

## REVIEW ARTICLE

# *Pseudomonas aeruginosa* dose response and bathing water infection

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## SUMMARY

*Pseudomonas aeruginosa* is the opportunistic pathogen mostly implicated in folliculitis and acute otitis externa in pools and hot tubs. Nevertheless, infection risks remain poorly quantified. This paper reviews disease aetiologies and bacterial skin colonization science to advance dose-response theory development. Three model forms are identified for predicting disease likelihood from pathogen density. Two are based on Furumoto & Mickey's exponential 'single-hit' model and predict infection likelihood and severity (lesions/m<sup>2</sup>), respectively. 'Third-generation', mechanistic, dose-response algorithm development is additionally scoped. The proposed formulation integrates dispersion, epidermal interaction, and follicle invasion. The review also details uncertainties needing consideration which pertain to water quality, outbreaks, exposure time, infection sites, biofilms, cerumen, environmental factors (e.g. skin saturation, hydrodynamics), and whether *P. aeruginosa* is endogenous or exogenous. The review's findings are used to propose a conceptual infection model and identify research priorities including pool dose-response modelling, epidermis ecology and infection likelihood-based hygiene management.

**Key words:** Acute otitis externa, dermatitis, folliculitis.

## INTRODUCTION

*Pseudomonas aeruginosa* is the most commonly identified opportunistic pathogen associated with pool-

acquired bather disease [1]. To better understand why this microorganism poses this protracted problem we recently appraised *P. aeruginosa* pool risk management. Much is known about the wider ecology of *P. aeruginosa* [2]. However, in contrast to ingested and inhaled pathogens, neither the preceding exposure conditions, the dermal route of infection, nor infectious doses, appeared well-defined. The goal of this follow-up review is to explore these knowledge gaps

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through a survey of data and theory on: (i) dose response, i.e. bodily responses arising from different microbial doses [3]; (ii) folliculitis and acute otitis externa (AOE) aetiology; and (iii) ecological processes preceding infection.

The review commences with an introduction to *P. aeruginosa* pool disease attributes, research drivers and knowledge gaps, and microbial dose-response theory. Next folliculitis and AOE aetiology relating to pools and experimental disease induction are reviewed, including uncertainties, notably whether *P. aeruginosa* might be autochthonous and whether folliculitis should be viewed as multiple localized infections rather than a systemic infection. Biophysical steps whereby *P. aeruginosa* migrates from the pool void to the epidermis are reviewed in the third section. How to progress dose-response theory bearing in mind aetiology and ecology is then analysed. Finally, using all information we discuss management implications, propose a conceptual framework and identify priority research.

### Pool infections and *P. aeruginosa* disease

Pool-acquired *P. aeruginosa* infection most commonly involves folliculitis, a rash then lesions around hairs, which progress from discrete follicular pruritic papules to erythematous papulopustules within a day [4]. Although usually localized and rapidly resolved, folliculitis can persist for several months [5]. *P. aeruginosa* is also associated with AOE, colloquially ‘swimmer’s ear’, an infection or inflammation of the external ear canal in rare cases progressing to necrotizing otitis externa [6]. Although other pathogens are associated with folliculitis and AOE, *P. aeruginosa* is viewed as the most common agent [6].

### Knowledge gaps and the need to quantify dermal dose response

Defining risk factors and dose-response mechanisms operating in pools is central to quantitative microbial risk assessment (QMRA)-based management. However, characterization of dermal and aural dose response, and exposure processes preceding infection, have been neglected. Rather, dose-response information on *P. aeruginosa* primarily pertains to ingestion, inhalation and subdermal inoculation [2]. This gap is also evident in reviews by Mena & Gerba [1] and Barna & Kádár [7] of *P. aeruginosa* and pool infection, respectively. The only dermal dose response

reported is a folliculitis minimum infective dose (MID) of 1000 c.f.u./ml [8].

To improve understanding and management of pool infection there is the need, first, for more sophisticated dose-response conceptualization implied by the Key Events Dose-Response Framework (KEDRF) proposal [3, 9]. Although folliculitis and AOE have been extensively documented since the 1980s [4, 10], many past studies were relatively unsystematic, e.g. statistically, by modern experimental standards. Therefore second, many knowledge gaps remain to be addressed, e.g. rarity of head immersion data accompanying AOE reports (an exception is [11]) and genomic studies, poor correspondence between outbreak frequency and water quality [1, 8, 10]. Finally there are diverse risk unknowns such as the safety of hydrotherapy pools for an ageing population (see also online Supplementary material).

Our review focuses on the first elements of the KEDRF framework (fig. 2 in [3]) which splits the dose-response process into: (i) intake/exposure; (ii) biological interaction/process; (iii) interaction/process (transport/distribution/excretion); (iv) interaction/process (metabolism); (v) target issue interaction; and (vi) ultimate effect: to support data evaluation, focus research, strengthen decision-making and advance dose-response assessment. Specifically, we explore KEDRF steps (i)–(iii), which correspond to the gaps we identified in dermal dose-response theory. These delineate infection from the better characterized illness induction processes, steps (iv)–(vi) [e.g. 12, 13].

### Dose-response theory and the epidermis

Modern dose-response characterization used in QMRA [14–16] reflects the introduction of the ‘single-hit’ exponential model of infection, i.e. a single viable propagule may cause systemic infection, with a probability reflecting exposure dose and infection site availability [Table 1, equation (6)]. Algorithm coefficients are typically derived from human exposure and foodborne disease outbreak studies where pathogen density estimates and attack rate data are concurrently available. This paradigm’s origin is credited to Furomoto & Mickey’s [17, 18] analysis of tobacco leaf mosaic virus (TMV) infection. Deviations from the exponential model have been addressed by introducing beta-Poisson, hypergeometric and other algorithm forms which allow better curve fitting [14–16]. These can incorporate secondary variance sources including between-strain variance and inoculum dispersion [16].

