WASTEWATER REUSE: CASE STUDIES IN MICROBIAL RISKS

N. J. Ashbolt
School of Civil and Environmental Engineering, University of New South Wales, Sydney NSW 2052, Australia

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Summary

Around the world, the reuse of wastewaters will rapidly increase over the next twenty years as other sources of freshwater diminish. Two areas of concern discussed in this article are pathogen risks from reclaimed wastewaters used to irrigation salad crops and the urban reuse of non-potable wastewaters. Current guidelines for these applications largely reflect what local communities deem to be achievable, rather than any estimate of disease burden or cost/benefit to the community. Furthermore, the current desire for a "virus-free effluent" or "no detectable coliforms", is simply not fully achievable (with modern detection methods and appropriate sampling). As a way forward, two case studies are presented which illustrate some of the issues and microbial risk assessment methods available to aid in community risk-based decision making.

1. Introduction

A previous article outlined various microbial and chemical risks potentially associated with wastewaters reused or recycled to communities (see Human health risks associated with water reuse). Two case examples are explored in this article, to examine microbial issues in more detail.

Around the world, communities irrigate crops with wastewater or poor quality water. Whereas the World Health Organization has long had guidelines for this practice, some emerging issues related to virus and bacterial risks are discussed in the first case study. The second case study focuses on risks associated with biofilms that develop with reclaimed wastewaters. Though dual reticulation systems are not commonly used in residential areas, the risks discussed here are also applicable to accidental ingestion of poor quality irrigation waters.

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Bibliography

Buswell C. M., Herlihy Y. M., Lawrence L. M., McGuiggan J. T. M., Marsh P. D., Keevil C. W., and Leach S. A. (1998). Extended survival and persistence of *Campylobacter* spp. in water and aquatic biofilms and their detection by immunofluorescent-antibody and -rRNA staining. *Applied Environmental Microbiology*, **64**(2), 733–741. [In water microcosm experiments, the survival times of *Campylobacter* isolates differed by up to twofold, as determined by culturing; this difference increased to fourfold when particular combinations of temperature and oxygenation were used. Immunofluorescent-antibody staining demonstrated that the pathogen persisted up to the termination of the experiments after 28 and 42 days of incubation at 30 ºC and 4 °C within biofilms.]

Flood J. A., and Ashbolt N. J. (2000). Virus-sized particles are concentrated and maintained within wastewater wetland biofilms. *Advanced Environmental Research*, **3**(4), 403–411. [The re-release of pathogens sequestered into environmental compartments (e.g. sediments) represents a potential health risk and is thus an issue of concern with relation to wastewater effluent release and reuse. Virus-sized (100 nm), fluorescent microspheres were one hundred-fold increase above the maximum water column density and persisted in biofilm for a period of at least seven months.]

Gilgen M., Germann D., Luthy J., and Hubner P. (1997). Three-step isolation method for sensitive detection of enterovirus, rotavirus, hepatitis A virus, and small round structured viruses in water samples. *International Journal of Food Microbiology.*, **37**(2–3), 189–199. [Control of drinking or bathing water quality in respect to viral contamination remains an unsolved problem. The application of the novel procedure to six river water samples revealed enterovirus in 6/6 (100%), rotavirus in 616 (100%), hepatitis A virus in 0/6 (0%), small round structured virus genotype I in 6/6 (100%) and small round structured virus genotype II in 2/6 (33%) of the samples.]

Hay J., Seal D. V., Billcliffe B., and Freer J. H. (1995). Non-culturable *Legionella pneumophila* associated with *Acanthamoeba castellanii*: detection of the bacterium using DNA amplification and hybridization. *Journal of Microbiological Methods*, **21**, 27–32. [Intracellular localization of *L. pneumophila* serogroup 1 within *A. castellanii* rendered the bacteria non-culturable on supplemented BCYE agar, but estimated 31% were viable.]

Itoh Y., Sugita-Konishi Y., Kasuga F., Iwaki M., Hara-Kudo Y., Saito N., Noguchi Y., Konuma H., and Jumagai S. (1998). Enterohemorrhagic *Escherichia coli* O157:H7 present in radish sprouts. *Applied Environmental Microbiology*, **64(**4), 1532–1535. [Viable enterohemorrhagic *Escherichia coli* O157:H7 not only on the outer surfaces but also in the inner tissues and stomata of cotyledons of radish sprouts grown from seeds experimentally contaminated with the bacterium. HgCl2 treatment of the outer surface did not kill the contaminating bacteria, which emphasized the importance of either using seeds free from *E. coli* O157:H7 in the production of radish sprouts or heating the sprouts before they are eaten.]

Lucena F., Araujo R., and Jofre J. (1996). Usefulness of bacteriophages infecting *Bacteroides fragilis* as index microorganisms of remote faecal pollution. *Water Research*, **30**(11), 2812–2816. [Multifactorial analysis of marine sediments and groundwater data clustered phages, viruses and *C. perfringens* in one factor and faecal bacteria in another. These data clearly suggest that *Clostridium*, phages of *B. fragilis* and, in some cases enteroviruses, are better index microorganisms of remote faecal pollution than present bacterial indicators.]

