre-commissioning for the scheme to commence on time in June 2008.

Figure 3. Water used for dust suppression.

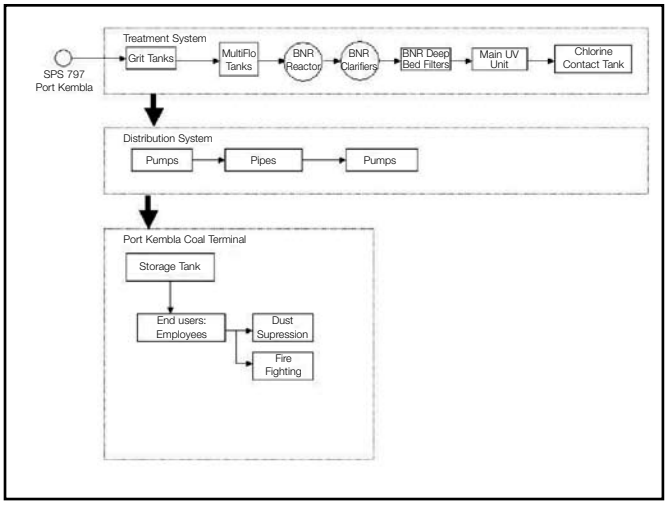

Figure 4. Schematic of treatment train and delivery of recycled water delivered to Port Kembla coal loading facility for dust suppression and fire fighting used to inform the quantitative microbial risk assessment. BNR – biological nutrient reduction.

Opportunistic pathogens in recycled water distribution system biofilms

Through the wastewater program of the Cooperative Research Centre for Water Quality and Treatment, a national survey of opportunistic pathogens in water delivery systems was undertaken [4] to better understand the risks they may pose and inform the operational management of recycling systems. Eight water utilities participated in the study, including Sydney Water, using seven drinking water and six recycled water systems as

Table 1. Log reduction rates obtained in the validation monitoring program and used in the risk assessment for the major pathogen groups (bacteria, virus and protozoa) in primary, secondary and tertiary treatment at Wollongong STP.

	Log (decimal) reduction		
	Bacteria	**Virus**	**Protozoa**
Primary/secondary			
Primary	0.25 (0-0.5)	0.05 (0-0.1)	0.25 (0-0.5)
Secondary	2.0 (1.0-3.0)	1.25 (0.5-2.0)	0.75 (0.5-1.0)
Tertiary			
Dual-media filtration	0.5 (0-1.0)	1.75 (0.5-3.0)	2.0 (1.5-2.5)
Chlorination	4.0 (2.0-6.0)	2.0 (1.0-3.0)	0.25 (0-0.5)
UV	3.0 (2.0-4.0)	> 3.0	> 3.0
Total (predicted)	**9.75**	**8.05**	**6.25**
Total (validated)	**14.3**	**9.3**	**>6.6**
Target [2]			
Municipal	4.0	5.2	3.7
Industrial	5.1	6.4	5.0
Fire fighting	5.3	6.5	5.1

study sites (Figure 5). With the exception of the Rouse Hill dual reticulation system, where water is designated for indirect human contact through toilet flushing, car washing and garden use, the recycling schemes used recycled water for industrial use as well as the irrigation of municipal landscape and recreational grounds. The University of New South Wales, CSIRO, the Australian Water Quality Centre and Sydney Water laboratories provided specialised research and analysis.

The study had three aims, namely to:

- Determine the incidence of opportunistic pathogens and faecal indicators and pathogens in potable and recycled water distribution systems.
- Undertake a preliminary (screening-level) qualitative risk assessment to estimate their significance within a water distribution system.
- Assess the efficacy of factors leading to their control and risk management.

Traditional faecal indicators *E. coli,* total coliforms and enterococci were quantified by standard methods for water testing in the recycled systems. Opportunistic pathogens tested for included *Acanthamoeba* spp., Aeromonads, Legionella, Mycobacteria and Pseudomonads. In addition to these organisms, the presence of Campylobacter (by culture) and Helicobacter (by PCR) were also assessed. Both water and pipe biofilms (Figure 6) were tested. To assess seasonal variability, samples were taken during a winter (June-August 2005) and a summer (January-March 2006) (Table 2). For each recycled water system investigated, an adjacent potable water distribution system was used to provide an estimate of relative risk between both systems.

In addition to the microbiological parameters, physical and chemical water quality parameters were also collected to assess their impact on the regrowth of bacteria in the water distribution systems. This information included the level of water treatment, age and condition of the distribution system, piping material, quantity and size distribution of particles, disinfectant type and concentration, nutrient concentration, pH, conductivity, temperature and hydraulic demand.

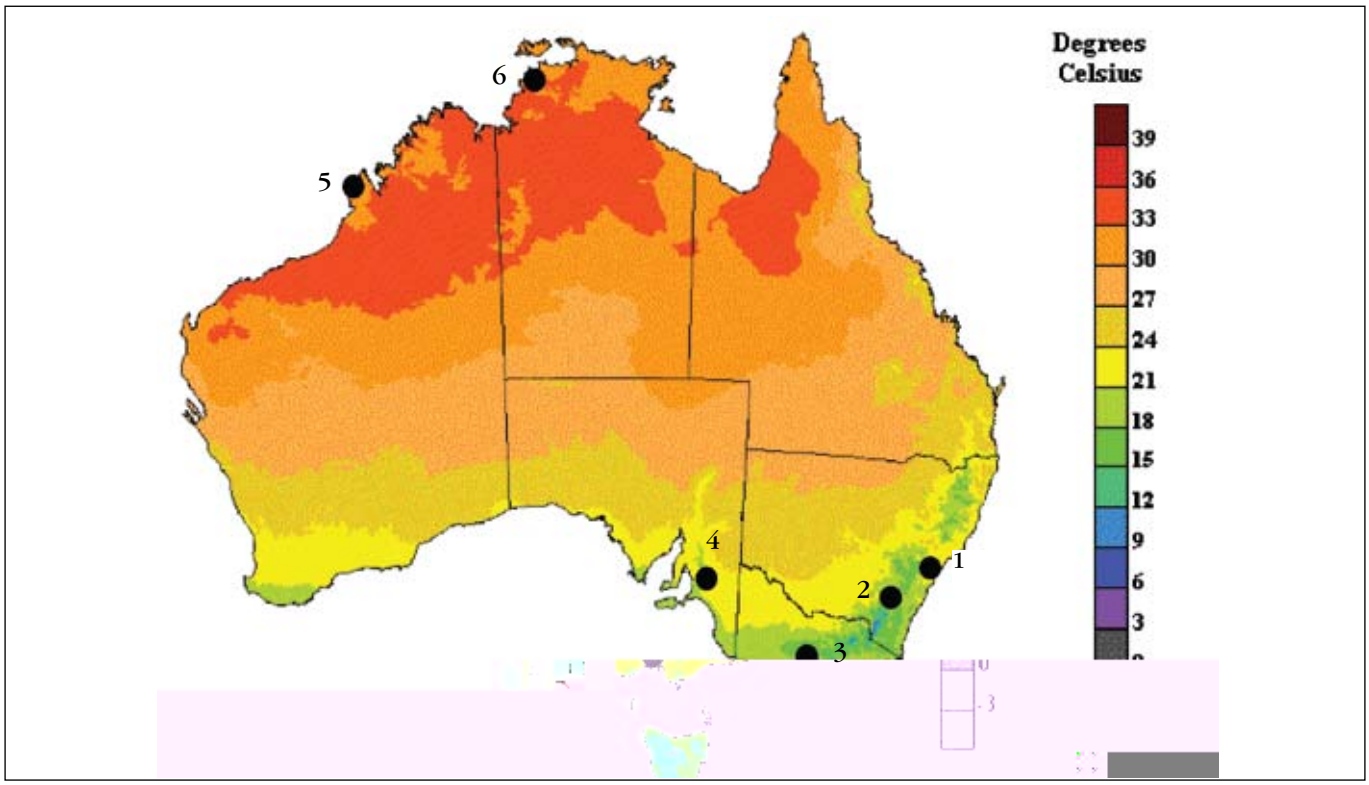

Figure 5. Project study recycled water scheme locations covered major climatic zones in Australia.
1. Rouse Hill Development Area, NSW (Sydney Water); 2. North Canberra Effluent Reuse Scheme, Fyshwick (ACTEW-AGL and Ecowise Environmental), ACT; 3. Eastern Treatment Plant, Carrum Downs (Melbourne Water), VIC; 4. Bolivar Recycled Water Plant (SA and United Water), SA; 5. Broome Recycled Water Scheme (Water Corporation), WA; 6. Darwin Recycled Water Scheme (Power and Water Corporation), NT. (Map courtesy of Australian Bureau of Meteorology).

Table 2. Incidence of opportunistic pathogens in recycled water distribution systems biofilms during summer (S: January – March 2006) and winter (W: June – August 2005) months.
1. North Canberra Water Reuse Scheme, ACT; 2. Rouse Hill Development Area, NSW; 3. Eastern Treatment Plant, VIC; 4. Bolivar STP, SA; 5. Broome Recycled Water Scheme, WA; 6. Darwin Recycled Water Scheme, NT. Results are expressed as colony forming units (cfu) in biofilms per square centimetre of pipe surface.

	1		2		3		4		5		6	
	S	W	S	W	S	W	S	W	S	W	S	W
Coliforms								NT				
E. coli								NT				
Enterococci								NT				
Somatic phage								NT				
F-RNA phage								NT				
Aeromonads								NT				
Pseudomonas								NT				
Legionellae								NT				
Burkholderia	NT	NT	NT	NT	NT	NT	NT	NT				
Campylobacter								NT				
Clostridia								NT				

NT – Not tested
<1000 cfu/cm²
Not detected
>1000 cf
<100 cfu/cm²

In this study, the water temperature, hydraulic demand and level of disinfectant residual were most correlated with the presence of opportunistic pathogens in recycled water systems. The detection of opportunistic pathogens in the environment is rarely associated with disease. The data obtained were used to perform a screening level (qualitative) microbial risk assessment for each scheme. Every scheme examined was shown to provide recycled water fit for purpose.

Acknowledgements

In addition to those groups mentioned in the article, the work described was carried out in close liaison with many groups in Sydney Water covering operations, planning and laboratory analysis.

Figure 6. Recycled water biofilm.

References

1. Metropolitan Water Directorate (2006) Metropolitan Water Plan, NSW Government.
2. The *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1)* (2006) Environment Protection and Heritage Council, the Natural Resource Management Ministerial Council and the Australian Health Ministers' Conference.
3. *Australian Guidelines for Water Recycling: Augmentation of Drinking Water Supplies* (2008) Environment Protection and Heritage Council, the National Health and Medical Research Council and the Natural Resource Management Ministerial Council.
4. Storey, M *et al.* (2008) Opportunistic pathogens in drinking and recycled water distribution systems *Water* 35, 38-45.

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Mark Angles and Michael Storey are microbiologists who are Project Managers in the Water Quality and Public Health team at Sydney Water. Both have extensive experience in microbial water quality and risk assessment. Mark managed a project in the Cooperative Research Centre for Water Quality and Treatment (CRC WQT) to assess nutrient impacts on biofilm development in drinking water distribution systems and has recently validated pathogen and water quality indicator removal in wastewater treatment process used to supply recycled water. Michael has previously worked with the Swedish Institute for Infectious Disease Control and CSIRO in the field of quantitative microbial risk assessment and microbial regrowth, and in particular with the legionellae. He currently advises Sydney Water on related matters, with particular emphasis on its recycled water schemes.

The safe use of recycled water

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Continued population growth, droughts and limited water storage capacity are placing ever increasing pressure on Australian water supplies. One of the responses to this pressure has been increased use of recycled water (Figure 1). However, increased use has to be balanced against protection of public health; the greatest risk is from enteric microorganisms. The separation of human drinking water supplies from wastewater has been the largest single contributor to improved population health in the developed world through reducing infectious disease and extending life expectancy [1, 2]. The new *Australian Guidelines for Water Recycling* (AGWR) [3] describe how recycled water schemes can be designed, operated and managed to ensure that they are safe. Reactive management based on end-point monitoring and using *E. coli* as a focus for assessing microbiological safety has been superseded by a preventive risk management approach.

