

## A risk assessment of *Pseudomonas aeruginosa* in swimming pools: a review

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### ABSTRACT

Despite routine monitoring and disinfection, treated swimming pools are frequently contaminated with the opportunistic pathogen *Pseudomonas aeruginosa*, which can represent a significant public health threat. This review was undertaken to identify the current understanding of risk factors associated with pool operation with respect to *P. aeruginosa*. The ecology and factors that promote growth of *P. aeruginosa* in the pool environment are complex and dynamic and so we applied a systematic risk assessment approach to integrate existing data, with the aim to improve pool management and safety. Sources of *P. aeruginosa*, types of infections, dose responses, routes of transmission, as well as the efficacy of current disinfectant treatments were reviewed. This review also highlights the critical knowledge gaps that are required for a more robust, quantitative risk assessment of *P. aeruginosa*. Quantitative risk management strategies have been successfully applied to drinking water systems and should similarly be amenable to developing a better understanding of the risk posed by *P. aeruginosa* in swimming pools.

**Key words** | HACCP, *Pseudomonas aeruginosa*, risk assessment, swimming pools

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### INTRODUCTION

The exercise and amenity that swimming pools provide is recognised as a major social service that promotes the health and wellbeing of people of all ages. In the UK, 1,770 public pools and leisure centres generated approximately revenues of £488M (Bullock 2003); there are currently an estimated 0.16, 0.32, 0.5 and 4.2 million private pools, respectively, in the UK, USA, Germany, and France. In the USA alone the revenue from sales of the pools is ca. \$3 billion per annum. Based on such numbers, the number of pool visits probably exceeds 1 billion per year (Zwiener *et al.* 2006) and such number excludes hot tubs, spas and hydrotherapy pools which are likely to represent a significant number of 'bathing' visits annually. Despite ongoing improvements in water quality and disinfectant monitoring, swimming pools still experience microbial contamination, representing a significant community health and economic risk.

*Pseudomonas aeruginosa* is one of the most frequently isolated opportunistic pathogens in pools and hot tubs. It can be derived from both human and non-human sources, grows rapidly on a wide range of substrates and is tolerant to chemical disinfectants, including chlorine (Wheater *et al.* 1980; Hardalo & Edberg 1997; Craun *et al.* 2005). These characteristics partly explain the occurrence of *P. aeruginosa* in the treated pool environment (Kush & Hoadley 1980; Ratnam *et al.* 1986; Price & Ahearn 1988; Uhl & Hartmann 2005; Zwiener *et al.* 2006). Its detection can lead to lengthy pool closures, increased maintenance costs, loss of revenue and negatively impact on user confidence. *P. aeruginosa* reservoirs within pool environments are often only partially defined at the time of outbreaks. In lieu of a comprehensive knowledge of the ecology and biodiversity of *P. aeruginosa*, pool management relies heavily on chemical (shock) disinfection and large-scale hygiene actions such as water dumping.

doi: 10.2166/wh.2012.020

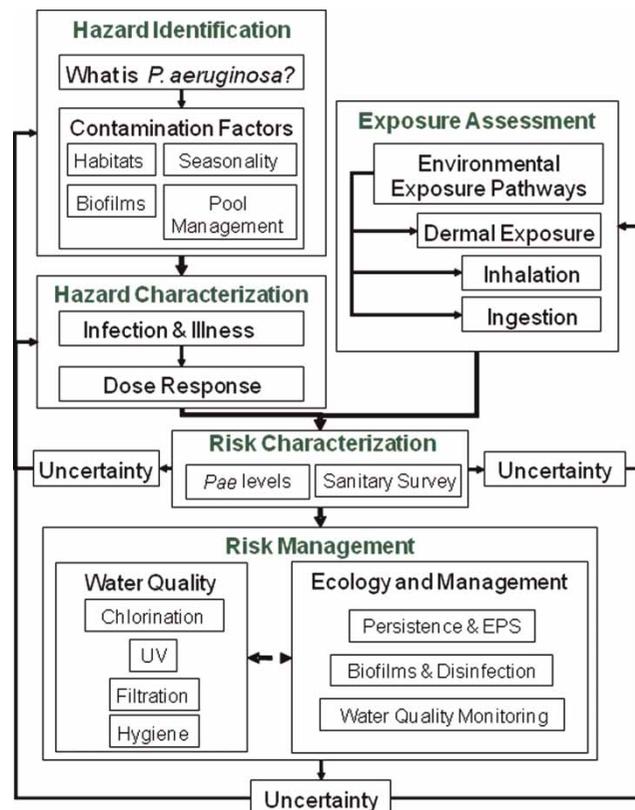
To improve the management of water-borne pathogens, the World Health Organization (WHO) is now widely promoting risk analysis, which is also being increasingly adopted as best practice by drinking water utilities (Fewtrell & Bartram 2001; World Health Organization 2006b). One notable feature of drinking water risk assessment has been a greater emphasis on quantifying the microbial ecology of drinking water (e.g. Brookes *et al.* 2004). The insights gained have been integrated with risk assessment and management under labels such as ‘Catchment to Consumer’ analysis (e.g. NHMRC/NRMMC 2008). In the treated pool environment its main application has been for characterising enteric and nosocomial pathogen impacts (e.g. Van Heerden *et al.* 2005; Schijven & de Roda Husman 2006). Accordingly, this review has been prepared with the aim of promoting a broader ‘Source to Bather’ risk assessment for the management of *P. aeruginosa* in swimming pools.