Table 1. Selected algorithms, which support dose-response quantification

Equation no.	Short description	Comments
(1): If $d < \text{MID}$ then $P_{\text{inf}} = 0$	Minimum infective dose	Algorithm is our formulation of the MID concept [9] which is imprecisely defined in the literature
(2): $D = \frac{k_b^0 k}{6\pi\eta a}$	Brownian diffusion rate	Stokes–Einstein equation. Units are $\text{cm}^2/\text{s}$ . Equation (2) in [40], equation (9) in [42]
(3a): $N_D = 1.13 C_0 (Dt)^{0.5}$	Particles colliding with a surface per unit area under ideal	Use equation (3a) where $<40\%$ of particles absorbed. Units are number/ $\text{cm}^2$ . Equation (9) in [40]
(3b): $N_D = C_0 h \left(1 - \frac{8}{\pi} e^{-(\pi^2 Dt/4h^2)}\right)$	Brownian motion	Use equation (3b) where $>28\%$ of particles are absorbed. Units are number/ $\text{cm}^2$ . Equation (10) in [40]
(4): $Sh \approx 0.5[1 + (1 + Pe)^{1/3}]$ , where	Sherwood number based on Péclet number	Sherwood number is the factor by which diffusive transport is increased due to local fluid motion. Equation (5) and p.104 in [42]
(5): $Pe = \frac{Ul}{D}$		
(6): $P_{\text{inf}} = 1 - e^{(-rd)}$	Exponential model	Limiting case is the maximum likelihood curve where $r = 1$ . Equation (9) in [17], equation (8.1) in [15]
(7): $P_{\text{inf}} = 1 - e^{(-r_c c)}$	Exponential model (density)	Variant proposed for pool <i>P. aeruginosa</i> . Equation (4) in [17]
(8): $d = \gamma C$	Dose: pathogen density ratio	Adapted from equation (1) in [17]
(9): $N_l = A \ln(1 + BC)$	Lesion number as function of microbial density model	Abstract and equation (15) in [17]
(10): $N_l = N_D Sh^{0.5} S_{\text{im}\%} I_{\%}$	Proposed dispersion-infection density model	$N_D$ is calculated from equation (3a)

$a$ , Particle radius;  $A$  and  $B$ , algorithm constants;  $C$ , density of pathogen;  $C_0$ , density of pathogen at time 0;  $\gamma$ , constant describing the relative infectivity;  $d$ , pathogen dose;  $D$ , Brownian diffusion rate;  $h$ , depth of fluid above surface;  $I_{\%}$ , infection efficiency;  $k_b$ , Boltzmann constant;  $l$ , characteristic length of a particle; MID, minimum infectious dose;  $N$ , number of lesions;  $N_D$ , number of particles absorbed under ideal Brownian diffusion assuming 100% adherence;  $N_l$ , lesions per unit area;  $\eta$ , viscosity;  $Pe$ , Péclet number;  $P_{\text{inf}}$ , probability of infection;  $r$ , constant;  $r_c$ , constant;  $S_{\text{im}\%}$ , per cent surface adherence + migration efficiency;  $Sh$ , Sherwood number;  $t$ , time;  $U$ , velocity from flow regimen.

‘Single-hit’ theory has proved a great advance over the MID concept [9] [Table 1, equation (1)] especially for inhalation and ingestion of water, food and aerosols. Unlike MIDs, algorithms are grounded in probability theory and experimental data, and support infection ecology and water and food-safety investigations. However, skin infection dose-response algorithms are few: Tamrakar & Haas’s [19, 20] application of dose-response theory to inoculation by scratches and bites, and Rose & Haas’s [21] equations for hand washing; neither of which considers whole body exposure. Nevertheless, these studies do incorporate several mechanistic factors, e.g. exposure time ( $t$ ), skin interfaces, bacterial growth dynamics, and the latter [21] endeavours to ground dose-response theory in pathogen–host biophysics.

## AETIOLOGY AND DOSE RESPONSE

### Water quality and infection likelihood

Folliculitis incidents and bathing water quality data (Table 2) point to high variance in the *P. aeruginosa*

density associated with outbreaks ( $0.01$ – $10^7$  c.f.u./ml) [1, 10]. Survey data also suggests that levels could reach  $10^3$  c.f.u./ml without outbreaks, consistent with Price & Ahearn’s [8] proposed threshold. These discrepancies make defining hazardous levels difficult and may reflect unknown disease induction factors. The value of outbreak reports is also limited by: (i) little concurrent reporting of *P. aeruginosa* densities [1, 10]; (ii) data being seldom systematic and statistically robust; (iii) context information paucity; (iv) delays between infection and water sampling; (v) analytical technology limitations; and (vi) limited strain virulence or genotyping data.

*P. aeruginosa* O:11 is suggested as the main serotype of concern in pools; however, other biotypes also cause infection [5, 22] and whether O:11 prevalence reflects virulence or environmental abundance is unclear. Subtyping of virulent isolates appears inconclusive [10, 22] and systematic [16] molecular typing has not been applied to *P. aeruginosa* bathing outbreaks.