Australian guidelines for water recycling

Limitations in guidance on safe and sustainable use were seen to be a significant barrier to the expanded use of recycled water [4, 5]. As a result, the development of new AGWR was commenced in late 2003. The first phase was published in 2006 and a second phase module on *Augmentation of Drinking Water Supplies* [6] was published in 2008. The second phase will be completed in early 2009. From a public health point of view, the major differences between the AGWR and previous guidelines include a focus on applying a risk management strategy to assure safety prior to use of recycled water. This is underpinned by a quantitative definition of microbial safety. The strategy includes methods for setting and meeting health-based targets for enteric bacteria, viruses and protozoa.

Figure 1. Signs indicating use of recycled water at Mawson Lakes.

Definition of safety

An essential first step is providing a quantifiable definition of safety. The guidelines do this using the metric of disability-adjusted life years (DALYs) to assess potential health impacts. An advantage of DALYs is that they recognise that not all pathogens are created equal, some only cause mild diarrhoea while others such as *E. coli* 0157 can cause more severe symptoms including haemolytic uraemic syndrome and death. DALYs reflect this variability by multiplying the frequency and severity of symptoms by duration to determine a burden of illness for individual pathogens. In the AGWR, safety is defined as being below a burden of 10^{-6} DALYs per person per year. This is equivalent to about one case of diarrhoea per 1000 people per year, which is well below the reported annual rate 0.8-0.92 cases of diarrhoea per person in Australia [7, 8].

Risk management

The definition of safety provides the goalposts that need to be achieved. The mechanism for meeting the goal is a risk management system. The guideline describes a purpose-designed system derived from the model included in the *Australian Drinking Water Guidelines* [9]. It incorporates hazard analysis and critical control point principles as well as features from other existing risk management systems.

At the heart of the system is risk assessment, identification of appropriate control measures and monitoring of those measures. For pathogens, quantitative microbial risk assessment (QMRA) is used to determine the likelihood of illness occurring from specific pathogens in sources of recycled water. This is then converted to health impacts using the DALY approach. It is not practical to do this for all pathogens, so Campylobacter, rotavirus and Cryptosporidium have been used as representatives of enteric bacteria, viruses and protozoa.

The guidelines provide default values for concentrations of pathogens in sewage and for exposures associated with typical end uses. Using QMRA, this enables calculation of reductions required to meet the goalpost of 10^{-6} DALYs per person per year (Table 1). These can be achieved by either reducing pathogen concentrations using treatment or by reducing exposure through mechanisms such as application controls (e.g. drip versus spray irrigation), applying buffer zones between points of use, and public access or crop restrictions (e.g. irrigation of fruit trees rather than lettuce).

Table 1. Log reductions for the safe use of treated sewage.

End-use	Exposure (L p.a.)	Required log reductions Viruses	Cryptosporidium & Campylobacter
Dual reticulation (toilet flushing, gardens)	0.67	6.5	5
Food crops	0.49	6	5
Irrigation of parks	0.05	5	4
Drinking water augmentation	700	9.5	8

Table 2. Achieving required virus reduction for irrigation of parks with treated sewage.

Log reduction required	Exposure reduction	Log reduction	Treatment	Log reduction
5			Secondary treatment Filtration Disinfection	5-6
5	No access Buffer zones Spray drift control	2 1 1	Secondary treatment Filtration Disinfection	2-3

The guidelines describe typical reductions achieved by various types of treatment and exposure controls. Table 2 demonstrates how both types of control can be applied to achieve virus log reductions required for safe irrigation of parks. This dual approach means that even sewage with relatively low levels of treatment can be used safely, provided appropriate end-use or on-site restrictions are applied. However, high exposure uses such as dual reticulation will always rely on high levels of treatment.

In a risk management approach, the focus of monitoring is to ensure that control measures work effectively. This is based on testing for surrogates and indicators of treatment performance and does not involve testing for *E. coli* or any other microorganism. For example, contact time with chlorine correlates with inactivation of enteric bacteria and viruses, while removal of turbidity by filtration correlates with removal of particles such as Cryptosporidium. Providing the relationship between the indicators and pathogens has been established, then monitoring the indicators can be used to demonstrate that pathogen reduction has been achieved. The advantage of the indicators is that many can be measured continuously using automatic monitoring devices connected to alarm systems. Schemes such as the dual reticulation supply at Rouse Hill (NSW) and the salad crop irrigation pipeline at Virginia (SA) use online monitoring devices to measure effective treatment and removal of pathogens. Traditional end-point monitoring and testing for *E. coli* is retained but it is not used as a short-term management tool and it is only one component of measuring microbial safety.

Conclusion

Australia has come a long way since the use of sewage farms in the late 19th and early 20th centuries. The AGWR describes how to apply a risk management approach to ensure that recycled water can be used safely. The guidelines provide a quantifiable definition of safety and mechanisms for reducing concentrations of enteric bacteria, viruses and protozoa to safe levels.

References

1. Pruss, A. *et al.* (2002) Estimating the burden of disease from water, sanitation and hygiene at a global level. *Environ. Hlth. Perspect.* 110, 537-542.
2. Cutler, D. and Miller G. (2005) The role of public health improvements in health advances: the twentieth century United States. *Demography* 42, 1-22.
3. Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, Australian Health Minister's Conference (2006). *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 1).* Commonwealth of Australia.
4. Rathjen, D. *et al.* (2003) *Recycling Water for Our Cities. Report to the Prime Minister's Science Engineering and Innovation Council.*
5. Radcliffe, J. (2004) *Water Recycling in Australia.* Australian Academy of Technological Sciences and Engineering.
6. Natural Resource Management Ministerial Council, Environment Protection and Heritage Council, National Health and Medical Research Council (2008). *Australian Guidelines for Water Recycling: Managing Health and Environmental Risks (Phase 2). Augmentation of Drinking Water Supplies. Commonwealth of Australia.*
7. OzFoodNet Working Group (2003). Foodborne disease in Australia: incidence, notifications and outbreaks. Annual report of the OzFood Network 2002. *Comm. Dis. Intell.* 27, 209-243.
8. Hellard, M.E. *et al.* (2001) A randomised, blinded, controlled trial investigating the gastrointestinal health effects of drinking water quality. *Environ. Hlth. Perspect.* 109, 773-778.
9. National Health and Medical Research Council, Natural Resource Management (2004) *Australian Drinking Water Guidelines.* Commonwealth of Australia.

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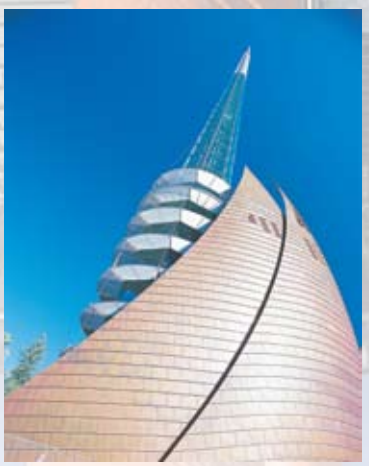

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A consensus: microbial source tracking (MST) in water

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Traditionally, water quality regulation and protection of public health has relied on culture-based methods that quantify faecal indicators such as the coliforms. Since *Escherichia coli* represents over 97% of the thermotolerant coliforms, it has been used extensively as a key indicator of faecal contamination in water testing industry. However the presence of *E. coli* or other coliforms (and more recently enterococci) does not provide any information regarding the source of contamination and therefore is not always an effective indicator of actual risk to humans. While human/animal faecal contamination of water can pose a serious health risk to public, the risk can be managed more efficiently and effectively if the source is known. In this respect, microbial source tracking (MST) can be used as an efficient tool by water managers to improve management of public health.

Indeed, there has been a growing interest in applying MST methods to identify the sources of human/animal faecal contamination over the past 10 years; more than 100 papers being published in this area over the last 3 years alone (Figure 1). These reports all demonstrate how MST methods have been utilised to differentiate groups of microorganisms, usually faecal indicator organisms, for the purpose of tracking sources of faecal pollution. Ecological studies using these methods, alone or in combination, have yielded varying results, sometimes contradictory to each other, leaving water management authorities wondering as to what extent they can rely on the outcome of these methods.

Surface waters are constantly receiving pathogenic microorganisms through defecation of humans (via septic tanks or due to sewage overflow) and animals. Identification of major sources of potential pathogens in water is therefore necessary to minimise the public health risks associated with such contamination. To trace the source of contamination, several MST methods have been used to establish a database of faecal indicator bacteria from known host groups (database-dependent methods). These methods, however, are either not sufficiently discriminatory to differentiate between indicator bacteria in the same species or are not sufficiently reproducible. In addition, some of the currently used methods are either complicated and require special trained personnel, or are costly and labour intensive, therefore not suitable for routine

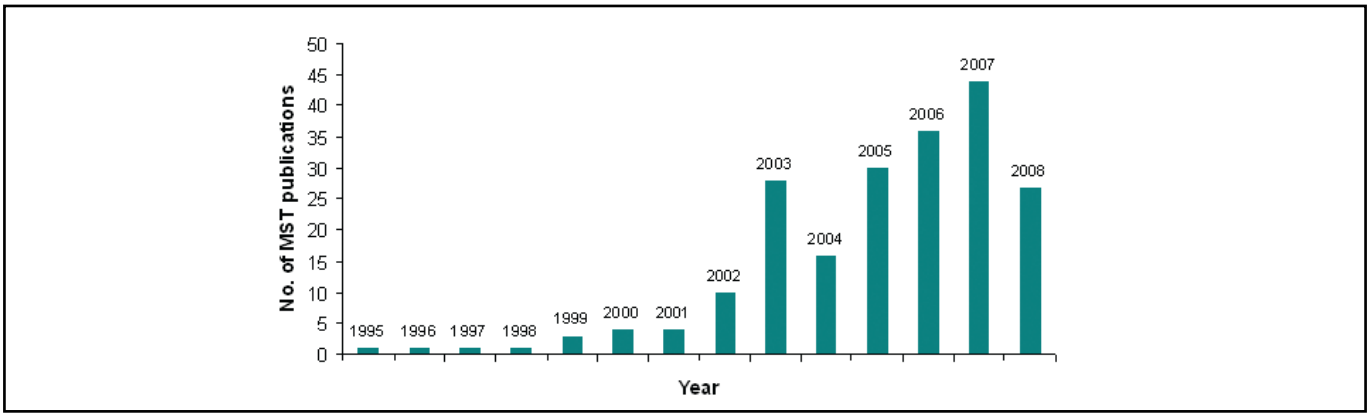

Figure 1. A representation of the increasing interest in microbial source tracking by the number of refereed publications.

water quality monitoring. The current literature also suggests that database-dependent methods require further evaluation in terms of their size and representativeness. Stability of faecal indicator bacteria in the environment is another important factor, which needs to be addressed. Finally, it is not known whether a database developed for a given catchment can be used in another catchment within the same geographical region.

Nonetheless, the objective of these methods is to overcome the limitations of traditional faecal indicator bacteria and more accurately identify the sources of faecal contamination (humans or animals or both). Indeed, some of these methods are designed to differentiate among animal species and to a large extent are capable of doing so [1]; however, these methods require more validation before they can be adopted as a standard tool.

The MST methods can be broadly categorised as microbial and chemical methods. Microbial methods can be further categorised as genotypic and phenotypic methods. Genotypic methods include ribosomal DNA genetic markers of bacteroides, *Enterococcus faecium* enterococci surface protein (esp) marker, ß-glucuronidase gene in *E. coli*, ribotyping, pulsed-field gel electrophoresis, repetitive extragenic palindromic polymerase chain reaction (REP-PCR), amplified fragment length polymorphism (AFLP), denaturing gradient gel electrophoresis (DGGE), terminal restriction fragment length polymorphism (T-RFLP), enterotoxin biomarkers and F^+ coliphages genotyping. Phenotypic methods include multiple antibiotic resistance analysis, carbon source utilisation and biochemical fingerprinting.

Chemical methods have also been used as indicators of human or animal faecal pollutions. These methods include faecal sterols, optical brighteners, caffeine and pharmaceuticals, with faecal sterols being the most commonly used chemical method to trace the source of faecal contamination in surface waters. All of these methods have pros and cons that need to be taken into consideration when setting out to answer a specific question.

The field of MST has been the subject of many recent reviews [2, 3], and the advantages and disadvantages of the existing methods have been summarised in other reviews [1, 4]. In Australia the application of MST for understanding water quality issues has been very much restricted to the research arena and the methods have not been routinely applied in a practical sense. However, there have been a number of studies carried out in south east Queensland to answer various questions for water authorities [5-8].