## RISK ASSESSMENT AND WATER-BORNE PATHOGENS

Human health risk assessment aims to reduce morbidity and mortality through a systematic analysis of hazards and their impacts. A key risk analysis tool used by the drinking water industry to identify system vulnerabilities is the concept of Hazard Analysis and Critical Control Points (HACCP). HACCP was developed to support spaceflight food and water protection and has subsequently been applied more generally to water/food-borne pathogens and chemicals. With HACCP style pathogen management in mind, the Codex Alimentarius Commission (1999) defines risk as ‘A function of the probability of an adverse health effect and the severity of that effect, consequential to a hazard(s) (in food)’. Risk assessment is defined as ‘A scientifically based process consisting of the following steps: (i) hazard identification, (ii) hazard characterisation, (iii) exposure assessment and (iv) risk characterisation.’ This information is used to inform and guide the development of risk management protocols. The HACCP approach has been adapted to the drinking water industry as ‘Water Safety Plans’ (Fewtrell & Bartram 2001; Haas & Eisenberg 2001; World Health Organization 2006a). In Australia, HACCP is also recognised in the generic national Health Risk Assessment

Guidelines (EnHealth Council 2002) and the Annapolis protocol based Natural Bathing Water Guidelines also promote HACCP (World Health Organization 2003).

In the case of swimming pools, risk assessment and management has historically focused on epidemiology and proximate impacts (Craun *et al.* 1996, 2005, 2006; Barwick *et al.* 2000). As a result, the sources of *P. aeruginosa* contamination in the pool environment and their relative importance tends to be unclear in pool surveys and outbreak studies. Based on observations such as these, and our own experience in natural bathing water risk assessment (Roser *et al.* 2006), we concluded: (i) treated pool Exposure Assessment and Risk Characterisation need to be better developed and (ii) reviewing the *P. aeruginosa* literature using a HACCP framework could facilitate the identification of current knowledge gaps. We have therefore structured this review based on the Codex Alimentarius Commission (1999) and EnHealth Council (2002) HACCP schemes (Figure 1).



**Figure 1** | Review structure showing issue relationships to a HACCP based conceptualisation of pool management. Pae, *Pseudomonas aeruginosa*.

## HAZARD ANALYSIS

### Hazard identification

#### Contamination factors

*Pool habitats.* *P. aeruginosa* can be derived from both human and non-human sources in substantial numbers (Hardalo & Edberg 1997; Wheater *et al.* 1980). Faecal and non-faecal shedding from humans is considered a major source of concern in the pool environments (Jacobson 1985; Hajjartabar 2004). Although the prevalence of colonisation of healthy adults outside the hospital is reportedly low (2.6–24%) (Rusin *et al.* 1997), this incidence is sufficient to generate infections in communal pools. It has been proposed that high water temperatures and turbulence in heated pools and tubs promote perspiration and desquamation. Such organic materials are likely to impact on disinfectant residuals and are a source of nutrients for microbial growth (Kush & Hoadley 1980; Ratnam *et al.* 1986; Price & Ahearn 1988). *P. aeruginosa* cells can be carried by fomites such as people's shoes (Fisher *et al.* 1985), towels, children's toys and inflatables (Buttery *et al.* 1998; Tate *et al.* 2003). Outdoor pools are additionally vulnerable to contamination from *P. aeruginosa* and organic matter from birds and rodents and windborne particulates (World Health Organization 2006b). Even relatively clean tap water, used in pool showers or even to fill pools, has an incidence of *P. aeruginosa* of 2–3% (Rusin *et al.* 1997; Leoni *et al.* 2001).

*Biofilms.* *P. aeruginosa* forms biofilms on virtually all surfaces, including soil particles, plant roots, leaves (Ercolani 1991), human tissues, e.g. skin, eyes, lungs and fomites (World Health Organization 2006b), and therefore, pool structures and surrounding damp surfaces such as decks, drains and benches can be sources of contamination (see Table 1) (Price & Ahearn 1988). Biofilms harbouring *P. aeruginosa* have been shown to accumulate in filters (Uhl & Hartmann 2005), on pool carpets (Hopkins *et al.* 1981), on shower floors (Leoni *et al.* 1999a), pool tools and toys. For example, inflatables appeared to be the source of *P. aeruginosa* in an outbreak in the UK (Tate *et al.* 2003).

*Seasonality.* Seasonality has been recognised as a significant risk factor for viruses in recreational water (Sinclair

*et al.* 2009); however, the seasonality of *P. aeruginosa* contamination in treated pools does not appear to have been well studied (Gibson *et al.* 1998; Ashbolt *et al.* 2010). Increased rates of gastroenteritis in swimming pools during the summer have been reported suggesting seasonal factors are important (Dale *et al.* 2009). Seasonal factors include solar visible and UV radiation (disinfection of outdoor pool environment), precipitation and community population density (increased disease burden) (Griffin *et al.* 1999; Rzezutka & Cook 2004). Higher temperatures should lead to higher evaporation of volatile disinfectants such as chlorine and higher bacterial and organic matter loading should also deplete available free chlorine, while the presence of urine, hair, skin, sweat and personal products should support bacterial growth (Uhl & Hartmann 2005; Liviac *et al.* 2010; Kanan & Karanfil 2011).

*Pool management.* Pool maintenance issues, which may not be detected by water quality monitoring, such as imprecise calibration of instruments measuring free chlorine and pH, may lead to inadequate chlorination and therefore facilitate blooms of *P. aeruginosa*. The drinking water industry has recognised that risk can be increased by multiple, simultaneous barrier failures or hazardous events (Risebro *et al.* 2007) and some case studies (see Table 1) suggest this may also occur in treated pools.