Mackay W. G., Gribbon L. T., Barer M. R., and Reid D. C. (1999). Biofilms in drinking water systems: a possible reservoir for *Helicobacter pylori*. *Journal of Applied Microbiology*, **85**, (Suppl S), 52S–59S. [A laboratory model system was innoculated with *H. pylori* (NCTC 11637) indicated detection in biofilm material for a period of up to 192 h in a viable state. Further investigations to assess the biological basis for the association of *H. pylori* with drinking water biofilms and the risk that this may pose to public health are being undertaken.]

Ortega Y. R., Roxas C. R., Gilman R. H., Miller N. J., Cabrera L., Taquiri C., and Sterling C. R. (1997). Isolation of *Cryptosporidium parvum* and *Cyclospora cayetanesis* from vegetables collected in markets of an endemic region, in *Peru. Amer. J. Trop. Med. Hyg.* **57**(6), 683–686. [Of the total vegetables examined, 14.5% contained *C. parvum* oocysts and 1.8% had *Cyclospora* oocysts. Thus, market vegetables may provide a route by which *Cryptosporidium* and *Cyclospora* can be transmitted and that washing vegetables does not completely remove them.]

Payment P., Siemiatychi J., Richardson L., Renaud G., Franco E., and Prevost M. (1997). A prospective epidemiological study of gastrointestinal health effects due to the consumption of drinking water. *International Journal of Environmental Health*, **7**, 5–31. [Second of Payment's studies to indicate the role of distribution systems in increasing waterborne disease.]

Petterson S. R., Ashbolt N. J., and Sharma A. (2000). Microbial risks from wastewater irrigation of salad crops: A screening-level risk assessment. *Journal of Environmental Science and Health Part B.* [A Monte Carlo simulation using a log-normal and a nonparametric kernel estimated PDF indicated that slight changes in the upper region of the PDF had a relatively low impact on the estimated infection rates. Predicted infection rates were much more sensitive to the decay rate of viruses (k) than occasional high virus numbers. The median and 99th percentile risks of infection from the overall model were 0.13 and 0.76 per 10 000 lettuce consumers respectively, indicating possible human health concern, and the necessity of a full MRA.]

Petterson, S.R. and Ashbolt, N.J. (2001). Viral risks associated with wastewater reuse: Modelling virus persistence on wastewater irrigated salad crops. *Water Science and Technology*, 43(12):23-226. [A model for virus decay on lettuce and carrot crops indicated the presence of a very persistent sub-population of viruses evidenced by an initial rapid phase of decay followed by a very slow phase. In addition, virus counts fitted a negative binomial rather than Poisson distribution indicating over-dispersion. Hence the data indicated that viruses were not uniformly distributed over the surfaces of both crops. When over-dispersion or clumping of viruses was accounted for, a significant increase in the heterogeneity in the risk estimates arose. Hence, both viral clumping and persistence sub-populations should be accounted for in future risk assessments of enteric viruses associated with wastewater reuse.]

Rababah A. A., and Ashbolt N. J. (2000). Innovative production treatment hydroponic farm for primary municipal sewage utilisation. *Water Research*, **34**(3), 825–834. [While no uptake of F-RNA bacteriophages were detected within lettuce leaves, uptake was apparent from spiked virus-sized particles (fluorescent 0.1 µm microspheres) and equivocal from spores of the faecal bacterium, *Clostridium perfringens*. Microbial data was used in a -Poisson dose response model and indicated that the probability of infection for a single ingestion event of NFT grown lettuce grown on primary treated municipal effluent was about 1.7% for viruses. Moreover, plants accumulated heavy metals in leaf tissues at concentrations higher than the maximum recommended levels for Australian and New Zealand food (As = 6.5, Cd = 3.8, Pb = 20 mg/kg).]

Steinert M., Birkness K., White E., Fields B., and Quinn F. (1998). *Mycobacterium avium* bacilli grow saprozoically in coculture with *Acanthamoeba polyphaga* and survive within cyst walls. *Applied Environmetnal Microbiology*, **64**(6), 2256–2261. [In addition to legionellae, here report the growth of another opportunistic pathogen within amoebae that may colonize pipe-biofilms and water systems.]

Szewzyk U., Manz W., Amann R., Schleifer K.-H., and Stenström T.-A. (1994). Growth and *in situ* detection of a pathogenic *Escherichia coli* in biofilms of a heterotrophic water-bacterium by use of 16S-

and 23S-rRNA-directed fluorescent oligonucleotide probes. *FEMS Microbial Ecology*. **13**, 169–176. [First conclusive evidence of growth of *E. coli* within pipe-water biofilms.]

Biographical Sketch

N. J. Ashbolt has been an Associate Professor in the School of Civil and Environmental Engineering, the University of New South Wales, Sydney, Australia since 1994. Prior to that time he was the principal microbiologist, Sydney Water Corp. His Ph.D. was undertaken on the microbial ecology of composting waste eucalyptus bark with biosolids and fish wastes (1984). Since then he as work in industry and government research organizations, covering microbial issues associated with sugarcane mill wastewaters, mineral leaching of sulphidic ores, hypersaline Antarctic lakes ecology and wastewater reclamation microbial risks. Current research direction is focused on molecular and conventional identification of environmental pathogens in waters, effluents, sediments and biofilms, and the interpretation of this data with state-of-the-art quantitative microbial risk assessment methods. Dr. Ashbolt has active research collaborations with the Swedish Institute for Infectious Disease Control (Stockholm) and the Institute for Medical Research (Kuala Lumpur) and is a member of the WHO microbial guidelines working group. He has published over 65 journal articles, 10 book chapters and holds two joint patents.