Implications for the water industry

The management, ie. regulation and legislation, of water quality totally focuses on enumerating faecal indicator organisms. Only recently some more specific pathogens or a wider group of indicator organisms have been added to the list. Water quality managers and those responsible for setting guidelines for water quality monitoring have largely lost sight of the actual pathogens.

Field and Samadpour [2] are sensible in their suggestion of a rational approach starting with epidemiological data that is available to identify the pathogens of concern and then use targeted pathogen monitoring, coupled with targeted faecal source tracking, to best manage water quality and public health. MST then would become a tool within a tool box that could be adapted and applied to answer specific questions. It is curious that this approach has been over sighted since the water industry in Australia and most developed countries have adopted a hazard analysis of critical control point (HACCP) approach to water quality management. MST goes a long way to identifying the critical control points in the water cycle by giving direct information about the source of the contamination.

The World Health Organization suggested that MST and analytical tools for measuring the infectivity and pathogenicity are priority research issues [9]. While the number of research papers has definitely increased since then (Figure 1), the uptake of the information in the water industry has been much slower. A drop off in the number of publications in MST in 2008 may also indicate that the interest, funding or both may not be sustainable in the research community.

Conclusion

MST has the potential to be an extremely useful tool for the management of public health for water supplies. Targeted

risk management approaches to deal with specific sources of contamination can save on resources by reducing the pathogen load at the source through catchment management practices. Other identified advantages of tracking the origin of water-borne pathogens include: orient control activities to priority areas; managing animal presence in catchments; understanding emerging and re-emerging disease; tracking disease to source; support out-break investigations and response.

The consensus on MST by the authors and a number of our colleagues is that MST tools (including database-dependent methods) should continue to be developed, validated and utilised in a tool box approach. More importantly, the routine practice of these methods in water industry, to trace the source of human or animal faecal contamination in a catchment, should be highly encouraged. The choice of methods in such practices may vary and should be primarily based on the questions asked by water industry.

References

1. Ahmed, W. *et al.* (2005) Faecal source tracking in surface waters: a brief review of faecal indicator microorganisms and current methods. *Environ. Hlth.* 5, 51-68.
2. Field K.G. and Samadpour M. (2007) Fecal source tracking, the indicator paradigm, and managing water quality. *Water Res.* 41, 3517-3538.
3. Stoeckel, D. M. and Harwood V. J. (2007). Performance, design, and analysis in microbial source tracking studies. *Appl. Environ. Microbiol.* 73, 2405-2415.
4. Meays, C.L. *et al.* (2004) Source tracking fecal bacteria in water: a critical review of current methods. *J. Environ. Manage.* 73, 71-79.
5. Ahmed, W. *et al.* (2006) Comparison of the efficacy of an existing versus a locally developed metabolic fingerprint database to identify non-point sources of faecal contamination in a coastal lake. *Water Res.* 40, 2339-2348.
6. Ahmed, W. *et al.* (2007) Sourcing faecal pollution: a combination of library-dependent and library-independent methods to identify human faecal pollution in non-sewered catchments. *Water Res.* 41,: 3771-3779.
7. Ahmed, W. *et al.* (2008) Evaluation of Bacteroides markers for the detection of human faecal pollution. *Lett. Appl. Microbiol.* 46, 237-242.
8. Stratton, H.M. *et al.* (2008) Determining the source of *E. coli* contamination in effluent ponds. CRC Water Quality and Treatment Report.
9. Cotruvo *et al.* (Eds) (2004) Waterborne zoonoses, identification, causes, and control. WHO http://www.who.int/water_sanitation_health/diseases/zoonoses/en/

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Microbial population changes during managed aquifer recharge (MAR)

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Managed aquifer recharge (MAR) is a technique that can be used to capture and store water in aquifers under managed conditions for later recovery and use for specific purposes [1]. There is a need to predict water quality changes during MAR, particularly when recycled water is used as the recharged water. An understanding of the interaction between the geochemistry of the aquifer and the microbial population dynamics in the groundwater is important for understanding any water quality changes. A study was undertaken to monitor the changes in the microbial population and link this to changes in the geochemistry. The results obtained showed that the recharge of recycled water to aquifers causes a change in microbial population structure which has direct links to corresponding changes in geochemistry.

There are a number of MAR methods and types of water that can be recharged. One major way MAR can be used is to assist in the recycling of water that would normally be lost or discarded to the environment. MAR has benefits for recycling water in that it can be a cheap form of storage which allows recycled water to be held prior to use. MAR can thus be used as a passive barrier in the water recycling scheme.

Additionally, MAR has been shown to be able to improve the quality of recycled water during passage through the aquifer and during storage [2], thus it may also be able to be used as a treatment barrier. However, to be able to be used as an active treatment barrier, it is important to be able to understand and manage any water quality changes that occur. Changes in the quality of the recharged water occur through a range of physical, chemical and biological processes within the aquifer. While the physical and chemical processes are generally well understood, much less is known about the biological processes. The biological processes occur through the activity of the autochthonous groundwater microorganisms. The role of these microorganisms has been shown to be important for the removal of microbial pathogens [3], trace organic compounds [4] and nutrients [3, 5]. An improved understanding of these biologically-based changes is essential for the prediction of water quality changes during MAR and to enable improved management of these schemes.

In order to gain a better understanding of autochthonous groundwater microbial populations, studies have been undertaken on the impact of changes in aquifer environments on these microbial populations. Groundwater studies have historically focused on contaminated aquifers which are segregated into discrete redox zones dominated by different physiologic microbial processes [6-9]. These and other studies have shown that numerous important geochemical processes in subsurface environments are carried out exclusively by enzyme controlled microbial processes. For example, ferric iron reduction can only occur via ferric iron-reducing bacteria [10]. Groundwater microbial populations are therefore clearly able to change the chemical nature of groundwater. Studies that have combined molecular techniques to describe microbial community structure with multivariate statistics to investigate groundwater microbial and geochemical characteristics have demonstrated an interdisciplinary approach which comprehensively explores these biogeochemical interactions [11, 12].

To further study the connections between geochemical reactions and changes in microbial populations during MAR, the bacterial population dynamics were studied in conjunction with changes in aquifer geochemistry [13,14]. This was done using multivariate statistics for two contrasting MAR techniques using secondary treated effluent at two different geographical locations (Perth, WA and Adelaide, SA). Variation in non-cultured groundwater bacterial population dynamics were studied in conjunction with changes in aquifer geochemistry. Principal component analysis (PCA) was used to investigate spatial and temporal changes in the overall 'chemical signature' of the aquifers using an array of chemical analytes which demonstrated a migrating geochemical plume.

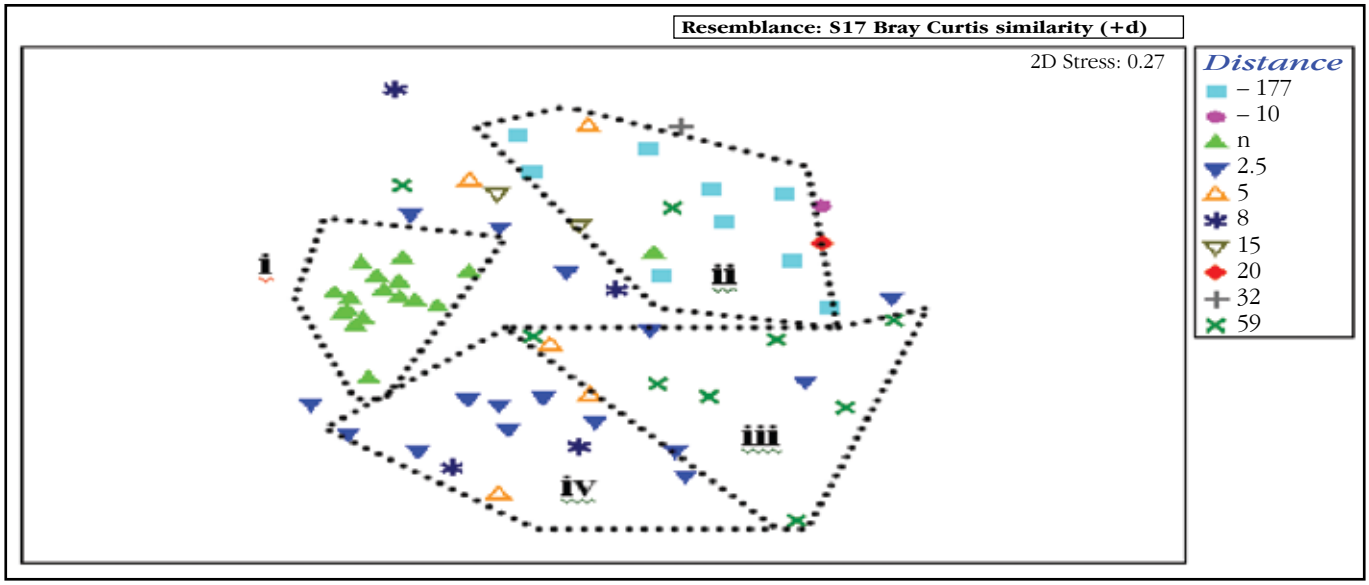

Figure 1. Example of a 2-D MDS plot for amplified microbial rDNA/DGGE banding patterns for all Perth MAR sulphate-reducing cultures demonstrating the clusters of similar population groups at different distances from the recharge site over time. Interpreted MDS clusters are i = Infiltration Galley; ii = background bore; iii = extraction bore; and iv = Monitoring bore set at 2.5 m from Infiltration Galleries

The PCA demonstrated that a migrating nutrient plume occurred in the form of a chemical gradient that varied with time. Denaturing gradient gel electrophoresis (DGGE) using DNA from cultures of groundwater bacteria and groundwater DNA extracts was used to detect spatial and temporal changes in population dynamics. Permutational multivariate analysis of variance (PERMANOVA), supported by multidimensional scaling (MDS) and principal coordinate (PCO), provided evidence of significant spatial and temporal differences in bacterial community structure. An example of these changes over time and distance can be seen in Figure 1 as the MDS plot of sulphate reducing bacteria population dynamics at one of the MAR sites studied.

Distance from the infiltration gallery (nutrient source) was able to be identified as the dominant factor that caused dissimilarities in microbial biodiversity. Distinct microbial populations developed in a distance-dependent successional manner concomitant with geochemical plume migration, suggesting that groundwater microbial populations responded to the chemical gradient. The results obtained also suggested that the groundwater bacterial populations responded to the migrating chemical gradient and to the changes in aquifer geochemistry caused by the MAR schemes. Additionally, the study showed that, at the Adelaide aquifer storage and recovery site, bacterial biodiversity was restored to background population structure when the aquifer geochemistry returned to ambient conditions during the recovery phase.

The outcomes of this study have also demonstrated that detailed microbial population changes may be able to be predicted based on observed changes in the aquifer geochemistry. Research is continuing to link the changes in microbial population dynamics and removal of nutrients, microbial pathogens and chemical contaminants.

References

1. Dillon, P. and Toze, S. (2005) *Water quality improvements during aquifer storage and recovery.* AWWA Research Foundation, Denver.
2. Dillon, P. *et al.* (2008) A critical evaluation of combined engineered and aquifer treatment systems in water recycling. *Wat. Sci. Technol.* 57(5), 753-762.
3. Toze, S. *et al.* (2004) Determination of water quality improvements due to the artificial recharge of treated effluent. *Wastewater Reuse and Groundwater Quality:* IAHS Publication 285, 53-60.
4. Ying, G-.G. *et al.* (2008) Decay of endocrine-disrupting chemicals in aerobic and anoxic groundwater. *Wat. Res.* 42, 1133-1141.
5. Dillon, P. *et al.* (2006) Role of aquifer storage in water reuse. *Desal.* 187, 123-134.
6. Stumm, W. and Morgan, J.J. (1981) *Aquatic Chemistry.* John Wiley. New York.
7. Lovely, D.R. (1991) Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* 55, 259-287.
8. Lovely, D.R. and Goodwin, S. (1988) Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments *Geochim. et Cosmochim. Acta* 52, 2993-3003.
9. Chapelle, F.H. (2001) *Ground-water Microbiology and Geochemistry,* 2nd ed. Wiley, New York.
10. Lovely, D.R. *et al.* (1991) Enzymatic versus nonenzymatic mechanisms for Fe(III) reduction in aquatic sediments. *Environ. Sci. Technol.* 26, 1062-1067.
11. Fahy, A. *et al.* (2005) Effects of long-term benzene pollution on bacterial diversity and community structure in groundwater. *Environmental Microbiol.* 7, 1192-1199.
12. Haack, S.K. *et al.* (2004) Spatial and temporal changes in microbial community structure associated with recharge-influenced chemical gradients in a contaminated aquifer. *Environmental Microbiol.* 6, 438-448.
13. Reed D. (2007) Spatial and temporal biogeochemical changes of groundwater associated with Managed Aquifer Recharge in two geographical areas. PhD Thesis, University of Western Australia.
14. Reed DA, Toze S and Chang B (2008) Spatial and temporal changes in sulphate-reducing groundwater bacterial community structure in response to Managed Aquifer Recharge. *Water Science and Technology* 57(5) 789-795.