#### Hazard characterisation

##### Infection and illness

One of the major health effects ascribed to *P. aeruginosa* disease outbreak is otitis externa or 'swimmer's ear'. The bacterium has been viewed as an infrequent component of the skin's microflora (Meyer-Hoffert *et al.* 2011), but is the predominant bacterial pathogen isolated from patients. Isolation from the external auditory canal is associated with injury, maceration, inflammation, or simply wet and humid conditions (Havelaar *et al.* 1983; Jacobson 1985; Centre for Disease Control 2000; Hajjartabar 2004). Of bathers who reported ear problems, 79% were positive for *P. aeruginosa* with symptoms ranging from earache to hearing loss (Hajjartabar 2004). *P. aeruginosa* associated folliculitis is another major pool concern (Washburn *et al.*

**Table 1** | Contamination levels and microenvironments associated with outbreaks

Pool type	Sample source	Density	Positive samples (%)	Reference
Hydrotherapy pool	Pool pump off-line for 3–4 days	$>10^5$ cfu/mL	NA	Aspinall & Graham (1989)
Residential and commercial whirlpools	Tiles at water line	$<1-10^5$ cfu/mL	NA	Price & Ahearn (1988)
	Filter swabs	NA	33	
	Pool hose (residual water)	$>10^5$ cfu/mL	NA	
12 public indoor swimming pools	Pool edge floor	$1-1.58 \times 10^2$ cfu/100 cm <sup>2</sup>	65	Leoni <i>et al.</i> (1999a, b)
	Shower floor	$1-5 \times 10^3$ cfu/100 cm <sup>2</sup>	79.5	
	Changing room benches	$0-3.5 \times 10^1$ cfu/100 cm <sup>2</sup>	19.2	
Indoor swimming pool	Carpet from the pool edge, post outbreak	$2.8 \times 10^7$ cfu/g of carpet	NA	Hopkins <i>et al.</i> (1981)
Whirlpool	Whirlpool water	$9 \times 10^0$ to $3.4 \times 10^3$ cfu/mL	3 of 3	Ratnam <i>et al.</i> (1986)
Public swimming pool	Inflatables	NA	90	Tate <i>et al.</i> (2003)
Whirlpool	Wooden bridge	NA	100	Havelaar <i>et al.</i> (1983)

NA: not available.

1976) and may be promoted by damage to the skin (e.g. burns wounds), high moisture in the ears of swimmers and the skin of hot tub users (Havelaar *et al.* 1983; Highsmith *et al.* 1985). *P. aeruginosa* has also been associated with a range of other infections, including eye, urinary and respiratory tract infections (Salmen *et al.* 1983; Watt & Swarbrick 2005; Mena & Gerba 2009). Details of *P. aeruginosa* exotoxins and virulence factors can be found in various reviews (Liu 1974; Deretic 2000; Sadikot *et al.* 2005).

In contrast to the prevailing notion that *P. aeruginosa* is either only a transient coloniser of the skin, or is an opportunistic pathogen, e.g. in burn and cystic fibrosis (CF) infections (Lyczak *et al.* 2000), recent evidence suggests that *P. aeruginosa* may be part of the normal flora (Hogan *et al.* 2004; Cogen *et al.* 2008). The presence of *P. aeruginosa* as a commensal may explain the high numbers of *P. aeruginosa* that were observed to develop on supersaturated skin (Hojyo-Tomoka *et al.* 1973). Because the source of *P. aeruginosa* impacts directly on the application of HACCP, it would be valuable to reassess whether *P. aeruginosa* is part of the natural flora or an external infectious agent.

**Dose response.** Ideally, quantification of a *P. aeruginosa* dose response would reflect the ‘Single-Hit’ theory, the concept that a single viable pathogen can cause infection with the likelihood of infection expressed as a simple exponential probability function (Haas & Eisenberg 2001; Gale

2003). This theory is well established for enteric and respiratory pathogens in pool environments (Van Heerden *et al.* 2005; Schijven & de Roda Husman 2006; Schets *et al.* 2011) and the algorithms used to enumerate risk likelihood are ideal for use in quantitative microbial risk assessment (QMRA) (Haas *et al.* 1999; Haas & Eisenberg 2001). The ‘Single-Hit’ approach has some limitations, including wide uncertainty boundaries of the algorithms due to the limited available data (e.g. Teunis *et al.* 2002), and factors such as particle aggregation, hazardous events, exposure time course and differences in virulence of pathogen subgroups (Haas 1997, 2002a; Teunis *et al.* 2002; Oscar 2004; Teunis *et al.* 2008). Despite potential limitations (Haas 2002b), the popularity of QMRA continues to grow because risk characterisation assumptions are clear, risk estimates are definitive, auditable and revisable, and illness probability can increasingly be translated into disease burden via the disability adjusted life year concept (Pruss & Havelaar 2001).

Unfortunately, the construction of dose response relationships for *P. aeruginosa* is not well developed, and unlike the classic enteric pathogens, dose response relationships for dermal exposure are not clear (compare Haas & Eisenberg 2001; Kothary & Babu 2001; Gale 2003). *P. aeruginosa* may enter the body through almost any ‘exposed’ tissue, including the skin, ears, eyes, urinary tract (Mena & Gerba

2009), lungs, and the gut (Kerckhoffs *et al.* 2011). Each orifice will likely have a different characteristic dose response, compounding the challenge of determining dose responses for *P. aeruginosa* (Mena & Gerba 2009).

The clearest existing dose response data are for ingestion and inhalation studies and suggests a relatively low risk to healthy humans and animals. The oral infectious dose for *P. aeruginosa* in drinking water have been estimated, using the beta-Poisson model based on feeding *P. aeruginosa* to human volunteers or mice, to be in the range of  $10^8$  to  $10^9$  colony forming units (cfu) for humans and animals (Buck & Cooke 1969; George *et al.* 1989; Rusin *et al.* 1997). Inhalation dose estimates for *P. aeruginosa* also do not appear to be available for humans (Cant *et al.* 2002). George *et al.* (1991) found that the intranasal LD<sub>50</sub> dose in mice for aerosolised *P. aeruginosa* was  $2.7 \times 10^7$  cfu, while an intranasal inoculation of  $1.6 \times 10^5$  cfu per animal was cleared from the body with no mortality or morbidity and that a sub-lethal dose of  $10^6$  cfu was also rapidly cleared (George *et al.* 1993). As the mass of a mouse is ca. 1/5,000th of an adult human, these data suggest the median inhalation dose required for infection of a healthy person should be greater than  $>10^7$  cfu.