Information on AOE is similarly deficient. Hajjartabar [23] reported an infection rate of 79% when

Table 2. *P. aeruginosa* densities reported in folliculitis pool outbreaks and contamination survey data

<i>P. aeruginosa</i> (log <sub>10</sub> c.f.u./ml)*	Attack rates	Exposure time	Comments	Reference†
>0·3 (14 of 16 homes)	6% (of homes) (3% users)	Unreported	Survey of hydrotherapy pools with a check for infection	[89]
>0·3	17%	Unreported	Hydrotherapy pool	[90]
<−2 to −1·5	53%	Unreported	Whirlpool and swimming pool, serotype O:11	McCausland & Cox, 1975 cited in [10]
2·7	24%	26 min average use	Mainly serotypes O:9 and O:11	[91]
6·9–7	88%	Unreported	Spa pool, serotype O:11	Stafford <i>et al.</i> 1982 cited in [10]
1–3·5 (follow-up)	50% (adults) 90% (children)	15 min 55 min	Whirlpool, serotype O:7	[10]
<−2 to −1·5 (follow-up)	52%	Unreported	Swimming pool and spa, serotypes O:4 and O:11	[92]
<−2 (4 days after outbreak conclusion) 7·4 (per g of carpet)	29, 88, 100% on three consecutive days followed by disappearance	Unreported	Indoor pool, serotype O:11	[93]
5·2	Not reported	(Wading)	'Hot foot' syndrome	[68]
0·3 to >3	No outbreak.	Survey of pools	Brief letter suggesting outbreaks are common but not well reported.	[94]
< −1·7 to >1·3, median −0·5	No outbreak	Water quality survey	Whirlpools, serotypes O:9 and O:11 = 23–38%	[95]
>1 (12–35% of spas)	No outbreak	Water quality survey	Hydrotherapy, spa and swimming pools	[96]
4–5, 5, 3, <0, 3–4, 2–5, 3–4	No outbreak	Water quality survey	Whirlpools, 14/15 samples positive	[8]‡
Increase to 4–6 in 24–48 h then decline		Unreported	Whirlpool, two household systems filled with water	[8]‡
Increase to 5–7 in 72–96 h then decline to 0–3		Unreported	Whirlpool, serotype O:6 dominant	[8]‡

\* For most outbreaks counts were taken several days later.

† Another seven references detailed attack rate and timing but not microorganism density.

‡ All commercial and disinfected by chlorine but poorly maintained. Bather load 1–6/h.

average pool densities were 13–18 *P. aeruginosa*/100 ml while Van Asperen *et al.* [24] reported infection odds of 15.5% for natural waters with 1–17 c.f.u. (median = 2) *P. aeruginosa*/100 ml. However, others [25, 26] report no strong correlation between ear infections and *P. aeruginosa* (medians <10, maximum reported  $10^3$ /100 ml,  $n > 500$ ) despite it being the dominant pathogen [6]. Critically, spa and pool surveys typically lack head immersion data and it is unclear whether simple water contact promotes infection [27].

### Experimentally induced skin infections

Verifying Koch's postulates for *P. aeruginosa* folliculitis has proved difficult despite the diverse characteristic symptoms reported in the literature (e.g. [5]). Forearm skin infection experiments [27] have shown that skin occlusion and super-saturation (plastic film  $\pm$  saturated dressing) induced blooms of *P. aeruginosa*, at the expense of the normally dominant Gram-positive skin bacteria [28], yet no folliculitis ensued. Leyden *et al.* [29] inoculated occluded and saturated skin with 13 different strains of *P. aeruginosa*, including six pool outbreak and four serotype O:11 representatives. Clingfilm-covered dry intact skin ( $10^6$  c.f.u./cm<sup>2</sup>), remarkably yielded only 0% and 5% clinical response in subjects after 2 and 7 days, respectively, despite undiminished *P. aeruginosa* numbers. Under super-hydration, however, 68% of volunteers developed a clinical response after 7 days. Contrary to the serotype O:11 high-risk hypothesis [22] there was no clear difference in virulence between strains eliciting papules, pustules or surrounding erythema (50–90% of volunteers). In further experiments [29], forearms ( $n = 112$ ) were scarified, inoculated with  $10^3$ – $10^9$  *P. aeruginosa*, dried and covered with semi-occlusive dressings. No clinical reactions were seen with  $\leq 10^5$  c.f.u. ( $n = 30$ ), although 60% of treated areas did react to a dose of  $10^7$  c.f.u. Folliculitis-like pustules were rarely induced, although oedema and erythema, resembling AOE symptoms [6], were induced. Consistent with these observations, folliculitis is more severe on bathing suit-occluded skin [10]. Although folliculitis appears more common on the torso and thighs [30], Maniatis *et al.* [5] reported folliculitis on the arms of 5/13 patients indicating forearms are a valid experimental system. Leyden *et al.* [29] concluded: (i)  $>10^4$  *P. aeruginosa*/cm<sup>2</sup> are required for dermal infection, (ii) super-hydration is essential with healthy individuals, (iii) pool isolates are not especially more pathogenic; and (iv) the

'epidemic skin infection' pool model may be too simplistic in view of the ubiquity of *Pseudomonas* (and) cuts and abrasions. These results suggest that infection is influenced by exposure duration and super-hydration as well as dose. Subsequent work [31] has not resolved whether strains vary in virulence.

### Autochthonous or exogenous pathogens?

As well as coming from contaminated water and soil [5, 32], *P. aeruginosa* could be a minor component of the normal ear microbiota whose growth is promoted by skin environment changes [27–29, 32]. Alternatively, autochthonous *P. aeruginosa* [33] may be transferred from elsewhere on the body. *P. aeruginosa* appears to be common around fingernails, leading in extreme cases to 'green nails' [1, 4, 29]. Although Gram-negative bacteria are normally minor components of the skin microbiota, ear canal community structure is unstable. High moisture promotes their growth [27–29] and *P. aeruginosa* have been isolated from 3% to 8% of healthy bather and non-bather ears [25]. These and other observations (see Supplementary material), raise the question of how often do AOE and folliculitis, reflect blooms of endogenous bacteria?

### A conceptual challenge for folliculitis dose-response algorithms

In the 'single-hit' infection model, the endpoint dose is a single pathogen inducing systemic disease. Folliculitis, however, appears localized [4] rather than systemic, and generally resolves without major therapy. This suggests folliculitis involves multiple independent infections analogous to those produced by *Trichobilharzia* ('swimmer's itch') [34] and epiphyte pathogens prior to systemic spread [e.g. 18, 35]. If so, dose-response algorithms should incorporate a relationship between water quality and numbers of infected follicles.