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Quantitative detection of pathogens in roof-harvested rainwater

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Roof-harvested rainwater is an alternative water source. Though generally considered acceptable for potable use, the presence of pathogens has been reported in research literature [1]. Various zoonotic pathogens are present in faeces of animals that have access to the roof and, following rain events, pathogens may be transported to rainwater tanks via roof runoff. The microbiological quality of water is traditionally assessed by enumerating faecal indicators such as *Escherichia coli* and enterococci [2]. Significant limitations in using faecal indicators include their poor correlation with pathogens and faecal indicator concentrations cannot be used to assess public health risk when compared to the direct monitoring of pathogens [3]. Polymerase chain reaction (PCR)-based techniques enable rapid and direct detection/quantification of pathogens in water that are otherwise laborious to culture using traditional microbiological methods.

In this study, the microbiological quality of roof-harvested rainwater was assessed by enumerating faecal indicators and detecting zoonotic pathogens in samples from rainwater tanks. The significance of this study stems from the fact that, instead of measuring faecal indicators, pathogens that are capable of causing illness were directly measured using quantitative PCR (qPCR) methods. The pathogen concentration data will be used to perform quantitative microbial risk assessment (QMRA). This work forms part of the development of a 'toolbox' of methodologies using qPCR-based methods which can be used to detect and quantify more than 35 microorganisms commonly found in water [more information on the qPCR 'toolbox' can be obtained from the corresponding author].

A total of 84 rainwater samples were collected from 66 residential houses in Brisbane and Gold Coast regions. Membrane filtration method was used for *E. coli,* and enterococci enumeration. For PCR/qPCR analysis, *Aeromonas hydrophila lip* gene, *Campylobacter jejuni mapA* gene, *Campylobacter. Coli ceuE gene, E. coli* O157 LPS, VT1, VT2 genes, *L. pneumophila mip* gene, *Salmonella invA and spvC* genes, *G. lamblia* β-giradin gene and *Cryptosporidium parvum* Cryptosporidium oocyst wall protein (COWP) gene were selected. Most of these genes were selected based on their virulent properties. In addition, priority was given to those genes which are single copy genes (where possible) so that gene copy numbers could be directly converted to cell counts. DNA extraction from rainwater samples, PCR amplification, the standards for qPCR and the primers used for this study are described elsewhere [4]. For each target pathogen, PCR reproducibility, limit of detection, detection efficiency and PCR inhibitory effects were evaluated.

For the samples tested, 57 (65%) were positive for *E. coli.* The concentrations were: 18 (20%) between 1-10 CFU/100ml, 16 (18%) between 11-100 CFU/100ml, 17 (19%) between 101- 1000 CFU/100ml, and 6 (7%) had >1001 CFU/100ml. For the 84 samples, 72 (82%) were positive for enterococci. The concentrations were: 16 (18%) between 1-10 CFU/100ml, 27 (31%) between 11-100 CFU/100ml, 20 (23%) between 101-1000 CFU/100ml, and 9 (10%) had >1001 CFU/100ml. The PCR positive results for potential pathogens are shown in Table 1.

Quantitative PCR assays were performed on selected pathogens considering their prevalence and infectious dose. Though *C. jejuni mapA* gene was detected in one sample, the concentration was below qPCR detection limit. *L. pneumophila*, Salmonella, and *Giardia lamblia* were detected in several samples (Table 1). *L. pneumophila mip* and *Salmonella invA* are single copy genes and were converted to cell numbers (i.e. 1 gene copy = 1 cell). *G. lamblia* β-giradin gene copy numbers were converted to cysts (16 gene copies = 1 cyst). Binary logistic regressions were also performed to identify the correlations between the concentrations of faecal indicator bacteria and the presence/absence of potential target pathogens (Table 2). The presence/absence of the potential pathogens did not correlate with any of the indicator bacteria concentrations.

Roof-harvested rainwater can be of poor microbiological quality.

Table 1. PCR positive results for potential pathogens.

Gene of target pathogen	**PCR positive results/ No. samples tested (% of sample positive)**	**Range of gene copies/100ml**
A. *hydrophila lip* gene	7/84 (8.3)	Not tested
Campylobacter coli ceuE gene	10/27 (37)	Not tested
C. jejuni mapA gene	1/84 (1.1)	Below qPCR detection limit
E. coli O157 LPS gene	0/84 (0)	Not tested
E. coli VT1 gene	0/84 (0)	Not tested
E. coli VT2 gene	0/84 (0)	Not tested
L. pneumophila mip gene	8/84 (9.5)	6-17
Salmonella invA gene	17/84 (20)	6.6-38
Salmonella spvC gene	0/27 (0)	Not tested
G. lamblia β-giradin gene	15/84 (18)	9-51
Cryptosporidium parvum COWP gene	0/84 (0)	Not tested

Table 2. The relationship between faecal indicators and the presence/absence of selected pathogens in samples from rainwater tanks.

Indicators vs. pathogenic microorganisms	**Nagelkerke's R square***	**P-value Δ**	**Odds ratio**
E. coli vs. *A. hydrophila*	0.055	0.460	1.00
E. coli vs. *C. jejun*	0.008	0.775	1.00
E. coli vs. *L. pneumophila*	0.006	0.640	1.00
E. coli vs. Salmonella	0.048	0.198	1.00
E. coli vs. *G. lamblia*	0.019	0.484	1.00
Ent vs. *A. hydrophila*	0.006	0.700	1.00
Ent vs. *C. jejuni*	0.001	0.943	1.00
Ent vs. *L. pneumophila*	0.007	0.555	1.00
Ent vs. Salmonella	0.016	0.388	1.00
Ent vs. *G. lamblia*	0.001	0.928	1.00

* Nagelkerke's *R* square, which can range from 0.0-1.0, denotes the effect size (the strength of the relationship); stronger associations have values closer to 1.0.

Δ *P*-value for the model chi square was <0.05 and the confidence interval for the odds ratio did not include 1.0. Greater odds ratios indicate a higher probability of change in the dependent variable with a change in the independent variable.

The presence of one or more pathogenic microorganisms along with faecal indicators represents a health risk to users. The pathogens had a poor correlation with faecal indicators. Currently we are performing QMRA using Monte Carlo analysis to determine the likely numbers of infections resulting from these exposures. These outcomes in terms of the impact of using roof-harvested rainwater on the disease burden of South East Queensland region of Australia will be interpreted.

Acknowledgements

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References

1. Simmons, G. *et al.* (2001) Contamination of potable roof-collected rainwater in Auckland, New Zealand. *Water Res.* 35, 1518-1524.
2. US Environmental Protection Agency (2000) Improved enumeration methods for the recreational water quality indicators: Enterococci and *Escherichia coli*. Office of Science and Technology, Washington, DC. EPA/821/R-97/004.
3. Hörman, A. *et al.* (2004) *Campylobacter* spp., *Giardia* spp., *Cryptosporidium* spp., Noroviruses, and indicator organisms in surface water in south-western Finland, 2000–2001. *Appl. Environ. Microbiol.* 70, 87-95.
4. Ahmed, W. *et al.* (2008) Real-time PCR detection of pathogenic microorganisms in roof-harvested rainwater in Southeast Queensland, Australia. *Appl. Environ. Microbiol.* 74, 5490-5496.

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Honorary Life Membership

Dr Warish Ahmed is a water microbiologist at the Queensland Department of Natural Resources and Water and Queensland University of Technology. His area of expertise includes faecal pollution tracking and detection and quantification of pathogens in environmental waters.

Ashantha Goonetilleke is a professor in water/environmental engineering at Queensland University of Technology.

Ted Gardner is a principal scientist with the Queensland Department of Natural Resources and Water and an adjunct professor at Queensland University of Technology.

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The re-use of water in agricultural settings

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Agriculture offers considerable opportunities for the safe and sustainable re-use of water, be that water sourced from humans or animals. A key point is understanding the differences in pathogen profiles between wastewater from humans as compared with that derived from animals. Agricultural re-use also offers the opportunity to appropriately match the treatment level of the used water with the planned end-use. There is no doubt that the re-use of water in agriculture will be an increasing focus as Australian agriculture adapts to the challenges of food security in a changing world.

Around the world, there is a recognition that water is a limited resource [1]. This recognition of the limited nature of water resources has resulted in an increasing interest in, and indeed use of, treated wastewater for agricultural applications [2]. Regardless of whether effluent comes from a sewage treatment plant or a piggery, a key issue is managing the pathogens potentially present in the water to be re-used. This means that there is a need to understand the type and level of pathogens present in the wastewater, to understand the efficacy of the treatment system used on the wastewater and to have an understanding of the type of re-use application.

Sampling pasture plots irrigated with piggery effluent.

'Oils ain't oils'

There is a difference between the range of pathogens present in wastewater arising from an animal production system as compared with human sewage. There are over 140 types of enteric viruses that can be present in human wastewater, including astrovirus, hepatitis A virus and norovirus [3]. In contrast, there are few, if any, viruses present in animal wastewater that could be regarded as realistic health risks. In the Australian context, there is no endemic presence of avian or swine influenza, two agents that are of considerable concern in other countries. There is some evidence that suggests that, possibly, rotaviruses (in pigs and cattle) [4] and caliciviruses (in pigs and cattle) [5] may be zoonotic agents. There is considerable evidence that pigs are a source of genotypes III and IV hepatitis E virus for humans [6]. However, with these few exceptions, wastewater from animal production systems does not contain viral agents of concern for human health.

There are pathogens of concern in animal wastewater. Based on the available literature, we have concluded that in both the pig and poultry industries, the only pathogens that pose a realistic public health concern in waste from these industries are *Salmonella* and *Campylobacter* [7, 8]. We have found that *Salmonella* was present in the final treatment ponds of four of 13 piggeries in South East Queensland, although at low levels (the highest level being 51 MPN per 100ml) [9]. In these same piggeries, the level of *Campylobacter* varied from none detectable (two of 13 piggeries) to a maximum of 930 MPN per 100ml [9]. We have performed similar studies on the levels of bacterial pathogens in effluent from Queensland coastal sewage treatment plants (STPs) [10]. In this study, *Salmonella* was detected in the final effluent of six of the 33 STPs, at levels that ranged from 0.7-110 MPN per 100ml [10]. The quantitative information gathered in our studies of STP effluents and pig effluent ponds is an essential basis that is required to develop methods and approaches that allow the safe re-use of these valuable resources.

Evaluating health risks

If water re-use schemes are to be widely adopted in agriculture, there is a need for a solid scientific basis that allows an informed public decision on the risks associated with these activities. We have looked at the survival of key pathogens in soil irrigated with piggery effluent [11]. We monitored the survival of *Arcobacter* and *Campylobacter* at different irrigation sites in South East Queensland over a summer and a winter. At the four sites, *Arcobacter* survival ranged from 7-14 days in summer and 7-42 days in winter. *Campylobacter* survival ranged from 0-4 days in summer and 0-7 days in winter. The rapid-die off of *Campylobacter* suggests that this organism is low risk in pathogen transfer scenarios involving the re-use of piggery effluent. However, *A. butzleri,* an emerging food-borne pathogen, was present in all piggery effluents and all irrigated soils, survived longer than *Campylobacter* and needs to be considered as a potential risk in piggery effluent re-use scenarios [11].

We have also used MS-2 phage (as a surrogate for human enteric viruses) to look at the risks associated with the use of chlorinated, stored effluent to irrigate commercial fruit trees and the potential for pathogen transfer to the environment and the fruit crop [12]. We spiked the holding ponds with MS-2 phage at high levels (1,000 times higher than the typical levels present in South East Queensland sewage effluent) in order to study phage die-off and phage movement in the environment. We found a 10-fold to 100-fold die-off in phage the spiked ponds (with no such die-off in control phage suspensions) within 72 hours. Additionally, we found only very low levels of phage in soil irrigated with the spiked effluent (around 100 phage per gm of soil). Overall, our use of MS-2 phage provided valuable new information on a operating re-use scheme that ensured appropriate guidelines were in place [12].