In the case of pool associated folliculitis outbreaks (Gustafson *et al.* 1983; Ratnam *et al.* 1986), the hazardous levels for healthy individuals have been suggested to be greater than  $10^5$  to  $10^6$  cfu/mL (Price & Ahearn 1988; Dads-well 1997). Note, this estimate is suggestive of the older dose 'threshold' concept (e.g. Kothary & Babu 2001) and it is unclear how such estimates translate into single-hit theory algorithms. Similarly, only limited quantitative dose response data are available for ear ailments. Hajjartabar (2004) studied the association between otitis externa and *P. aeruginosa* in indoor and outdoor pools and showed that *P. aeruginosa* contamination averaged 0.13 and 0.18 most probable number (MPN) per mL in the two sets of pools sampled during the peak use period. Infection likelihood was reportedly correlated with usage rates (591 and 857 bathers/day), average residual chlorine (1.3 and 1.7 mg/L) and typical time spent in pools (6–12 h/week). Of bathers who reported ear problems, 79% had positive *P. aeruginosa* ear swabs, whereas *P. aeruginosa* was found in only 4% of the control group (Hajjartabar 2004). This work also indicated that most infections rapidly resolved

themselves and only 5.6% of cases resulted in hearing loss. Studies in the Netherlands of the correlation between otitis and *P. aeruginosa* numbers in natural bathing waters also suggested relatively low infectious doses for aural infection/illness (Van Asperen *et al.* 1995; Schets *et al.* 2010).

A further limitation of current dermal and aural data is that they are based on associations with organism concentrations in pool water rather than controlled experimental studies involving well-defined doses. Though 'swimmer's ear' is a common complaint, studies have focused on attack frequency and illness consequences rather than causation for otitis externa (Reid & Porter 1981; Gustafson *et al.* 1983; Havelaar *et al.* 1983; Jacobson 1985; Beers & Abramo 2004). The limitations of ingestion and inhalation estimates are that few biotypes were compared, inoculated cells were washed (George *et al.* 1993) or diluted in another carrier media (milk) (Buck & Cooke 1969), probably reducing the influence of exotoxins, virulence factors, and the number of subjects challenged was small. The issue of infection is clearly complex as indicated by the otitis studies suggesting infectious doses of  $<1$  *P. aeruginosa*/mL in contrast to the  $>10^5$ /mL estimated for folliculitis (Price & Ahearn 1988).

A clear message from the dose response studies presented above is that there is no absolute number of *P. aeruginosa* that is important for infection, but rather the infectious dose is related to the specific type of infection. Further, dermal dose response is likely to be a function of contact time, a factor that is not included in the current beta-Poisson and exponential models. Such differences in dose response therefore must be incorporated in the hazard assessment process to develop a complete risk assessment and the associated risk mitigation strategy.

## Exposure assessment

### Environmental exposure pathways

The populations at greatest risk from *P. aeruginosa* and other opportunistic pathogens include those whose immune systems are compromised, including the elderly, young children and pregnant women. Other populations of concern include those who use pools frequently or for an extended period of time, e.g. swimming instructors and

athletes (Reid & Porter 1981; Rusin *et al.* 1997; Rose *et al.* 1998; Mena & Gerba 2009). There are many possible pathways that could result in contamination of the pool or infection of users and we suggest the conceptual model in Figure 2 to describe pathways that could lead to:

- epidermal contact and infection through the skin including the ears and eyes;
- inhalation of aerosol; and
- direct ingestion of water.

**Dermal exposure.** The most frequent infections associated with treated pools appear to be those leading to epidermal invasion leading to folliculitis and otitis externa (Gustafson *et al.* 1985; Havelaar *et al.* 1985; Ratnam *et al.* 1986; Beers & Abramo 2004). As well as high densities of *P. aeruginosa* found in pool water and the surrounding environment (Table 1), risk factors increasing the chance of folliculitis include being female, extended exposure and *P. aeruginosa* serotype (Reid & Porter 1981; Highsmith *et al.* 1985; Hudson *et al.* 1985; Birkhead *et al.* 1987). Risk factors considered to increase the occurrence of otitis externa related to water exposure include: (i) time spent in the water; (ii) age (less than 19 years); (iii) a history of previous ear infections; and (iv) repeated exposure to water (Seyfried & Cook 1984; Van Asperen *et al.* 1995; Schets *et al.* 2011). An information gap is data on the physiological mechanisms that facilitate epidermal adherence and invasion, analogous to that available for pathogenic *Escherichia coli* (e.g. Kaper *et al.* 2004). Also absent in the literature is a mechanistic description of the pool ecology processes driving *P. aeruginosa* behaviour and risk (e.g. biofilm attachment and detachment) comparable to that developed for other environments such as aquifers (Foppen & Schijven 2006).

**Inhalation.** In addition to needing to better understand the infectious dose for *P. aeruginosa* when inhaled (see section above), it is important to know the volume that is typically inhaled or aspirated during swimming. Armstrong (2005) estimated numbers of bacteria inhaled in pool environments and 'bacterial water to air partitioning coefficients' ( $PC_{bwa}$ ), the ratio of bacteria or particulates in air to those in water. The  $PC_{bwa}$ s for *Legionella* in hot tubs was calculated to be  $1.6 \times 10^{-5}$  to  $3.1 \times 10^{-5}$  L (of water)/m<sup>3</sup> (of aerosol) (Rose *et al.* 1998), while the  $PC_{bwa}$  for shower aerosols was  $<5 \times 10^{-5}$  to  $3 \times 10^{-4}$  L/m<sup>3</sup>. Chen *et al.* (2008)