That folliculitis pustule density could correlate with water quality and exposure time raises the question of what lesion densities correspond to clinical folliculitis? As we found no such data we estimated lesion density from outbreak report photographs which arguably represent what is clinically considered folliculitis (for calculations see Supplementary material). Minimum and geometric means were 200 and 1000 follicular lesions/m<sup>2</sup>, respectively. This indicated only a small proportion of follicles ( $120000$ – $300000$ /m<sup>2</sup>) [36, 37] are typically infected.

## THE ECOLOGY OF INTIMATE CONTACT

### Traversing ‘the void’

To induce infection of follicles and abrasions, exogenous dermal pathogens must: (i) cross the pool ‘void’ to the skin; (ii) intimately, but not irreversibly, interact with the skin interface; and (iii) migrate to final invasion sites; or (iv) collide with them directly from the void. Sattentau [13] explored how gut pathogens could avoid the ‘void’ (intestinal lumen) and subsequent excretion through re-infection while Spagnuolo *et al.* [38] modelled how *Vibrio cholerae* traversed the intestinal lumen. With this ‘void’ concept in mind, we reviewed how bacteria traversed the pool ‘ocean’ to bathers’ skin and ears. No comprehensive analysis was identified, but germane studies were found on: (i) microbial diffusion and motility; (ii) plankton interaction; (iii) (plant leaf) pathogen infection; (iv) biofilm rheology and life-cycle theory.

Movement of *P. aeruginosa* towards the skin appears driven by a combination of Brownian diffusion ( $D$ ) and ‘superdiffusive Levy processes’ [39], e.g. settling, turbulence and flagella swimming. We use the term ‘dispersion’ to denote these combined processes and distinguish them from Brownian diffusion alone.

Using the Stokes–Einstein equation [Table 1, equation (2)] Valentine & Allison [40, 41] developed two formulae relating diffusion to surface attachment [Table 1, equations (3a) and (3b)] for poxviruses and 0.69  $\mu\text{m}$  latex particles. Where attachment approached 100%, experiment matched Brownian theory within  $\pm 20\%$  (tables 1 and 2 in [40]). Surprisingly, agitation had little effect on adsorption kinetics, with dispersion being dominated by Brownian diffusion. Diffusion also dominates viral and bacterial interactions in large water volumes [42]. The limited effect of mixing (table 2 in [40]) appears to be explained by shear and Sherwood numbers [Table 1, equations (4) and (5)], which quantify increases in transport over Brownian diffusion. Dispersion is calculated by multiplying diffusion by the Sherwood number. For bacteria and viruses, Sherwood numbers are small (about 1 and 1.5 for sinking and swimming bacteria, respectively) [42].

Some studies [43–45] suggest that bacterial motility increases dispersion velocities by 100-fold ( $\sim 2 \times 10^{-13} \text{ m}^2/\text{s}$  under ideal Brownian diffusion *vs.*  $1\text{--}2 \times 10^{-9} \text{ m}^2/\text{s}$ , noting particle velocity increases as the square root of dispersion). These rates may reflect experimental system design and scale. Dispersion coefficients of Wei *et al.* [44] and Kim [45] were measured in

chemotaxis experiments with stable (e.g. capillary) gradients while Mueller’s [43] dispersion rates were measured in a chamber tens of micrometres in depth, comparable to bacterial free run distances, where the randomizing effects of tumbling and Brownian motion [46] and shear forces were likely suppressed. Conversely, larger scale experiments report substantially lower dispersion. Pseudomonad infection experiments [35, 47] suggest dispersion increases of only  $2\times$  to  $10\times$ . Liu *et al.* [48] also reported intermediate dispersion enhancement with porous media. Separately, Bartumeus *et al.* [39] concluded from predator–prey modelling that ‘Lévy motion’ does not lead to significantly higher encounter rates than Brownian strategies except for scarce, small, and slow target scenarios, the opposite of the bathing situation. Murray & Jackson [42] similarly concluded that although bacterial motion can be rapid over short time scales, dispersion is diffusion dominated over large scales. We provisionally concluded that bacterio-plankton scenarios [42] most likely describe the pool situation, and Brownian diffusion [Table 1, equations (2), (3a), (3b)], with modest Sherwood number corrections, provides a first-cut mechanistic description of bacterial dispersion towards bathers’ skin, recognizing that other dispersion and diffusion formulations need incorporation, e.g. [43].

### Epidermal deposition and adhesion

Biofilm models view surface interaction as a two-stage process of reversible then ‘irreversible’, biofilm-mediated, adherence [49]. Biofilm micro-colonies then evolve mediated by quorum sensing, the regulation of gene expression in response to fluctuations in cell-population density [50–52]. Given the need to reach follicles or abrasions to initiate infection, *P. aeruginosa* must either contact such sites directly or adhere sufficiently reversibly for horizontal dispersion, but not irreversibly or so weakly they are scoured back to the ‘void’.

Adherence is a well-documented virulence factor and potential control point [12, 53] involving many surface attachment-mediating factors including pili, fimbriae, flagella and capsular material [32, 53] whose action is likely mediated by motility, colloids and other ‘stickiness’ factors [48, 54].

Reversible adherence involves weak electrostatic and hydrophobic interactions [49] whose formulation is termed the extended Derjaguin, Landau, Verwey and Overbeek (DLVO) theory. It describes how

particles closely approaching a surface reach a free-energy minimum where binding is loose [54]. Factors controlling subsequent interaction are diverse and include physical/rheological (Van der Waals, opposite charge attraction, free energy of surface, surface tension, hydrophobicity, surface roughness, topography), chemical (hydrogen liaison, ionic pair and triplet formation, inter-particulate bridges), biochemical (cellular surface dehydration, membrane fusion) and biological (competition, flagella function, surface coatings, e.g. mucin) [49, 55].