Sampling effluent pond.

Acknowledgements

The authors acknowledge our collaborators – Ted Gardner, Alison Viertiz and Jim Sands – in our studies. The funding of the Australian pig industry via Australian Pork Limited has been an important support of work on pig effluent.

References

1. Mandilara, G.D. *et al.* (2006) Correlation between bacterial indicators and bacteriophages in sewage and sludge. *FEMS Microbiol. Lett.* 263, 119-126.
2. WHO (1989) *Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture.* World Health Organization.
3. Bitton, G. (1994) *Wastewater Microbiology.* Wiley-Liss.
4. Martella, V. *et al.* (2008) Detection of a porcine-like Rotavirus in a child with enteritis in Italy. *J. Clin. Microbiol.* 46, 3501-3507.
5. van Der Poel, W.H. *et al.* (2000) Norwalk-like calicivirus genes in farm animals. *Emerg. Infect. Dis.* 6, 36-41.
6. Zheng, Y. *et al.* (2006) Swine as a principal reservoir of hepatitis E virus that infects humans in eastern China. *J. Infect. Dis.* 193, 1643-1649.
7. Blackall, P.J. *et al.* (2000) Pathogens and piggery effluent – using science to guide safe, re-use practices. In *2nd National Pig Environmental Conference.*
8. Blackall, P.J. *et al.* (2002) Pathogens and poultry litter – a risk assessment approach. In *7th World Poultry Science Association Pacific Federation Conference/12th Australian Poultry and Stockfeed Convention.*
9. Chinivasagam, H.N. *et al.* (2004) Microbiological status of piggery effluent from 13 piggeries in the south east Queensland region of Australia. *J. Applied Microbiol.* 97, 883-891.
10. Thomas, R.J. *et al.* (2000) Indicator organism levels in effluent from Queensland coastal STPs. *Water* 27, 38-45.
11. Chinivasagam, H.N. *et al.* (2006) Occurrence and survival of *Campylobacter, Arcobacter* and *E. coli* in soil following application of piggery effluent to pasture. In *12th Australian Food Microbiology Conference.*
12. Chinivasagam, H.N. *et al.* (2008) The use of F-specific coliphages to assess effluent treatment and re-use schemes. *Environ. Technol.* 29, 515-524.

Nalini Chinivasagam is a senior research scientist working at the Animal Research Institute, Department of Primary Industries and Fisheries. Her research interests involve studying the survival and spread of food-borne pathogens in and around intensive animal production systems and the re-use of by-products from these systems.

Pat Blackall is a senior principal research scientist working at the Animal Research Institute, Department of Primary Industries and Fisheries. His research interests cover bacterial respiratory diseases of pigs and poultry, molecular epidemiology, antimicrobial resistance, rapid identification and typing of bacterial pathogens and on-farm aspects of food safety.

Enteric bacteria build-up in effluent irrigated plantations

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Australia uses more than 70% of re-used effluent as irrigation in playgrounds, parks, golf courses and race courses [1]. This land irrigation is preferred over other methods (wetlands, tertiary treatment and aquifer storage) for being the economical, practical and vastly applicable option [2, 3]. Bacteria (*Escherichia coli*, and *Salmonella* spp.), protozoa (*Giardia* spp. and *Cryptosporidium* spp.), viruses (Poliovirus, Coxsackie virus and Norwalk virus) and helminths (tapeworms and hookworms) are the major pathogens present in municipal effluent. These enteric pathogens have the potential to enter the food chain and cause health risks. Although enteric pathogens start dying once in contact with aerobic environment, bacterial build-up as well as decay rate should be probed periodically.

The Centre for Plant and Water Sciences at CQU studied bacterial build-ups down to 1m depth under seven municipal effluent-irrigated agroforestry systems thrice at Yeppoon, Capricorn coast QLD. These plantations had either mono or mixed crops of Ma bamboo (B), pangola grass (P) and flooded gum (E), and were irrigated at the rate of 1.42ML ha^{-1} yr^{-1} via two irrigations per week (a small irrigation as compared to cotton/wheat irrigation). The primary objective of this study was to determine if agroforestry systems would have an attenuation effect on enteric microbes.

Periodic enumerations, made over 2 years, revealed that MPN of enteric bacteria increased significantly in comparison to the initial status, irrespective of season and soil depth. The MPN of total bacteria, across times and soil layers, ranged from 33-69x10^3 cfu g^{-1} dry soil under different agroforestry systems (Figure 1). Generally city councils irrigate public sites with effluents, and often the irrigation rates are higher than the one selected for this study. Thus, the effluent irrigated sites could have enteric pathogens present all the time. The mere presence of a pathogen does not cause a health hazard because the health risks are associated with quality of 'exposure to pathogens' as well [2]. However, councils should periodically monitor microbial populations and modify access to those sites to minimise exposure to the pathogens.

Enteric bacterial populations persisted in the soil, irrespective of the season, due to frequent effluent irrigation (every third day). Groundflora, provided with frequent effluent irrigations, was sufficient to foster microbial populations by promoting congenial growth conditions (shade, temperature and humidity). The compositions of tall plants did not affect microbial populations. The *E. coli* populations are known to persist in a subtropical riverbed environments characterised by warm and moist conditions with cyclic periods of wetter and drier weather, all of which are conducive to *E. coli* growth outside the mammalian gut [4]. The microbiota have been reported to persist from 15 days to several weeks after effluent irrigation [5]. Also, spatial and temporal variations in microbial populations are common [6] and

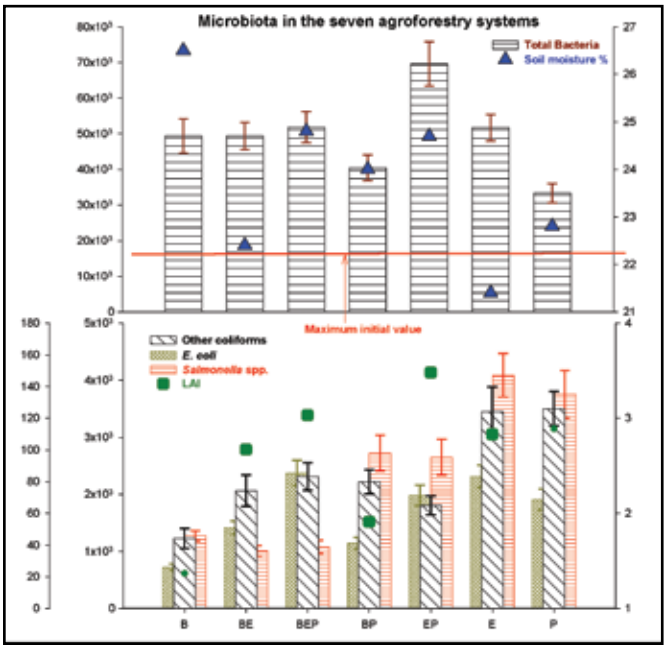


Figure 1. Average MPN of microbial populations in seven agroforestry systems. The data are an average of soil layers and seasons. Leaf area index and soil moisture content of the AF systems are also presented. Maximum initial value is marked by a horizontal line in the A graph (upper).

may require periodic monitoring to assess if different microbial populations prevail. Farmers growing pastures, root crops and bamboo shoots with effluent irrigation should determine the safe frequency of irrigation to avoid enteric microbes as well as minimise their associated health hazards.

The process of predicting the health hazard associated with effluent irrigation is complex but is based on the presence of *E. coli* alone. At times, the MPN of typhoid-causing *Salmonella* spp. was observed, increasing in deeper soil depths where *E. coli* was found declining. The agroforestry systems that had more shade (indicated by leaf area index – LAI), particularly the flooded gum and Pangola plantations, sustained higher *Salmonella* spp. (Figure 1B). Thus, enteric bacteria, other than *E. coli,* may also be included in the process of declaring effluent disposal site 'safe' for grazing or public access. Regional studies in the natural die-off rate of microbes may provide further insight into survival of microbiota under different environmental conditions, and increase the effectiveness of the practices followed in effluent irrigated plantations.

Acknowledgement

Efforts and inputs from Prof David Midmore and Ms Charmain Elder (both from CQU), and Mr Daniel Toon, Mr Bill Van Wees, and Mr Robert Effeny (all from Livingston Shire Council, Yeppoon) are kindly acknowledged.

References

1. DPI (2000) *Agricultural Water Recycling Background Study, Study Report – 2.* Queensland Water Recycling Strategy June 2000. Department of Primary Industries, GPO Box 2454: Brisbane 4001 Qld, Australia, p.64.
2. Rynne, F.G. *et al.* (1998) Effluent reuse – current practices and presentation of a reuse decision tree. In *WaterTECH Conference during 27-28 April 1998* at Brisbane, pp. 14, Australian Water and Wastewater Association.
3. Crites, R.W. *et al.* (2001) Applying treated wastewater to land. *Biocycle* 42, 32-36.
4. Desmarais, T.R. *et al.* (2002) Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment. *Appl. Environ. Microbiol.* 68, 1165-1172.
5. Feachem, R.G. *et al.* (1983) *Sanitation and Disease: Health Aspects of Excreta and Wastewater Management.* p 501, John Wiley and Sons, Chichester.
6. Gupta, S.R. *et al.* (1998) Effects of secondary treated sewage effluent application on the populations of microfauna in a hardwood plantation soil: Bolivar HIAT trial. *Geoderma* 84, 249-263.

Dr Ajay Sharma researched the changes in soil physics, chemistry, microbiology and hydrology under seven municipal effluent irrigated agroforestry systems for his doctoral research at Central Queensland University. These days he is Dy. GM (Environment and Farm Forestry) with A.P. Paper Mill Ltd., India.

Dr Keith Harrower is an a/prof in microbiology at Central Queensland University. He has been editor-in-chief of the international CSIRO journal *Australasian Plant Pathology* since 2005, and editor-in-chief of the new electronic international CSIRO journal *Australasian Plant Disease Notes* since August 2006.

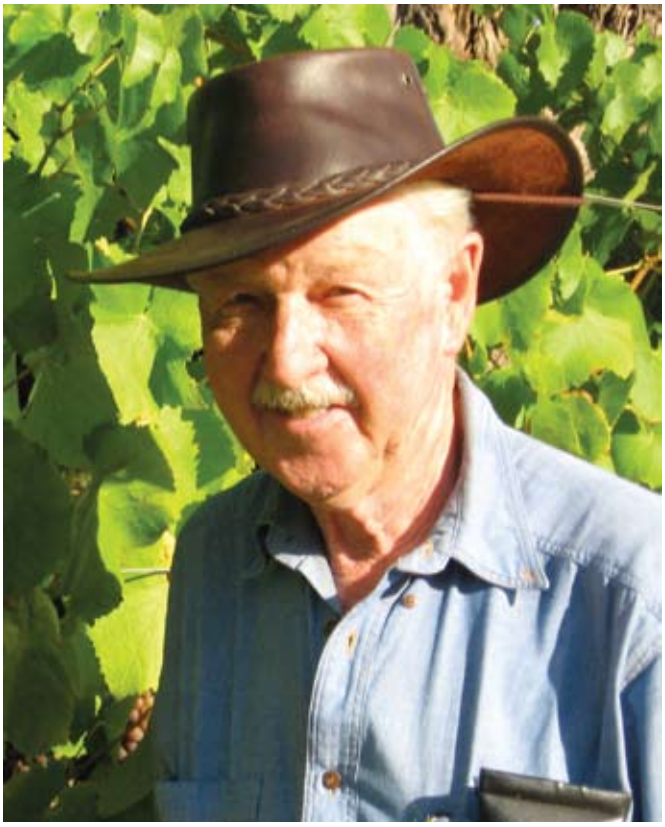
Dr Nanjappa Ashwath is an a/prof in life sciences at Central Queensland University. He is an expert in using native plants for mining heavy metals (phytoremidiation) as well as stabilising soil caps in landfills (phytocapping).

New ASM Honorary Life Member Ian Holmes

Ian Holmes was one of the first graduates in the newly established BSc(Hons) course in microbiology at Melbourne University under the supervision of Professor Sydney Rubbo. Since Prof Rubbo wanted his department to include all areas of microbiology, he suggested training in virology at the Australian National University with Dr WK Joklik in Professor Frank Fenner's department, where Holmes completed a PhD on poxviruses. After a brief return to the Melbourne department which was about to move into a new building and acquire an electron microscope, and noting an interest in photography, Prof Rubbo encouraged him to use a travelling scholarship to learn electron microscopy in Glasgow with Drs Peter Wildy and Douglas Watson, before returning as a staff member.