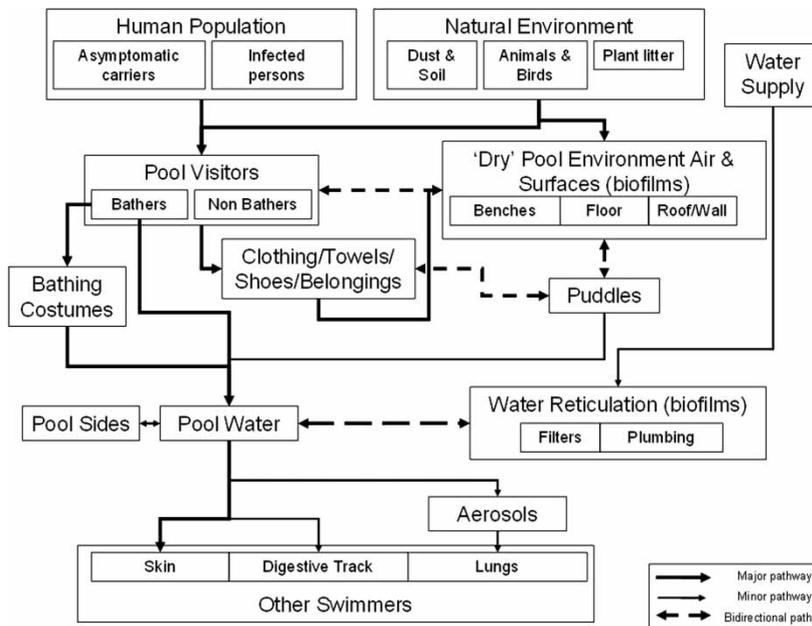
estimated a somewhat higher  $PC_{bwa}$  of  $10^{-3}$  L/m<sup>3</sup>, based on aerosol and water concentrations of endotoxin at a swimming park. These figures are comparable to partitioning estimates reported by Angenent *et al.* (2005) and Medema *et al.* (2004). Overall partitioning data indicate a conservative pool  $PC_{bwa}$ s of  $10^{-3}$  to  $10^{-4}$  L/m<sup>3</sup>. When combined with moderate intensity of activity, inhalation rates (average/95th percentile 1.8/2.4 m<sup>3</sup>/h, respectively) (USEPA 2009) pool water inhalation appear to be ca. 0.2–2 mL/h.

**Ingestion.** The amount of water ingested by swimmers and pool users, which directly impacts on the delivery of an infectious dose, will depend upon a range of factors, including experience, age, skill, exposure duration and type of activity. Early QMRA studies (Crabtree *et al.* 1997; Van Heerden *et al.* 2005) proposed an ingestion volume of 30 mL per exposure. Subsequent studies (Dufour *et al.* 2006) collected urine samples from swimmers who had used a pool disinfected with dichloroisocyanurate and analysed the concentration of cyanurate in urine to quantify ingestion. Average water intakes were higher for children (37 mL) than adults (16 mL). The upper 95th percentile intake for children was approximately 90 mL. These average volumes are in the same order of magnitude as those determined in questionnaire studies for swimming pools (Schets *et al.* 2011) and divers (Schijven & de Roda Husman 2006). The latest USEPA (2009) swimming ingestion standards for risk assessment reflect these data (medians for adults/children 16/37 mL and 95th percentiles of 53/154 mL per event, respectively). These estimates also compare well with older assumptions and child worst case estimates of ca. 100 mL (e.g. NH&MRC 2008).

## Risk characterisation

### *P. aeruginosa* levels

Despite frequent water monitoring, risk estimates for *P. aeruginosa* in treated pools, analogous to that possible for enteric pathogens (Van Heerden *et al.* 2005; Schijven & de Roda Husman 2006; Schets *et al.* 2011), is not yet possible. This appears due to a range of data gaps, e.g. (i) limited data on the complex exposure pathways by which bathers can come in contact with *P. aeruginosa* (Figure 2); (ii) the diversity of illnesses and population sensitivities; and (iii) a lack of studies



**Figure 2** | *Pseudomonas aeruginosa* exposure pathways.

correlating pool *P. aeruginosa* numbers with disease incidence compared to natural bathing (e.g. Prüss 1998).

Some provisional conclusions regarding *P. aeruginosa* levels can be drawn from consideration of the Hazard Characterisation and Exposure Assessment literature. The commonly used benchmark of  $<0.01$  cfu/mL (e.g. Department of Health NSW 1996) is well below recognised levels of concern in respect to folliculitis ( $>10^5$ /mL), although for otitis externa the level of concern appears in some reports to be  $<1$ /mL (Van Asperen et al. 1995; Hajjartabar 2004; Schets et al. 2010).

Provided a *P. aeruginosa* benchmark of 0.01 cfu/mL is largely achieved, ingestion and inhalation appear to pose little risk to health bathers. Where *P. aeruginosa* numbers reach  $10^6$  cfu/mL, inhalation doses could approach the infectious dose observed for mice (George et al. 1991, 1993), and at such levels, virulence factor secretion and a  $PC_{bwaS} > 10^{-3}$  L/m<sup>3</sup> in vigorously aerated whirlpools, could increase the risk of illness.

Overall the folliculitis, ingestion and inhalation risk data suggest that *P. aeruginosa* detection in pools at intermediate levels ( $>0.01$  to  $10^2$  cfu/mL) should remain a trigger for timely management action but may not represent levels that are immediately a risk to the public or basis for litigation. Having an advisory message reflecting this intermediate pool state between a full public health warning

and an 'all is safe' may prove a useful management tool for preventing over-reaction to *P. aeruginosa* detection.

### Sanitary surveys

Sanitary surveys involve a qualitative or semi-quantitative evaluation of the occurrence of risk moderating factors. In the case of natural bathing these include sewage contamination, stormwater and bather shedding. The results of water monitoring and sanitary surveys are then integrated to define a waterbody's suitability for use on a five-point scale of very good to very poor. The process and outcomes are flexible and auditable and the integration focuses managers away from monitoring statistics onto the central issue of overall risk.