Fletcher's [56] experiments indicate, consistent with Langmuir isotherm theory, that the number of bacteria attached is a function of time and cell density, and can exceed  $5 \times 10^{10}/\text{m}^2$ . Adherence rates vary with surface, bacteria and hydrodynamic conditions. With *P. aeruginosa*, adherence of  $5\text{--}50 \times 10^6$  cells/ $\text{m}^2$  per minute has been observed at liquid densities of  $\sim 10^6/\text{ml}$  [57]. Sand experiments suggest the reversible adherence free energy is low, up to  $15 \text{ mJ}/\text{m}^2$  [54]. By contrast, biofilm adhesive strength can be  $100\text{--}1000 \text{ mJ}/\text{m}^2$  where flow is  $0.6\text{--}1.6 \text{ m/s}$  [58]. Bacteria, including motile cells, can then orient themselves horizontally and accumulate at interfaces even in the absence of chemotaxis [59].

### Horizontal dispersion

Dispersion of *P. aeruginosa* horizontal to the epidermis likely involves diffusion and motility. Four types of surface motility: walking, crawling, swimming and spinning, can propel bacteria at velocities of  $10\text{--}100 \mu\text{m/s}$  [60]. Follicle entry could be facilitated by spinning motility and induced changes in the viscosity of blocking polymers [61]. The possession of a single flagellum by *P. aeruginosa* may influence its dispersion. Monotrichous bacteria cannot tumble, but Brownian diffusion may substitute [59]. Moreover, a polar flagellum could reverse direction, improving the potential to explore a structured surface and avoiding excessive circular motility [62]. Conceptually, chemotactic attraction to nutrients escaping follicles could facilitate infection. *P. aeruginosa*'s genome has 46 genes potentially involved in chemotactic responses toward amino acids, inorganic phosphate, phospholipids and fatty acids [63] and it can respond strongly to  $<100 \mu\text{M}$  of attractant [64].

Irreversible adherence would self-evidently initially immobilize bacteria and inhibit infection. However, after biofilm formation propagules could subsequently 'creep' to infection sites [65].

### Ultrastructure and infection

Skin and ear canal anatomy and physiology will likely influence interface interactions, infection ecology, and folliculitis and AOE dose responses. Three distinct structures provide different microbial habitat and infection points, the epidermis proper, sweat glands, and hair follicles, including shafts and sebaceous glands. Discounting micro-topography, the area of bather skin available for colonization totals  $1\text{--}2 \text{ m}^2$  [37]. Nano-particle delivery studies suggest follicles are favoured infection points [66]. Toll *et al.* [67] showed that  $0.75\text{--}1.5 \mu\text{m}$  particles migrate from 'stripped' skin into skin follicles during drying. The centrality of follicles is supported by 'hot hand/foot' syndrome's rarity and distinct aetiology [68].

Macroscopically, folliculitis is most common on the lower trunk and thighs even though hair follicle density is  $10\text{--}25$  times lower than on the forehead and scalp ( $\sim 12\text{--}30$  vs.  $300/\text{cm}^2$ ). This difference cannot be explained solely by degree of immersion, as torso and thigh follicle densities are comparable to arms and calves [30, 36, 37] but reportedly less commonly infected. Other factors could include epidermis thickness, hair morphology [36, 37], varying microbial communities [28], occluding surface debris [67] and bathing suit material [10].

Insights into AOE can be gained from similarly considering the structure of the ear canal. The volume and surface area of each adult outer ear canal are about  $1 \text{ ml}$  and  $6 \text{ cm}^2$ , respectively [69]. Ear canal volume indicates exogenous *P. aeruginosa* densities of concern  $>50 \text{ c.f.u./100 ml}$  depending on water retention and colonization rates, consistent with infectious doses [23, 24], and represents  $\sim 0.1\%$  of the total skin available for infection ( $12 \text{ cm}^2$ ).

### Adherence and follicle invasion efficiency

Many factors likely reduce the efficiency of skin, follicle and ear canal colonization such as dissolved protein, and adherence is typically much less than  $100\%$  (e.g.  $7\text{--}33\%$  in [41]). In addition there is desorption probability ( $3\text{--}84\%$  depending on motility and substrate for *P. aeruginosa* and *P. fluorescens* [43]), competition and interaction with normal microbiota [28, 33] and follicle blockage ( $<25\text{--}87\%$  [67]). However, *in vivo*, obstruction could be overcome if *P. aeruginosa* can 'drill down' through mucin [60, 61]. Separately, infection could be constrained by migration distance. Arms, torso and thighs/upper legs have median follicle densities of  $17\text{--}32 \text{ cm}^{-2}$  and orifice diameters of

80–130  $\mu\text{m}$  [36], indicating a median of 700–1000  $\mu\text{m}$  from contact to a follicle's boundary (for estimation see Supplementary material).

### Hydrodynamic scouring and infection

Conceptually, surface hydrodynamic forces (shear, turbulence) could dislodge bacteria prior to infection. A likely critical point is where water movement parallel to the epidermis exceeds the critical velocity where flow changes from laminar to turbulent, associated with a rapid increase in Reynolds number [65]. Hydraulics theory indicates that loose particles in the 2–200  $\mu\text{m}$  range are mobilized at flows of 0.1–0.4 m/s [70] while biofilm induction is inhibited by water velocity increases of 1.4–2.7 m/s [71].