Thus began a very fortunate scientific life in that he was always able to follow his interests, and each topic seemed to lead naturally on to the next, as in the book *A trail of research* which he read as a graduate student and never forgot. The identification and reclassification of rubella virus drew him to electron microscopic studies on arboviruses, especially the 'unclassified' ones previously isolated by Ralph Doherty in Queensland. These were mostly bunyaviruses and orbiviruses, and these interests led to a year's study leave in Venezuela in 1970.

In 1973 Ruth Bishop and Geoff Davidson at the Royal Children's Hospital obtained duodenal biopsies from babies with acute gastroenteritis and, since no bacterial cause could be identified, Dr Alan Ferris suggested collaboration with Ian Holmes and Brian Ruck at the University of Melbourne, where EM studies quickly identified a new virus. The fact that at first sight rotaviruses looked so like orbiviruses was the greatest luck and assisted a rapid start on rotavirus characterisation. Rotaviruses turned out to be amazingly common and widespread, occurring in a wide range of animals and even birds, and became a new genus in the family Reoviridae. Structural, chemical, serological, molecular



epidemiological, genetic and vaccine research on rotaviruses occupied the next 27 years up to his retirement in 2000.

Although Ian retains a lively interest in rotavirus research, especially in the area of receptors and receptor-blocking agents, he is happy to leave this in the capable hands of Dr Barbara Coulson. Since he now lives in Red Hill on a property with a small vineyard, he has renewed enthusiasm for following his old role model Louis Pasteur into experiments on winemaking, which now take up most of his time.

Student Special Interest Group

The following are abridged extracts from the ASM Student SIG Newsletter. The complete newsletter is available on line at http://www.theasm.com.au/sigs

Message from the Student SIG Convenor

As the convenor of the Student Special Interest Group (SIG) I welcome you to the inaugural edition of the ASM Student SIG Newsletter! The re-establishment of the Student SIG in October 2008 signifies the importance of the student body within ASM. The Student SIG is a division within ASM dedicated to promoting activities relevant to all students. Currently, there are 366 student members within Australia and abroad who share a common interest in microbiology.

The future goals and visions for the Student SIG are to:

- Increase student participation in ASM activities.
- Recognise outstanding students through scholarships and prizes for achievements in research, teaching and/or leadership.
- Promote student research in the form of scientific articles or reviews through the journal *Microbiology Australia* and our newsletters.
- Organise scientific sessions relevant to student members (e.g. careers workshops).
- Increase networking between students across the country.

Finally, with the world constantly facing problems associated with emergent pathogens, unconquered infections, and imminent microbiology issues threatening our environment, it is an exciting time to be a budding microbiologist. Your talent and continued research efforts will help unravel some of these microbiological challenges. I firmly believe that ASM students are the future leaders in microbiology and I hope that this Student SIG will bring all of you closer together through establishing an enriched and collaborative learning environment.

Si Ming Man, Student SIG Convenor

Message from the Committee

This year commemorates the 50th anniversary of ASM, a generational landmark for Australia's largest biological society. This first edition of a quarterly newsletter confirms the re-establishment of the ASM Student SIG. This SIG signifies the importance of undergraduate and postgraduate students in ASM and their important role in the future of this society, as well as the future of microbial research and advancements in Australia. It is the hope of the committee that this SIG will not only provide students with their own niche but also establish an environment where students can voice their opinions, get answers to their questions, showcase their research studies, and be active in establishing their career in the world of microbiology.

What can I expect?

This quarterly newsletter is only the beginning to what this SIG has to offer. Where it goes and what it becomes rests in the arms of the student members of ASM. Without active members this SIG can not exist. So what is in this newsletter? Regular features will include:

Microbe review of the month and *Microbe image of the month* – this is where you, the researcher, can share with others the work you are doing for your degree.

Careers advice – in each issue, two professionals in the area of microbiology will share their highs, lows and insights of what they do and provide us with some career advice. This will help you determine if an area of work is for you or not.

Students' perspective – as a student in microbiology, you are not alone. This is where other students share their experiences of being a budding microbiologist.

As this is the first issue of this newsletter, your feedback would be greatly appreciated. Submissions for *Microbe review of the month* and *Microbe image of the month* are now open and a cash prize may be awarded to the most preferred review or image depending on funding availability from ASM. If there is anything that you would like to see in this newsletter, want removed, or done differently, let us know for the next issue. We are interested in what interests you and what ideas you have to share with ASM and its student body.

David J. Speicher, Student SIG Committee Member

About the Student SIG Committee

Si Ming Man

School of Biotechnology and Biomolecular Sciences, University of New South Wales, NSW

Si Ming graduated with a Bachelor of Medical Science (Hons I) and the University Medal in Microbiology from the University of New South Wales (UNSW) in 2007. His Honours research investigated the role of Campylobacter and Helicobacter species in children with idiopathic inflammatory bowel disease (IBD), which includes Crohn's disease and ulcerative colitis. Si Ming's doctoral research aims to examine the pathogenicity of mucus-associated bacteria in paediatric IBD. In addition to his PhD, Si Ming is an academic tutor/demonstrator for 2nd year undergraduate science and

medical students at UNSW and a reviewer for the journal *Inflammatory bowel diseases.* His leadership role also extends beyond the classroom as he is a peer mentor for both the Smith Family and UNSW, enabling him to facilitate the development of students' management and problem-solving skills in their first year of study, and helping them to cultivate a sense of responsibility for their own learning.

David Speicher

Griffith Institute of Health and Medical Research (GIHMR), Griffith University, QLD

David received his BSc (Hons) from Redeemer University College (Canada) in 2003 and his MSc (Hons) from Griffith University through the Sir Albert Sakzewski Virus Research Center (SASVRC) in 2006. He is currently a Griffith University PhD student in the new Griffith Institute of Health and Medical Research (GIHMR) laboratory on the Gold Coast in Queensland. His doctoral thesis aims at comparing the Australian human herpesvirus-8 (HHV-8) strain and its seroprevalence in a cohort to HIV-infected patients to findings from India and Kenya.

While most of his time is spent on research efforts, David is also an avid outdoorsman and can often be found fishing the Australian Bass Tournaments (ABT) Skeeter bass pro series or camping and bushwalking in remote areas. David's outdoor adventures became infamous when was apart of a group of seven who spent 50 hours bushwalking in Lamington National Park and had to get rescued by the state emergency services. David is also involved with the ACET International Alliance, which provides HIV/AIDS education and medical services in third world countries.

Rajat Mittal

Faculty of Medicine, University of New South Wales/ University of Sydney, NSW

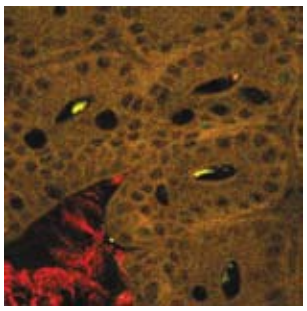
Rajat is a medical doctor currently undertaking a Masters of Surgery through UNSW. Rajat is aiming to finish all his lab work in 2008 and start his thesis writing in 2009. He will be based in Nowra as an orthopaedic registrar next year. Rajat is also concurrently completing a Masters of Medicine to further his research interests at the University of Sydney. Rajat's main interest lies in biofilms and his current project involves investigating biofilm formation in *Staphylococcus epidermidis.* His previous research project examined biofilm-forming Pseudomonas.

Be a part of the Student SIG Committee!

The ASM Student SIG committee is looking for new committee members, so if anyone feels they would like to contribute to the running of the SIG including its newsletter please let us know.

We would like to hear about your research!

Applications for *Microbe review of the month* and *Microbe image of the month* are now open.



In *Microbe review of the month,* we would like to hear about your interesting research. The subject must be relevant to microbiology, stemming from the current research topic within your Honours/Masters/PhD. It will involve no more than a 1,000 word write up that includes an introduction, results, discussion and conclusion. The introduction should provide an overview of the topic for a general audience and where your work fits within the relevant literature. The article must have a clear conclusion, showing how the research contributes to further the understanding within that area of microbiology.

In *Microbe image of the month,* we would like to see some of the interesting images derived from your research techniques such as electron microscopy, light microscopy, confocal microscopy, or any other method that produces a form of visual media that aids our understanding of microorganisms such as the microbial processes/physiology, and/or microbial interaction with other forms of life. The image should be accompanied by text (no more than 250 words) explaining the image and outlining the contribution to our understanding of microbiology.

The work from winners of *Microbe review of the month* and *Microbe image of the month* will be published in the *Student Special Interest Group Newsletter* and *Microbiology Australia,* and awarded a cash prize of $100. Non-ASM members are welcome to apply. A complimentary student membership will be awarded to winners who are not members of ASM. Please submit your application to Si Ming at s.man@student.unsw.edu.au

Careers advice

What's next after my Honours, Masters, or PhD?

Are there jobs other than research?

What career paths are available to graduates with a microbiology background, and which of these are suitable for me?

Do I need to undertake further study to achieve my goals?

In this issue of the *Student SIG Newsletter,* we invited two professionals with a microbiological background to give us some insights into their respective career paths. We caught up with Professor Hazel Mitchell, who is a medical microbiologist at the University of New South Wales. She is heavily involved in the teaching of 2nd and 3rd year microbiology courses at UNSW as well as the UNSW undergraduate medical program. Her research laboratory is interested in the role of the human pathogen *Helicobacter pylori* in gastric disease, as well as mucus-associated

bacteria in inflammatory bowel diseases. We were also fortunate to have Mrs Jenny Brown from In Vitro Technologies to tell us what life is like working for a scientific company, how she applies her scientific knowledge as a territory manager and the career benefits of entering the world of sales, including opportunities to travel overseas! In Vitro Technologies is a company with 75 years of experience dedicated to the distribution of world class products to the New Zealand research, diagnostic, biotechnology manufacturing and Healthcare and related service industries.

> *"Nothing is more exciting than seeing one of your students graduate and become one of Australia's future microbiologists"*

> *"It can be really exciting to see a product which I have sold to a customer being used to conduct ground-breaking experiments"*

Academia: Professor Hazel Mitchell

What made you become interested in the study of microbiology?

When I was growing up in Scotland we lived with my grandmother who was a medical doctor. Amazingly she actually had a microbiology laboratory in our house where she cultured specimens from her patients. This early introduction to infectious disease and bacteriology kindled my interest in this area and led me to enrol in a degree in microbiology at the University of Strathclyde in Glasgow, Scotland.

Tell us a little bit about your research interests

The focus of my research for over 20 years has been the gastric pathogen *Helicobacter pylori.* Initially this work focused upon the epidemiology of *H. pylori* as at that time *H. pylori* had only just been discovered and little was known about the prevalence and transmission of this bacterium. These early studies were conducted in China and resulted in an enduring collaboration with researchers in Guangzhou. This collaboration has focused upon the pathogenesis of *H. pylori* related disease. Currently in collaboration with researchers in Malaysia and Singapore we are investigating the role of *H. pylori*, host and environmental factors in the aetiology of gastric cancer.

My second area of research is inflammatory bowel disease (IBD). Although it is known that microorganisms play an important role in the initiation of IBD, the identity of these microorganisms remains unclear. We are investigating, in collaboration with researchers at Sydney Children's Hospital and in Canada, the role of intestinal mucus associated bacteria, including lower bowel Helicobacter species and non-jejuni Campylobacter species in the aetiology of IBD in children.

What is the most exciting part of being an academic?

One of the most exciting things about being an academic is that you have the opportunity to inspire and motivate students in the area of microbiology, as well as provide them with opportunities to learn how exciting and rewarding scientific research can be. Another exciting aspect of an academic's life is being able to interact, collaborate and build up friendships with research scientists from around the world. Finally, nothing is more exciting than seeing one of your students graduate and become one of Australia's future microbiologists.

What is the worst aspect of being an academic?

Finding enough time to complete the many administrative duties that are required of an academic.

What advice would you give to undergraduate or postgraduate students of ASM who would like to pursue a career in academia?

To undertake a career in academia you have to be passionate about research and teaching. There is no doubt that research and teaching go hand in hand, with each one informing the other. As well as finding an area of research that really 'turns you on', it is very important to find a good mentor who can advise and help you through your early years as a researcher and post-doctoral fellow.

What approach would they need to take in order to get where they want to be?