It appears to be standard practice to undertake a sanitary survey after a pool related outbreak (e.g. Washburn et al. 1976; Hopkins et al. 1981; Aspinall & Graham 1989) and treated pool guidelines (e.g. Department of Health NSW 1996; World Health Organization 2006b) document an array of survey considerations. Despite this, explicit 'sanitary surveys' and 'risk characterisation' of the kind developed for natural waters are not currently part of the recommendation for pathogen risk assessment of treated pools. This could reflect a perception that sanitary surveys are so commonplace that full exposure pathway/HACCP

style risk assessment is unnecessary. However, given that outbreaks of *P. aeruginosa* remain a problem, we suggest that a broader characterisation of pool ecosystems and pool built environment (Figure 2) and a more formalised sanitary surveys system may be warranted.

## Risk management

Favero *et al.* (1971) showed that *P. aeruginosa* can grow from 1 to 10 cfu/mL to average concentrations of  $10^6$ – $10^7$  cfu/mL within 3–4 days in distilled water in hospitals, illustrating why continuous effective disinfection of pools is critical. In addition, the ability of *P. aeruginosa* to form biofilms enhances its potential to persist in the pool environment. Thus, its complete elimination from the pool environment is impractical and disease prevention needs to be based on improved management. The dominant protective barriers which reduce the density of *P. aeruginosa* in most swimming pools are chlorination, UV treatment and filtration. A summary of these barriers and their effectiveness is presented in Table 2.

## Water quality

**Chlorination.** Generally, free-living, planktonic *P. aeruginosa* are unable to survive in pool water that is adequately chlorinated. Conversely, chlorine levels tend to be depressed in poorly maintained systems and therefore chlorine levels serve as a good indicator of general pool health. Survey data from a large number of pools have found that the incidence of *P. aeruginosa* increased when free chlorine residual dropped below 0.4 (Seyfried & Fraser 1980) and 1.0 mg/L (Esterman *et al.* 1984) or when free chlorine was below 0.3 mg/L in whirlpools (Havelaar *et al.* 1983). The relatively high sensitivity of planktonic *P. aeruginosa* to low concentrations of free chlorine noted in the pool survey data have also been confirmed using well controlled laboratory studies. Aspinall & Graham (1989) showed that 0.5 mg/L of free chlorine resulted in a 99.5% reduction of *P. aeruginosa* within 5 min. Similarly, Seyfried & Fraser (1980) demonstrated that 0.4 mg/mL reduced the number of *P. aeruginosa* by 99.8% within 1 min, however, as water becomes more alkaline, the efficiency of chlorination decreased, which is in agreement with observations that high levels of

*P. aeruginosa* in the presence of high free chlorine can largely be accounted for by periods when the pool water pH was greater than 8 (e.g. Seyfried & Fraser 1980).

The bactericidal efficiency of combined chlorine is also significant, however, higher doses and/or longer contact times are generally required than for chlorine alone. In the presence of 1 mg/L of combined chlorine, Ward *et al.* (1984) showed that 99% inactivation of *P. aeruginosa* required 4 min at pH 6, and 9–9.5 min at pH 9. One ppm of active chlorine (in the presence of 0.3–1.5 mg/L of urea) reduced *P. aeruginosa* by 4 orders of magnitude in 10–20 min at pH 7, while 150 ppm hydrogen peroxide or silver ions were ineffective (Borgmann-Strahsen 2003). Inactivation times in real pool situations can be comparatively longer since disinfection is constrained by the presence of other compounds in water and high temperatures. For example, Fitzgerald & Der Vartanian (1969) showed that the time required to inactivate 99.9% of *P. aeruginosa* in swimming pools (at 0.5 mg/L chlorine), varied between a few minutes and 3 h, depending on pool design (indoor/outdoor), water conditions and time of the day.

**UV disinfection.** While inactivation rates of *P. aeruginosa* under natural solar conditions in pools or other recreational waters are not available, such data are available for experimental systems, e.g. transparent drinking water bottles exposed to both natural and artificial sunlight (e.g. Lonnen *et al.* 2005; Dejung *et al.* 2007). The time required for 1 log<sub>10</sub> inactivation of *P. aeruginosa*, at 1.69 mW/cm<sup>2</sup> of UV-A (320–405 nm), was 1.2 h, which was 17–26% longer than observed for *E. coli* (Dejung *et al.* 2007). The lower efficiency for inactivation of *P. aeruginosa* by UV was also noted by Hassen *et al.* (2000), who showed that *P. aeruginosa* was more tolerant to UV treatment than most non-spore forming indicators (faecal coliforms and faecal streptococci). At UV doses of 54, 108 and 162 mJ/m<sup>2</sup>, 1.0–1.1 log<sub>10</sub> reductions of *P. aeruginosa* were noted, compared to a 3 log<sub>10</sub> reduction of standard indicator organisms. The relative tolerance of *P. aeruginosa* suggests that UV disinfection procedures which are commonly based on meeting indicator targets (e.g. *E. coli*) may not be adequate to eliminate *P. aeruginosa* (Hijnen *et al.* 2006). This reduced efficacy is exacerbated when *P. aeruginosa* grows as a biofilm, where it was shown that *P. aeruginosa* in an extracellular polymeric matrix (EPS) was protected