Conversely, agitation reportedly has a moderate impact on adherence [40, 72]. Moreover, the skin has a complex topography of primary and secondary furrows 5–100  $\mu\text{m}$  in depth [73] which could protect bacteria from scouring [74]. Where follicle/abrasion invasion does not immediately occur, infection could be facilitated long term by furrow accumulation [66, 73, 75, p. 339]. Removal by superficial (e.g. towel) drying after pool exit is probably limited unless abrasion is vigorous [75, p. 351] while air drying may draw surface bacteria deep into the epidermis [67]. Overall, shear driven remobilization of *P. aeruginosa* seems plausible but its extent remains to be assessed.

Epidermal hydrodynamics considerations also raise the question of whether bulk water velocity influences adhesion. Filter turnover times of 2–6 h [76, 77] indicate that even pool inlet to outlet distances of 2 m, should generate bulk flow >100  $\mu\text{m}/\text{s}$  exceeding *P. aeruginosa* velocities [64]. Body movement arising from buoyancy changes (>1000  $\mu\text{m}/\text{s}$ ) and swimming (0.5–2 m/s), also exceed flagella enhanced dispersion. Flagella-enhanced dispersion may be more important where water is occluded (ear canal, under bathing garments). For ears interesting questions include how well bacteria adhere and are flushed during bathing? Dispersion theory should provide estimates. Following adherence bacteria would be difficult to dislodge due to surface topography [74] and the rapid establishment [72] and resilience of biofilms [65, 71].

### Quorum sensing

Attachment of *P. aeruginosa* could also depend on between strain differences and virulence factor regulation via quorum sensing. The genetic diversity of *P. aeruginosa* and its implications are now better

understood and >16 major serotypes are distinguishable [22]. Sequenced strains have genomes of  $\sim 6$  Mbp, 10% of which may regulate virulence [78]. This large genome could also aid *P. aeruginosa*-colonizing diverse habitats, organs and organisms including nematodes, insects and plants [79]. One key regulatory system involves the production and secretion of small, diffusible 'signals', called acylated homoserine lactones (AHLs). When signals accumulate, in high concentrations, *P. aeruginosa* respond by inducing virulence genes, such as elastase. In swimming pools, quorum-sensing-regulated genes would likely be inactive due to signal dilution in water but could still accumulate in biofilms. In the confined space of an epidermal follicle or ear canal, biofilms and signals might also accumulate, activating the quorum-sensing system and further facilitating signal accumulation and infection [52].

### Time dependency of infection induction

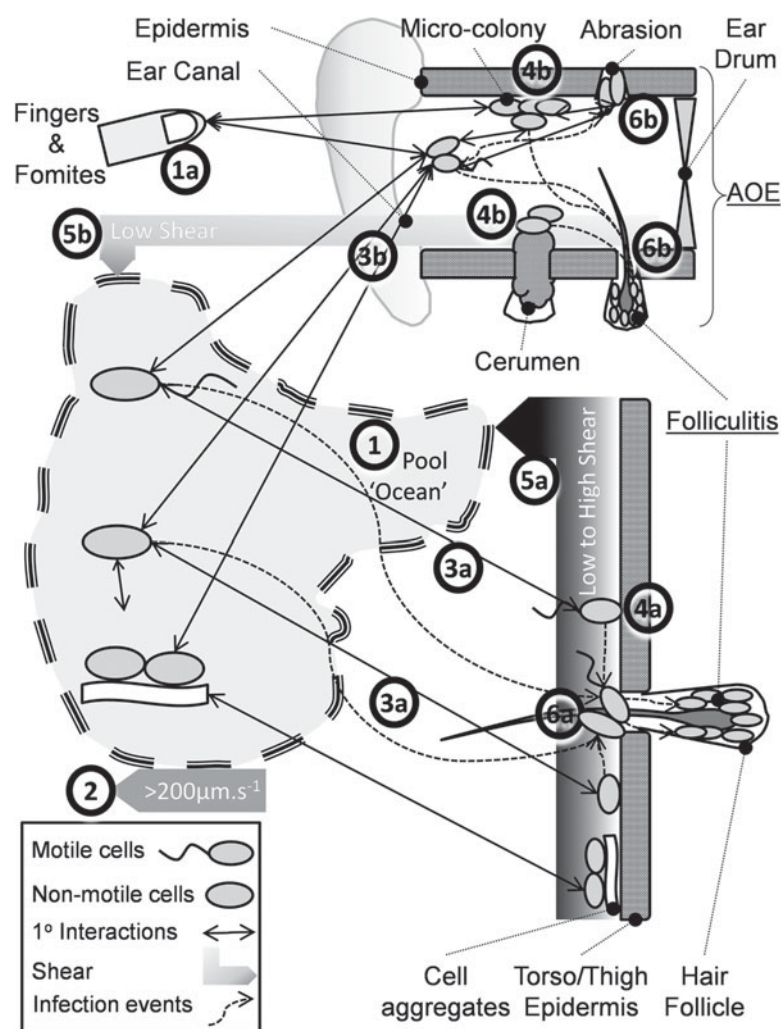
Although exposure time is not included in traditional 'single-hit' dose-response formulation, there is increasing interest in this variable [21, 80] and several reasons why exposure time likely affects folliculitis and AOE dose response. Pool 'voids' of tens to hundreds of centimetres would require many minutes to traverse for *P. aeruginosa* swimming at 2–4 mm/min [42, 64]. Valentine & Allison (fig. 1 in [40]) showed surface attachment increases markedly over a timescale of minutes as does plant leaf infection [35, 47]. Time will be required for bacteria to adhere [56], migrate to infection points and express virulence factors, especially if a biofilm is required. Skin super-hydration leads to marked changes in ultrastructure again over a period of minutes to hours [81], potentially influencing pustule or rash development. Finally, folliculitis frequency and severity reportedly increases with exposure time [82].