There are many approaches that can be taken to get where you want to be. However, no matter what path you take, a PhD as well as a post-doctoral period in a well-respected research laboratory, if possible outside of Australia, is in most cases essential. It is also important to publish as many papers as possible in high impact journals. During your PhD candidature and as a Post-Doctoral Fellow it is important to get experience in teaching, including tutoring and lecturing. Attending national and international conferences and being an active member of a scientific society like ASM is also an excellent way of meeting potential collaborators and also contributing to your discipline.

If you weren't a microbiologist, what would you be instead?

I would really love to be an opera singer. I enjoy music and have over many years been a great fan of the opera. In my next life I hope I will be gifted with a much better voice and then maybe I can follow my dream!

Commerce/science: Mrs Jenny Brown

What position do you hold and what is your role?

My position with In Vitro Technologies is territory manager. My role is to visit with laboratory staff, as well as laboratory leaders, purchasing officers and stores managers, to discuss the products which our company supplies and help our customers with their problems that our products may be able to solve.

How do you apply your knowledge in microbiology in your job?

As many of my customers are microbiologists, I am able to utilise my knowledge and experience in microbiology to help solve their problems and suggest products within our range which can help them out.

What made you become interested in becoming involved in commerce/sales associated with science?

I have always been a 'people person' and had worked in retail sales all through my time at university. I found that working in a laboratory I was constantly frustrated by the routine, and I tried a number of different roles before I moved into sales. Being involved in sales with In Vitro keeps me involved in science, and I still use my scientific experience and knowledge daily.

What is the most exciting part of your job?

It can be really exciting to see a product which I have sold to a customer being used to conduct ground-breaking experiments. It's also very exciting for me when I am able to help people out with new products that can solve problems they may have had for a long time. There are also some opportunities to travel both within Australia and internationally, and one of my most exciting experiences was attending training at the Nunc facility in Denmark.

What is the worst aspect of your job?

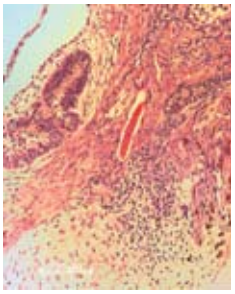
Occasionally things go wrong, either in the delivery of a product being delayed, or an administration error that can result in the wrong product being sent to a customer. Because most of our products are brought in from the USA or Europe, it can take a long time to get the correct product to the customer. This is always very frustrating and we work hard to prevent these errors, but unfortunately it happens from time to time and can result in some unhappy scientists!

What advice would you give to undergraduate or postgraduate students of ASM who wish to have a career combining science and commerce? What approach would they need to take in order to get where they want to be?

If you are interested in a job in sales, the best piece of advice I would give is talk to the reps in your area. I began working at In Vitro because I knew my local rep and she gave me a good idea of what it might be like to work in this company. I believe in the quality of products that In Vitro sells, and when a job came available, she encouraged me to apply for it. If you talk to your local reps you will know if you want to represent their company and products, and they will also be able to give you a better indication of what it's like to work in sales in your area.

Students' perspectives

No matter what situation you face in your research, whether amazing or completely horrid, remember that many other graduate students have been right where you are.



Students' perspectives is a section where other students share their experiences of being a budding microbiologist. In this issue of *Students' perspectives,* our very own Mr David Speicher from the SIG Committee shares his thoughts on how to 'drive your research'. David received his BSc (Hons) from Redeemer University College (Canada) in 2003 and his MSc (Hons) from Griffith University through the Sir Albert Sakzewski Virus Research Center (SASVRC) in 2006. He is currently a Griffith University PhD student in the new Griffith Institute of Health and Medical Research (GIHMR) laboratory on the Gold Coast in Queensland. His doctoral thesis aims at comparing the human herpesvirus-8 (HHV-8) strain and its seroprevalence in a cohort of HIV-infected patients in Australia to those in India and Kenya.

We also spoke to Miss Katharina Filarsky about her experience of what it is like to live and study in Australia and how this experience Down Under makes her a better microbiologist. Katharina is 3rd year biochemistry student from the University of Regensburg, Germany and currently completing an exchange study at the University of New South Wales.

There are so many new people I got to know, a different culture I experienced, and of course this amazing country has a beautiful countryside and many places that I haven't had the opportunity to discover...

Driving your research – David Speicher

It was during a PhD thesis writing workshop that I became aware that many of the horror stories you hear about the difficulties many PhD students face are real. As I looked into this further, I realised that many of these horror stories are not due to university policy or supervision, as many PhD students meet with their supervisors weekly or fortnightly, but actually from simply following their supervisor's directions for their research and hoping things work out perfectly. While many postgraduate students (Masters or PhD) have the luxury to do their research in established facilities, other students pursue the same goal in small or newly established facilities. Wherever you study or whoever you study under difficulties will arise, but here are four key points to help you reach a successful finish.

- *You are not alone.* No matter what situation you face in your research, whether amazing or completely horrid, remember that many other graduate students have been right where you are. After all this is science and, when exploring the unknown, things never work as they should the first time. Every graduate student has their share of highs and lows and it's important not to let circumstances slow down progress. If you need help ask any post-doc, senior PhD student or supervisor… they've been there.

- *Supervisors are not 'super', they only 'advise'.* Many students maintain the undergraduate mentality when they enter graduate school of "This should be easy. As long as I follow my supervisor's instructions, I'll graduate easily with flying colours". The truth is often quite the opposite. Research in microbiology and other hard sciences is often original in that it explores the unknown. While our supervisors are experts in the field because they have been there much longer than we have, all they can do is advise based upon what they have previously seen. Many of the methodological difficulties we face our supervisors have seen many times because of repeat use, kind of like driving a car, but halfway through a PhD your knowledge of the field should be more current than that of your supervisor's, making you the new expert in the field.
- *This is your research. Be motivated, disciplined, and get the most you can out of it.* As seen previously, there will come a point when your knowledge surpasses that of your supervisor's. While they are busy with lectures and other facets of their career, your full-time job is to study that one area. Many supervisors are so busy that they can only provide guidance once a week, making it your job to determine what needs doing, prepare a plan, and work it to completion. I'm not saying to ignore or bypass your supervisor; in fact for my PhD I have four supervisors. They are all from different facets of the field I study and I think of all five of us as a team with a common goal – my PhD project. They all provide different views and insight to where I should be going, but ultimately the responsibility is on me. I can listen to all their advice but in the end it's my decision what gets done. It's my responsibility to determine what needs doing for the confirmation, how my thesis should look, what and where I'm going to publish.

  It can be daunting having to make some really difficult choices, but as a colleague once told me "a PhD is not about what you prove or fail to prove the purpose is to make you think things through. Determine what you are doing, what could go wrong, and why you do what you do and why things turn out the way they did. Ultimately a PhD is designed to make you think". Many PhD students find themselves in trouble because they overlook some major tasks, no matter how many times they meet with their supervisor. Apart from bearing the responsibility, you also get your share of the reward when you begin publishing as first author.
- *Realise when to stop (keep focused and take breaks).* Many PhD students try to do too much that they spread themselves so thin that while their thesis looks big it lacks a single strand of cohesion. Keep focused. It's only a PhD and not the next Nobel Prize, although that may be to come if you work hard and are lucky enough. You should focus on determining the answer to one simple problem and show where it fits into the big picture. If you do too much you'll get disjointed and loose relevance as you become so busy you don't have time to reflect and think. Most of the 'ah-ha' moments and great ideas and insights come when we take our mind off our work to do something more relaxing. This gives the brain time to think things through. So if you feel like you are up against a brick wall... take a step back, breathe and relax, and when the answer comes, move forward focusing on the task at hand.

I agree that trying to accomplish a PhD can be a daunting task, especially when you reach submission time and realise that you failed to do one critical step. Hopefully, by keeping these four key points in focus you will be able to run with your own project, realising that you are the driving force in a team of researchers, and when the difficult times come don't worry you are not alone, there's a whole team behind you. It is only when things get horrid that you can step back, breathe, realise that you are not the only one in this predicament, and that the whole purpose of a PhD is to make you think. Don't try to wrack your brain too much because, when you least expect, it the answer will find you.

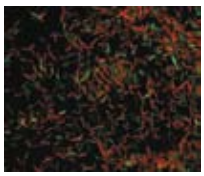
Life of an overseas exchange student – Katharina Filarsky

How did you come to a decision of undertaking an exchange study in Australia?

It was clear from the beginning of my studies that I wanted to go on an international exchange experience. One factor for doing an overseas exchange program was employability. I think the ability to speak a different language and show motivation by doing research in a foreign country are important attributes that employer consider for future employees. In science, you can go almost anywhere worldwide, even if you cannot speak the local language, as most labs communicate in English. One aspect that encouraged me to do research in Australia was the partnership formed between my host university (University of Regensburg) and UNSW as well as the willingness of UNSW to offer admission in my desired program. I also considered if I would be able to have fun whilst on the exchange program. For a successful exchange program you need to choose a country you would really like to visit so you can discover new things while enjoying your stay. In my case, all these factors helped me decide to undertake an exchange program in Australia.

How is your exchange program structured and how does this exchange contribute to your degree in Germany?

I'm staying in Australia for 7 months. During that time, I'm working in the lab for 6 months and then travelling for an additional month. My studies in Germany require me to work in different labs in different scientific fields so that I can learn as many scientific techniques as possible, including microbiological techniques. In each of the labs I have to stay for at least 5 weeks. The 6 month stay here in Australia directly contributes to my degree back in Germany.



What type of experience or skills related to microbiology were you hoping to get out of your exchange study?

My programme in biochemistry is relatively fixed. The opportunity to work in different labs has helped broaden my mind and ability to use different methods and different research areas encompassing biological sciences. Although I already had some experience in microbiology from my university, I was hoping that I could get a more in-depth view by going to an exchange program in a medical microbiological laboratory. Through this program I was able to learn techniques such as fluorescent in situ hybridisation, dead/live viability staining and confocal microscopy, and bacterial infection assays, all of which are relevant to modern molecular microbiology.

Was funding an issue? How did you fund your stay in Australia?

As students are usually poor, funding will always be a big issue for all students as it was for me. In Germany, I worked a lot in restaurants and different companies to earn the money needed for this exchange program. But that's not always possible because many students don't have the time to work in addition to their studies.

I also applied for scholarships to fund my studies. The first rule is to try everything! Often students don't apply for scholarships because they think they are not good enough, but grades are often not the most important thing when determining whether a scholarship is awarded or not. To find out which scholarships are available, you should ask the student office and other students who have applied for scholarships as well as your professors. It's also very important to do some research on the price of rent in the city you will visit. Take into account that you will spend more money than at home because you want to explore places.

What has been the highlight of your Australian exchange experience?

It's really hard to pick one thing as a highlight. There are so many new people I got to know, a different culture I experienced, and of course this amazing country has a beautiful countryside and many places that I haven't had the opportunity to discover. My time here is not over yet and I'm sure there will be more chances to see the beauty this country has to offer. If I have to choose one highlight it would be the Oktoberfest celebration at the university as this occasion is really famous back home in Munich. The Australian Oktoberfest has a different feel to the ones in Germany, but it was fun and I met so many nice people.

Are there many exchange students studying in Germany? What advice would you give to future students thinking of doing an exchange in Germany?

When you go to a foreign country you always have to take into account the time required to find an apartment, to get to know the people, and to settle in to the lab. Also watch out at which time of the year you want to undertake an exchange program as university semesters are not aligned between different countries. You don't have to speak German to go to Germany, but of course some basic language is necessary for the daily life. In comparison to Australia, the student fees are really low. We pay about 600 Euro per semester (which is about A$1200) depending on where you study. The semesters are in the summer from April to August and in winter from October to February. There is lots of student accommodation organised by the so-called 'Studentenwerk'. To search for suitable accommodation you should apply for one of the apartments as soon as possible, but it is also very common to live in private shared houses. The price of rent depends on the city you live. For example, Munich is quite expensive compared to smaller cities like Regensburg. The cost of food across Germany is very similar. Many exchange students visit from Europe or Asia but we welcome everyone! I hope to see you in Germany.

Interested in becoming an ASM Student Member or renewing your membership?

Benefits of student membership include:

- Membership of the largest biological society in Australia.
- Access to society publications, a video library and the society's journal, *Microbiology Australia.*
- Networking with students from across Australia.
- A programme of continuing education via lectures and workshops organised at Branch level.
- A programme of continuing education via the Visiting Speakers' Programme which brings out five to six eminent and world-recognised microbiologists and research scientists to tour around the states of Australia.
- A five day Annual Scientific Meeting which is held each year, rotating around the states of Australia.
- The opportunity to participate in a variety of annual awards, fellowships and scholarships.
- Access to over 26 Special Interest Groups, including the Student Special Interest Group and other interest groups covering most sub-disciplines of microbiology.