**Table 2** | Sensitivity of *P. aeruginosa* to swimming pool treatment processes

Pool/study type	Isolate	Temp (°C)	Disinfectant type or barrier	pH	Dose	Time	Reduction	Reference		
Laboratory study	Serotypes 8, 10, 11 and other pool isolates	NA	Free chlorine	7.4	0.4 mg/L	1 min	>99.9%	Seyfried & Fraser (1980)		
				8	0.4 mg/L	1 min	89.8%			
				7.4	0.2 mg/L	1 min	>99.1%			
Laboratory study	NA	NA	Free chlorine	NA	0.25 mg/L	20 min	4.6 log <sub>10</sub>	Aspinall & Graham (1989)		
					0.5 mg/L	5 min	4.6 log <sub>10</sub>			
					1.0 mg/L	<10 sec	4.6 log <sub>10</sub>			
Laboratory study using pool and potable water	Mucoid cells SG41	NA	Free chlorine	7.3–7.8	0.11–0.57 mg/L	5 min	0.4–4.3 log <sub>10</sub>	Grobe <i>et al.</i> (2001)		
						0.5 min	0.2–2.5 log <sub>10</sub>			
						5 min	0.9–5.3 log <sub>10</sub>			
Laboratory study using potable water	ATCC 9721	22	Chloramine	6	1 mg/L	4 min	99%	Ward <i>et al.</i> (1984)		
						8	1 mg/L		9–9.5 min	99%
						6	3 mg/L		1.5 min	99%
Laboratory study	6A-11983	25	Free chlorine + urea	8	3 mg/L	3.5–4.5 min	99%	Borgmann-Strahsen (2003)		
						8	3 mg/L		<2 min	99%
						8	3 mg/L		8.5 min	99%
Laboratory study	DSMZ 939	25	Free chlorine + urea	7	1 ppm	30 s	2.6 log <sub>10</sub>	Borgmann-Strahsen (2003)		
						10 min	4.0 log <sub>10</sub>			
						20 min	4.1 log <sub>10</sub>			
Water samples collected from an open-air pool at the end of a hot, sunny day	2F5 strain of <i>P. aeruginosa</i>	Warm	Free chlorine	7.3	0.5 mg/L	30 min	0.34 log <sub>10</sub>	Fitzgerald & DerVartanian (1969)		
						30 min	0.34 log <sub>10</sub>			
						30 min	0.34 log <sub>10</sub>			

(continued)

Table 2 | continued

Pool/study type	Isolate	Temp (°C)	Disinfectant type or barrier	pH	Dose	Time	Reduction	Reference
Water samples collected from an open-air pool at midday on a cool day		Cold				<1 min	99.9%	
Water samples collected from an indoor pool under nominal cool conditions		Cold				1–4 min	99.9%	
Water samples collected from an indoor pool at the end of a hot day		Warm				>3 h	99.9%	
Pilot UV disinfection unit	<i>P. aeruginosa</i>	NA	UV-A		54–162 mW/ s cm <sup>2</sup>		1.0–1.1 log <sub>10</sub>	Hassen <i>et al.</i> (2000)
Drinking water bottles exposed to full sunlight	<i>P. aeruginosa</i>	<44	Sunlight	6.3	16.9 W/m <sup>2</sup>	1.2 h	1.0 log <sub>10</sub>	Dejung <i>et al.</i> (2007)
Drinking water bottles exposed to artificial sunlight	ATCC 9027	<40	Solar simulator	6.5–6.6	870 W /m <sup>2</sup> in the 300 nm–10 μm range, 200 W/ m <sup>2</sup> in the 300–400 nm UV range	2 h	5.0± (0.2) log <sub>10</sub>	Lonnen <i>et al.</i> (2005)
Swimming pool filtration	<i>P. aeruginosa</i>	NA	Rapid sand and diatomaceous earth filters				>99%	Leoni <i>et al.</i> (1999a, b)

from exposure to UV-A, UV-B and UV-C at levels consistent with solar radiation (Elasri & Miller 1999). UV treatment has been trialled on water distribution plants and was found to either not substantially reduce bacterial numbers or that bacterial numbers increased in UV treated systems (Långmark *et al.* 2007). These reports reflect our own experience where UV exposure killed >99.5% of planktonic bacteria in contrast to <90% of biofilm bacteria formed on a reverse osmosis membrane (unpublished observation). Another limitation of UV disinfection is that it does not retain any residual activity, so that, despite initial killing of planktonic or biofilm cells, bacteria can rapidly regrow after the UV treatment is removed or after they pass the UV treatment unit (e.g. as in a flowing system) (Guo *et al.* 2009). Conversely, when the UV treatment is combined with standard chlorine treatment, the combination treatment can show greater killing of both planktonic as well as biofilm grown *E. coli* (Murphy *et al.* 2008).

**Filtration.** Filtration can significantly improve water quality by removing *P. aeruginosa* dramatically, however, filters can also harbour opportunistic pathogens if poorly maintained. Rapid sand filters and diatomaceous earth have been shown to remove >99% of *Pseudomonas*, however, similar high removal was not observed for mycobacteria and total bacterial counts (Leoni *et al.* 1999a, b). Granular activated carbon beds may promote *P. aeruginosa* contamination (Uhl & Hartmann 2005) by accumulating nutrients on the activated carbon surface and in turn encourage biofilm growth. It has been demonstrated that *P. aeruginosa* can reach up to  $10^7$  cfu/g on packed beds of glass beads confirming that filtration media, such as sand filters, can harbour high numbers of bacteria (Liu & Li 2008) and analysis of fast sand filters has shown that biofilms are complex communities, including *Pseudomonas* spp., that can reach up to  $10^5$  cfu/g (El-Masry *et al.* 1995). While there are currently few data on the microbiology of sand filters in pool systems, such data would be important for a complete understanding of the sources of *P. aeruginosa* in the treated pool environment.

**Hygiene.** In the USA and Europe, many public pools require users to shower prior to entry in an effort to reduce the introduction of pathogens and organics into the pool. However, proper hygiene requires a shower with soap rather than a simple rinse and such procedures are difficult to enforce. Hygiene is likely to be most important

under conditions of high bather load, where pre-swim showering may reduce the shedding of skin and human organic substances such as dead skin cells into pool water that can deplete the available free chlorine and accordingly increase bacterial loads.