## TOWARDS DOSE-RESPONSE MODELS FOR *P. AERUGINOSA* POOL INFECTIONS

### Applicability of 'single-hit' theory

With aetiology and ecology in mind we first assessed the applicability of conventional QMRA algorithms to bathing risk models. Furumoto & Mickey [17, 18] developed their 'single-hit' hypothesis using plant leaves dipped into suspensions of TMV, an experimental system analogous to the pool+*P. aeruginosa*





**Fig. 1.** Conceptual model of the pathways preceding folliculitis and acute otitis externa (AOE) by exogenous *P. aeruginosa*.

+bather skin scenarios. In addition to the single-hit formulation, they derived two other relevant algorithms. First, they described a variant of the exponential model [Table 1, equation (6)], relating microorganism density, rather than dose, to infection probability (cf. equations (1) and (9) in [17]). In their model, ‘dose’  $a$ , was defined by the pathogen density  $V$  and a correction factor  $c$  [ $\gamma$  in our equation (8)]. Coefficient  $r$  and ‘dose’  $d$  in the exponential ‘single-hit’ model are replaced by pathogen density  $C$  and a probability coefficient analogous to  $r$  we suggest be designated  $r_C$  [Table 1, equation (7)].

The second algorithm [equation (15) in [17], reproduced as equation (9) in Table 1], relates lesions per unit area to pathogen density using an infectivity-dilution function. Two coefficients ( $A$  and  $B$ ) are quantified by least squares fitting. The form of this equation indicates lesion numbers should be

proportional to pathogen density and equation (9) (Table 1) might be used to relate folliculitis lesion density to *P. aeruginosa* density where lesion and count data are obtained simultaneously. These algorithms could also apply to *Trichobilharzia* (‘swimmer’s itch’) [34].

### Single-hit theory caveats

Consideration of folliculitis and AOE aetiology and ecology suggested qualifications should be attached to empirical applications of ‘single-hit’ theory. Equations (6) and (7) in Table 1 estimate systemic infection probability and seem applicable to AOE as this disease involves *P. aeruginosa* multiplication potentially from a single locus. Folliculitis papules, however, generally appear localized, although systemic disease is possible [4]. Another limitation of Furumoto

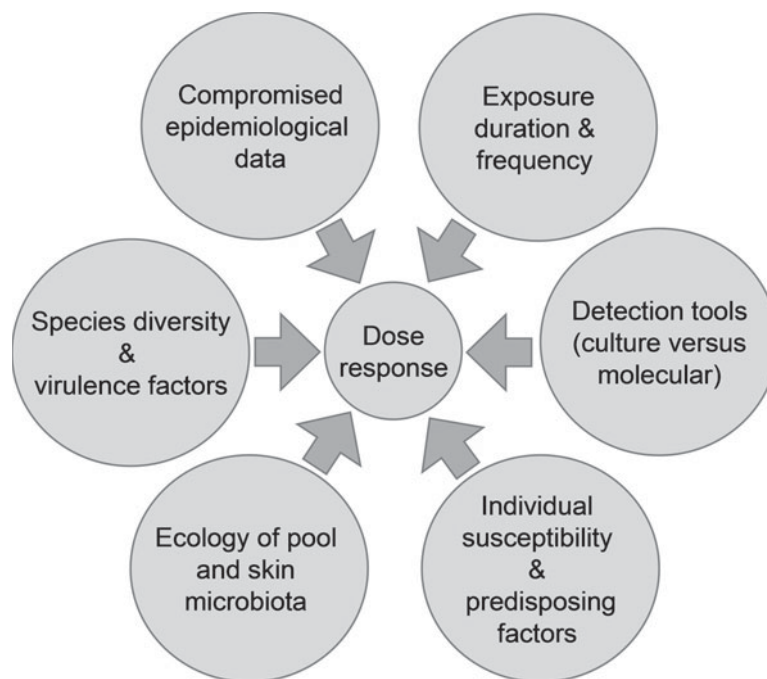


Fig. 2. Constraints associated with developing a dermal dose-response model for *P. aeruginosa*.

& Mickey's [17] algorithms is that none incorporate exposure time, probably because plant defence mechanisms were reactivated (<2 min) following inhibition by leaf abrasion, and lesion density rapidly plateaued. This contrasts with pool infections where exposure time seems important and raises a tangential question of how exposure time influences gut infections? Spagnuolo *et al.* [38] considered this issue across multi-second scales, although not the hour scales over which exposure occurs during digestion. An interim solution may be to use Furumoto & Mickey's implied assumption that exposure time variance is relatively small compared to other factors and might be discounted. Discounting is supported by the plateauing of lesion density observed with plant pathogenic pseudomonads (fig. 5 in [35], fig. 2 in [47]). A different uncertainty is how far *P. aeruginosa* should be viewed as an exogenous pathogen, perhaps more adapted for another eukaryotic host (cf. [83]) or an endogenous skin inhabitant out of control [27, 33].

### 'Third-generation' mechanistic modelling of folliculitis

The information reviewed suggested that folliculitis lesion density might be estimable by combining: (i) 'void' crossing; (ii) surface adsorption/interaction; and (iii) follicle invasion likelihoods. Sufficient theory appears available to start framing follicle invasion and surface adherence in terms of attachment rates while

dispersion equations appear to describe the 'void' crossing. In this conceptual model, folliculitis lesion density should be estimable as the product of dispersion, the probability of *P. aeruginosa* interacting with the skin boundary layer, and the probability of a follicle being invaded.