The Student Membership Application is at http://www.theasm.com.au/membership. If you have any membership queries please contact Lina Raco: lina@theasm.com.au

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Feel free to contact any of the committee members if you have any questions or suggestions concerning our newsletter or the ASM Student SIG.

2009-2010 meetings

Contributions listing relevant meetings are welcome. Please send to: editor@theasm.com.au

2009 – Golden Jubilee Year

13-15 March 2009
Sydney Convention & Exhibition Centre NSW
XXV World Congress of Pathology and Laboratory Medicine

www.rcpa.edu.au/PathologyUpdate

24-26 March 2009
Melbourne VIC
13th Australian Food Microbiology Conference

The Australian Institute of Food Science and Technology Incorporated: "Back to Basics and Beyond"
Members of ASM can attend at the AIFST member rate
www.aifst.asn.au/templates/bbb_content.aspx?pageID=513

25-28 March 2009
Cypress Lakes Resort, Hunter Valley NSW
ASID 2009 – Australasian Society for Infectious Diseases (ASID) Annual Scientific Meeting

www.asid.net.au

30 March – 2 April 2009
Harrogate International Centre, UK
SGM 164th Meeting

5-7 April 2009
Seoul, South Korea
BIT Life Sciences' 2nd Annual World Congress of Industrial Biotechnology-2009

A dedicated event on industrial biotechnology, with a theme of *Innovative Biotechnology for Sustainable Bio-economy.*
http://bit-ibio.com/program.asp

7-9 May 2009
The Carrington Hotel, Katoomba, Blue Mountains, NSW
Viruses in May

Australia's only meeting focused specifically on the clinical, diagnostic & management aspects of viral infections.
Programme themes:
- Principles of clinical virology
- Congenital infection
- Paediatric infection & vaccination
- Blood-borne viruses
- Hepatitis

Convenors: Professor Bill Rawlinson & Dr Monica Lahra
Conference Management: Australian Society for Microbiology
Contact: Meg Lukies, Event Coordinator
www.virusesinmay.com

10-13 May 2009
Buenos Aires, Argentina
VTEC 2009 – 7th International Symposium on Shiga Toxin (Verocytotoxin) – Producing *Escherichia coli* Infections

www.vtec2009.com.ar

17-21 May 2009
Philadelphia, PA, USA
109th General Meeting of American Society for Microbiology

www.asm.org

21-25 June 2009
Hamilton Island QLD
10th International Symposium on Double-Stranded RNA Viruses

Coordinators: Barbara Coulson & John Taylor
www.dsrna2009.org

28 June – 2 July 2009
Goteborg, Sweden
FEMS 2009 – Third Congress of European Microbiologists: Microbes and Man – Interdependence and Future Challenges

www2.kenes.com/fems-microbiology/Pages/home.aspx

6-10 July 2009
Perth Convention Centre, Perth WA
ASM 2009 Perth – Annual Scientific Meeting & Exhibition

Australia's largest microbiology event for 2009 celebrating ASM's 50th Golden Jubilee Year

19-24 July 2009
Manchester, UK
24th International Conference on Yeast Genetics and Molecular Biology

www.yeastgenetics.org

26-30 July 2009
Toronto, Canada
SIM 2009 Annual Meeting and Exhibition

http://www.simhq.org/

25-28 August 2009
Christchurch, New Zealand
26th NRL Workshop on Serology

Workshop Secretariat: National Serology Reference Laboratory
Contact: (03) 9418 1117
Email alison@nrl.gov.au
www.nrl.gov.au

29-31 October 2009
Hamilton Island, QLD
Mycology MasterClass IV
[1 November 2009 – Additional MasterClass Workshop for laboratory staff]

Convenor: Associate Professor David Ellis
Conference Management: Australian Society for Microbiology
Contact: Janette Sofronidis, Conference Manager

2010

28 June – 1 July 2010
Melbourne Convention and Exhibition Centre, Melbourne VIC
11th International Symposium on the Genetics of Industrial Microorganisms

Chair: Ian Macreadie
www.gim2010.org

4-8 July 2010
Darling Harbour Convention Centre, Sydney NSW
ASM 2010 Sydney

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Authorship

All authors must make a substantial contribution to the manuscript and will be required to indicate their contribution. Participation solely in the acquisition of funding, the collection of data or supervision of such does not justify authorship. All participating authors must be acknowledged as such; proof of authorship may be requested by the editors. The first-named author is responsible for ensuring that any other authors have seen and approved the manuscript and are fully conversant with its contents. If the author wishes to reproduce copyrighted work, it is the responsibility of that author to obtain written permission from the copyright holder and to submit the original copy of that permission to the editor with the work as it is to be copied.

Conflict of interest: It is the responsibility of the submitting author to disclose to the Editor any significant financial interests they may have in products mentioned in their manuscript. Conflicts of interest should also be disclosed within the manuscript before the References section.

Ethics

Investigations in human and animal subjects must conform to accepted ethical standards. Authors must certify that the research protocol was approved by a suitably constituted ethics committee of the institution within which the work was carried out and that it conforms to the Statement on Human Experimentation or the Statement on Animal Experimentation by the NH&MRC.

Manuscript type

Microbiology Australia has a very large and broad readership; it is now released to science writers for further communication to the public. Articles should be written in a style that is attractive to this general audience while keeping your peer group informed of the latest developments and their impact.

In Focus articles

In Focus articles are major, review-type articles on a theme chosen by the Editorial Board which should attract interest and understanding from those in all disciplines of microbiology. They are published in each issue of the journal, should be about 1500 words in length and include 2-3 graphics or colour pictures. We would like you to discuss your own work in the context of other important work undertaken in the same field. It is important to acknowledge other work, since it helps paint a broader picture of your subject.

Under the Microscope articles and Laboratory reports

These are short reports of between 500 and 1000 words dealing with a current aspect of the topic. The opening paragraph should include the major points being made. State your conclusions up front, then discuss how they were arrived at. Concentrate almost entirely on the significance of the work being reported, rather than reporting detailed results. Articles, which should include a graphic or colour photograph, may be solicited by the Editorial Board or offered by members of ASM.

Preparation of manuscripts

Manuscripts are to be no more than 1500 words and include an abstract of no more than 250 words. Use double spacing with Times Roman 12 font and margins 2.5cm. Title page to include title of manuscript, author's names, qualifications and affiliations, corresponding author's details including email address and contact phone number, total word count and up to five key words. Include title of work on the abstract page and first page of introduction. Include key points on what is already known on the topic and what your manuscript contributes. Define abbreviations in the summary and on first mention in the text. Avoid abbreviations unless terms are used repeatedly and abbreviating them will enhance clarity. Additionally, photograph(s) of the author(s) must be included in the submission and should be in .jpeg format.

Tables and figures are to be presented on separate pages, one per page. Tables should be clearly typed, showing columns and lines. Number tables consecutively using Arabic numerals in the order of their first citation in the text and supply a brief title for each. Place explanatory matter in footnotes, not in the heading. Explain in footnotes all non-standard abbreviations used in each table.

Figures must be submitted on separate pages. Photographs of the highest quality may be included in the submission and should be in .jpeg format. Legends for any figures supplied must be typed in sequence on a separate page(s). Illustrations and figures must be clear, well-drawn and large enough to be legible

when reproduced. Titles of illustrations should be supplied on a separate piece of paper, not in the figure or illustration. Each figure must include its place, its number, and the orientation of figure. Patients or other individual subjects should not be identifiable from photos unless they have given written permission for their identity to be disclosed; this must be supplied.

Referencing guidelines

The referencing format is based on the Vancouver style, the main feature of which is the use of numbers at the point of reference so as not to interfere with the flow of words. Each number corresponds to a single reference provided in the reference list at the end and, once assigned a number, a reference retains that number throughout the text, even if cited more than once. If more than one work is quoted in a reference, each work must be assigned a number. That is, at any point in the text, the reference may be one [1] or several [2-4] numbers.

References are set out in the following style. Only include listings for up to two authors (for more than two, list the first followed by *et al.*) and cite both the first and last page numbers. For authors using citation managing tools, follow the style of *Trends in Microbiology*.

1. Fisher-Hoch, S.P. *et al.* (1985) Pathophysiology of shock in a fulminating viral infection (Ebola). *J. Infect. Dis.* 152, 887-894.
2. Groseth, A. *et al.* (2005) Hemorrhagic fever viruses as biological weapons. In *Bioterrorism and Infectious Agents* (Fong, I. and Alibek, K., eds), pp. 169-187, Plenum Press.

Personal communications, unpublished observations and manuscripts in preparation should be square-bracketed and italicised in the text – for example: [personal communication].

Submission of manuscripts

Manuscripts are only accepted as an electronic submission with an attachment as a Word document. All tables, figures and photographs are to be included in the one attachment. Please ensure image files are no larger than 700kb. The manuscript must be accompanied by a covering letter indicating that the manuscript has not been submitted elsewhere and transferring copyright to the Journal.

Manuscripts are submitted electronically:

- Go to the publisher's website: www.cambridgemedia.com.au
- Click on Manuscript System
- Login
- Create and account if first time using the system – this will be retained for future enquiries and submissions
- Enter your personal details – *Microbiology Australia* requires all fields to be completed
- Confirm your details

Follow the steps for submitting an article

- Step 1 – Type the title, type of paper and abstract. *Microbiology Australia* requires an abstract for all submissions. Select publication – *Microbiology Australia*.
- Step 2 – Confirm author. Add co-author details (all fields) if applicable.
- Step 3 – Upload files. Only Word documents are accepted by *Microbiology Australia*. Please ensure your document contains the required information and is formatted according to the author guidelines.
- Step 4 – Add any comments for the editor.
- Step 5 – Review your information then click submit.

Once submitted, the manuscript is reviewed by the editor and, if acceptable, sent for peer review. You will be notified by email once your manuscript has been selected for peer review.

Peer review process

All manuscripts are initially reviewed by the Editorial Board and those deemed unsuitable (insufficient originality, serious scientific or methodological flaws, or a message that is too specialised or of limited interest to a general medical audience) are returned to the author(s), usually within 4 weeks. If the manuscript does not conform to the submission guidelines, the author will be asked to amend prior to peer review.

All manuscripts are reviewed by content and writing peers for relevance, construction, flow, style and grammar. All reviewers spend considerable time in reviewing the manuscripts and providing feedback to the authors. The length of time of the publication process can vary and depends on the quality of the work submitted. Several revisions may be required to bring the manuscript to a standard acceptable for publication. The Editorial team undertake the final review and often have different questions for the author/s to consider.

When time permits, proofs of articles about to be published will be sent to the corresponding author for review. This requires rapid response; if such a response is not forthcoming, the article will be published irrespective of the author's reply. Providing facsimile numbers facilitates this process. The final decision about publication is made by the Editor.

The peer review process is managed online. Decisions are communicated by email to the corresponding author. Authors without email are contacted by phone, fax or post. Submitted manuscripts are acknowledged by email.

Publication deadlines

All materials for publication must be in the hands of the Editor by the following dates for 2009/10. Please note that due to the editorial review process there is no guarantee of when accepted papers will be published.

15 April 2009	August 2009 issue
15 July 2009	November 2009 issue
15 October 2009	February 2010 issue
15 January 2010	May 2010 issue

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- **Bioterrorism**
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- **Water recycling—infectious disease risks**

ON THE PANEL

- Nobel Laureate Prof Barry Marshall AC
- Rita Colwell USA
- John MacKenzie AO
- Keryn Christiansen
- Peter Collignon

FACILITATOR

- Dr Norman Swan

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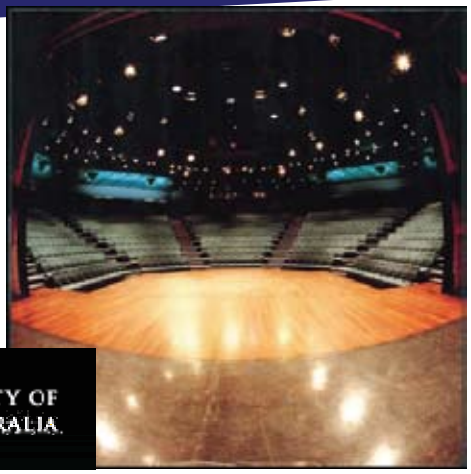

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