## Ecology and management

**Persistence and EPS.** The growth of mucoid strains, those that overproduce EPS material and hence are more tolerant to chlorine than non-mucoid forms, represents a significant challenge to the control of *P. aeruginosa*. Mucoid strains show enhanced survival at free chlorine concentrations commonly used for the disinfection of swimming pools (0.11–0.57 mg/L), and thus may account for the presence of *P. aeruginosa* even in those pools with a properly maintained chlorine residual (Grobe *et al.* 2001). Given that free chlorine at *in vitro* levels of ca. 0.5 mg/L is very effective against non-mucoid *P. aeruginosa*, it is possible that pool environments provide a selective pressure for the promotion and growth of chlorine tolerant mucoid strains. This may be particularly exacerbated by shock treatments of high chlorine which further select for strains with higher tolerance.

**Biofilms and disinfection.** Excess production of EPS by bacteria is often associated with biofilm formation, and this is particularly true for *P. aeruginosa* (Goeres *et al.* 2004). Biofilm formation, which is increasingly viewed as the primary mode of existence of bacteria in the environment, is relevant to microbial control in swimming pools because biofilm bacteria are protected from a range of stresses, including UV light, chlorine, natural predators (e.g. protozoa) and antibiotics (Høiby *et al.* 2001; Jefferson 2004; Buckingham-Meyer *et al.* 2007). For example, biofilms of *P. aeruginosa* were up to 10,000-fold more tolerant to quaternary ammonium chloride or chlorine treatment compared to planktonic cells (Buckingham-Meyer *et al.* 2007). The ability to resist biocidal treatment means that shock disinfectant application may only kill planktonic populations, leaving the biofilms largely unaffected. Thus, the biofilm may represent a significant reservoir or sink of *P. aeruginosa* that can seed planktonic cells into the water column in between shock chlorine treatments. *P. aeruginosa* has been shown to form biofilms in the presence of 1–3 mg/L

of chlorine and shock treatments of 10 mg/L of chlorine were required to reduce biofilm numbers (Goeres *et al.* 2004). Similarly, Vess *et al.* (1993) demonstrated that EPS production facilitated biofilm formation by *Pseudomonas* and enhanced survival at chlorine levels of 15 mg/L. Together, these observations suggest that *P. aeruginosa* biofilms may not be adequately managed under current pool management regimes.

**Water quality monitoring.** Traditional water quality monitoring of swimming pools based on viable cell counting has many limitations (World Health Organization 2006b), e.g. small sample sizes, assay completion time. Further, biofilms may not be detected until large numbers of free-living cells are shed into the planktonic phase. For a pool holding one megalitre of water, it would only require  $10^8$  *P. aeruginosa* cells (ca. 0.1 mg of cell material or a single colony on an agar plate) to achieve a concentration of 10 cfu/100 mL, biofilms are not uniformly distributed over surfaces and swab samples would not account for biofilms formed in the pool plumbing system. Therefore, traditional water sampling (i.e. grab samples of the water column) is insufficient for monitoring risks. For these reasons, managing pool contamination and disease risk must account for and monitor biofilm development.

## CONCLUSIONS

Water and water security issues are emerging as one of the key global challenges for the 21st century, where the goal is to ensure safe water supplies that are pathogen and chemical free. While this is mostly viewed from the drinking water perspective, it also applies to recreational waters. This is particularly true given the significant role that recreational waters (e.g. pools, spa, reservoirs) receive over 1 billion visits per year. Despite routine maintenance, testing and regulatory guidelines, swimming pools and spas remain prone to *P. aeruginosa* contamination. The consequences of such events can be temporary pool closures, discharge the pool water for cleaning (2,500,000 L for an Olympic size pool) as well as community-based infections (e.g. ear infections, eye infections and folliculitis). Therefore, there is a clear need to develop new and to refine existing tools to enable pool operators to manage these systems optimally.

The drinking water industry has adopted and benefited from a risk management based approach, based on HACCP. We suggest this system should also be applied to treated pools systematically. To achieve this, several aspects of the problem must be defined, including: (a) the factors facilitate growth of *P. aeruginosa* within pools, based on its natural ecology as well as factors within the built environment; (b) types of infection caused by the pathogen and quantitative dose responses; and (c) what are the key exposure routes. Such information can then be used to characterise the risk within treated pools by comparing such information with the prevalence of *P. aeruginosa* within pools and identification of the primary reservoirs that inoculate *P. aeruginosa* into the pools. The integration of such data should then provide the basis for developing pool risk based management plans, which include not only monitoring of bacterial levels and chlorine residuals, but also bather numbers, temperature, filter behaviour and biofilm development.

Based on the existing literature, it was possible to identify literature estimates for some aspects necessary to develop the HACCP-based approach, but there were considerable gaps in data necessary to fill all aspects of the HACCP approach. Key information gaps included dose responses algorithms, especially between strains and disinfection rates. Interpretation of the available data was complicated by the fact that *P. aeruginosa* causes multiple diseases. The factors that lead to barrier failure in the treated pool environment appear incompletely understood or quantified, e.g. how chlorine concentrations, ambient temperatures, bather loads, organic materials and pH interact. It has only recently been appreciated that the formation of biofilms represents a key survival strategy for bacteria, significantly increasing their resistance to disinfectants, including chlorine and UV treatments. The biofilm issue is important to understand since it impacts on sampling methods, where biofilms would be responsible for shedding planktonic bacteria into the water column, and can be present anywhere in the water system of the pool, including pool and pipe surfaces, fomites and filters. Therefore, there is a need to develop a detailed understanding of the ecology and epidemiology of *P. aeruginosa* in the treated pool environment, e.g. the contribution of *P. aeruginosa* from the skin as a commensal versus introduction from the environment, to

facilitate the operation of treated pools and improve bather safety.

## ACKNOWLEDGEMENTS

The authors recognise the support provided by The City of Sydney, NSW Australia. The authors declare they have no actual or potential competing financial interests.

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