We propose equation (10) (Table 1) as the first step in mechanistic model development, recognizing that other migration and attachment formulations exist [43, 56, 57] in addition to that of Valentine & Allison [40]. Equation (10) describes the number of lesions per unit area of skin  $N_I$  as the product of (i) the number of particles absorbed under ideal Brownian diffusion assuming 100% adherence  $N_D$  (using equation (9) in [40]); (ii) the Sherwood number  $Sh^{0.5}$ ; (iii) the per cent surface adherence + migration efficiency  $S_{im\%}$ ; and (iv) the infection efficiency  $I_{\%}$ .  $N_D$  is derived from the Stokes–Einstein–Brownian diffusion theory and assumes <40% removal of suspended particles [40].  $Sh^{0.5}$ , the increase in transport over diffusion [42] is square-root-transformed because the diffusion coefficient is similarly transformed in calculating  $N_D$  [40].  $S_{im\%}$  and  $I_{\%}$  could be estimated experimentally using existing procedures, e.g. [35, 47].

Third-generation models have significance beyond folliculitis. 'Second-generation' 'single-hit' dose-response algorithms have proved a major advance over 'first-generation' MIDs [9]. However, they are still grounded in empirical curve fitting (e.g. [16]),

and do not provide full insight into infection processes. Further, model validation and coefficient estimation depends on rare, seldom comprehensive, outbreak data and some human and animal feeding/dosing studies [19]. By comparison ‘third-generation’ mechanistic models could address these constraints and provide predictions and checks for comparison with second-generation algorithms, disease aetiology and epidemiology. The only other prototype mechanistic models we are aware of are the schemes of Rose & Haas [21] and Spagnuolo *et al.* [38].

### Exogenously induced AOE

AOE is reportedly associated with ear infection when *P. aeruginosa* occurs at densities of 10–100 c.f.u./100 ml [23, 24] whereas folliculitis appears associated with  $>10^5$  c.f.u./100 ml [8]. Despite this, folliculitis outbreaks can occur with little or no concurrent AOE, e.g. [30]. We suggest this indicates that the role of exogenous *vs.* endogenous swimming pool *P. aeruginosa* in AOE needs attention.

Resolving this puzzle will likely require a mechanistic analysis of dose response leading to understanding of how infection, skin saturation and ear canal microbial ecology interact [27–29, 84]. Improved understanding is also needed of interactions between hydration, bacterial strains, mechanical disturbance (cotton tips, fingernails, hair clips) and cerumen (ear wax), the mixture of ceruminous and pilosebaceous glands secretions, epithelium squames, dust and other foreign debris [85]. Cerumen had been thought to protect against infection, but disinfection capacity is now disputed [86] and wet, impacted cerumen is associated with ear disease [85]. Further, cerumen function could be modified by ear cleaning, excessive water exposure, acid neutralization by swimming pool alkalinity. AOE likelihood could be increased by ear canal epithelium disruptions, e.g. maceration, high humidity, psoriasis, eczema, hearing aids, exostoses, and genetic predisposition associated with blood type A [87].

## A FRAMEWORK FOR EXOGENOUS FOLLICULITIS AND AOE

### Pool management and dose-response algorithms

Our review raises many questions pertaining to pool management such as: whether the epidemiology, infection ecology and biophysics pictures are consistent;

are current water quality guides satisfactory, over-conservative or too lax; and can dose-response models account for the folliculitis *vs.* AOE contrast? Exposure duration appears central to infection severity and likelihood. Diffusion/dispersion theory promises estimates of the minimum water quality posing folliculitis and exogenous AOE risk. Conversely the aetiologies of folliculitis and AOE are not well grounded, and *P. aeruginosa* management is currently based on empirical hygiene principles rather than quantitative satisfaction of Koch’s postulates.

Presently these pool management questions and propositions should be viewed as provisional. To resolve them and confirm or disconfirm major pool infection causes, we consider the next step should be infection modelling and experiments. Such work could also provide a reality check on this review’s conclusions and the proposed models. As an aid we now propose a conceptual framework for exploring folliculitis and AOE.

### Conceptual model

Steps (i)–(iii) of the KEDRF scheme (fig. 2 in [3]) propose targeted research of intimate processes leading to infection initiation. We reviewed the analogous ‘key events’ during *P. aeruginosa* bathing infection. Motile, non-motile and aggregated bacteria disperse through pool ‘voids’ (Fig. 1, process 1) driven by bulk water movement, swimming and agitation (Fig. 1, process 2). A few approach the epidermis (Fig. 1, processes 3a, 3b) where dispersion leads to reversible attachment (Fig. 1, processes 4a, 4b). Where shear forces are high ( $Re > \sim 1000 \approx 1$  m/s) bacteria detach and return to the ‘void’ or bind irreversibly and potentially multiply (Fig. 1, processes 5a, 5b). Some cells disperse horizontally and infect follicles and abrasions (Fig. 1, processes 6a, 6b), or infect from the ‘void’. Infection is promoted by prolonged epidermal water retention, low shear (ear canals, occluded skin) and drying and possibly microbiome changes. Abrasion (scratching, clearing ear canals) (Fig. 1, process 1a) directly inoculates pathogens (Fig. 1, process 4b).

### Next steps

Guidelines on managing *P. aeruginosa* in pools (p. 45 in [88]) suggest that cause-and-effect relationships are well understood. Our analysis suggests otherwise. To resolve matters we recommend the following research

based on the framework in [Figure 1](#) and themes in [Figure 2](#):

- (1) Develop and test mechanistic and empirical dose-response model options for opportunistic pathogens using constructed pool environments as study models.
- (2) Basic research into: (i) bacterial biodiversity and distribution in the recreational water environment; (ii) interactions in skin and ear model systems covering motility, skin adherence and saturation, hydrodynamics, blooms, chemotaxis, biofilms and quorum sensing; (iii) (limited) human experiments [29]; (iv) AOE aetiology and the risk posed by contaminated water.
- (3) Pool and bathing management research covering: (i) pool and filter ecology; (ii) impacts of bather load and occurrence of individuals shedding *P. aeruginosa* into the pool environment.

#### SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0950268813002690>.

#### DECLARATION OF INTEREST

None.

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