Looking deeper

The Journal of the Water Safety Forum

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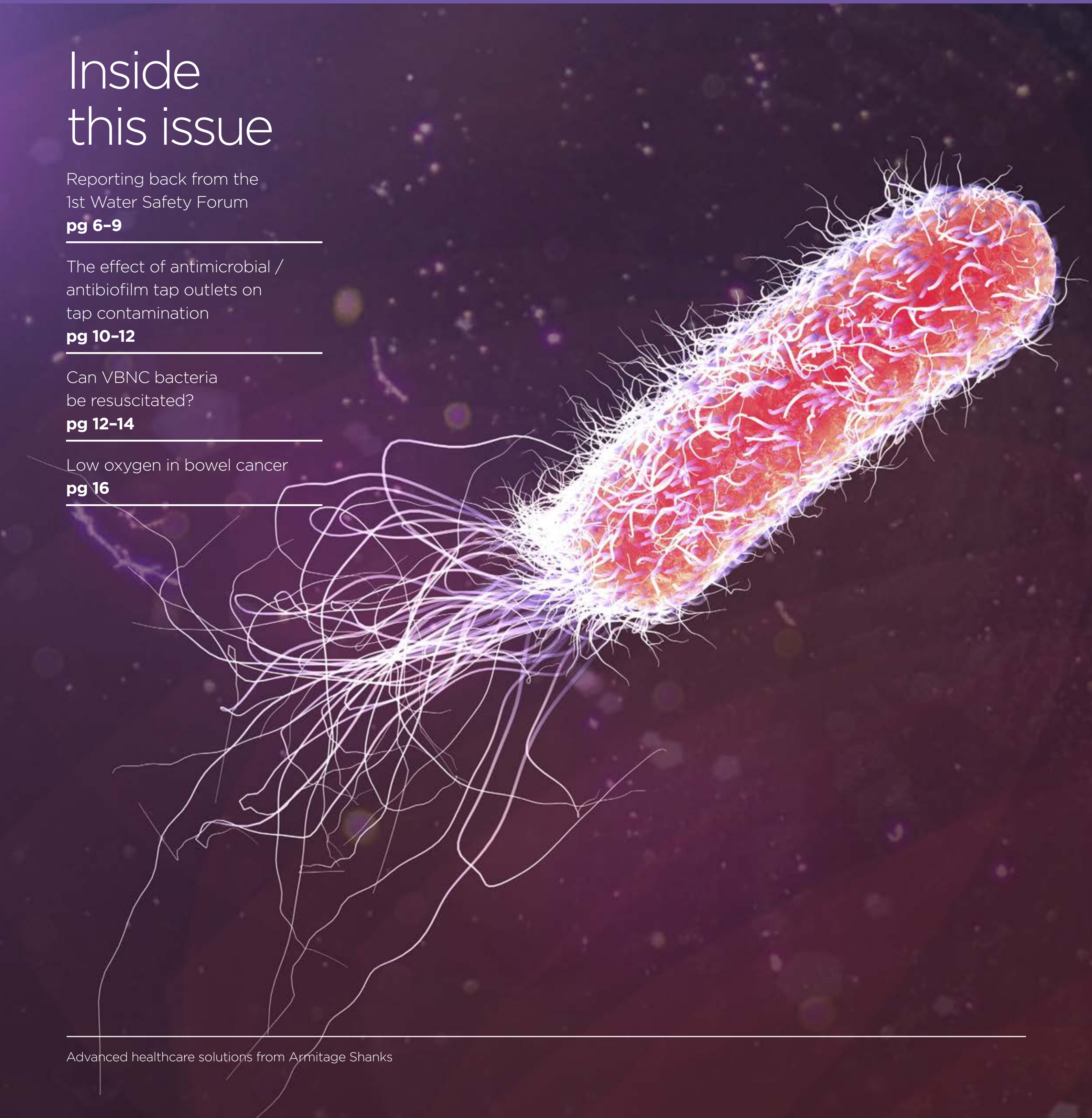

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A flying start for the Water Safety Forum Round Table

Looking Deeper Editor, **Susan Pearson**

In Issue 1 of Looking Deeper we put forward our aim of creating an interactive conversation on current thinking on water safety and infection control, particularly in relation to healthcare fixtures and fittings — and we got off to a flying start with our first Water Safety Forum Round Table discussion in Tamworth in May — see our report on pages 6–9.

Everyone involved in the very lively session felt that this had been a constructive exercise and the feedback was very positive:

"It was great to sit in a room and discuss the different drivers for design and to talk about in-use experience."

"It was good to hear the different views and priorities of other professionals to focus on what the requirements of future water delivery for hand washing might be."

An interesting theme that emerged was the degree of impact of human behaviour.

This becomes clear in paediatric settings where visiting siblings and other family members are accessing ward wash hand basins (WHBs). It is important to encourage visiting youngsters to engage in hand hygiene, but they are less likely to follow instructions, making prevention of contamination of these outlets from hand contact more problematic than in adult settings.

Another discussion point was the need for more staff training on the risks from waterborne diseases and how cross-contamination can occur in water outlets — although the panel recognised how hard it is to find time for this.

For example, splashing is increasingly seen as a cause of transmission events. While design of WHBs may play a part in this issue — and this has been acknowledged in at least one new WHB design — the panel identified that clinical personnel may not always fully appreciate the microbiological dynamics involved.

Another Water Safety Forum Round Table will be held later in the year or early in 2019 based on the outcomes from this initial event.

If you are interested in joining the Round Table panel or contributing on an ad hoc basis, please contact Elise Maynard at **elise@elisemaynardassociates.com** and include a CV and a summary of why you would like to be considered for the panel.

In the meantime we hope you find the content in Looking Deeper useful and constructive, and we welcome your feedback on what's been discussed so far or other issues you feel we should address.

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Editorial Contributions

Susan Pearson

Susan is an independent journalist and communications specialist with a background in biology, medical research and publishing.

She has been writing on medical issues for over 25 years and on water-borne infection and water management since 2010. She has been a frequent contributor to IHEEM's Health Estate Journal and WMSoc's Waterline.

Elise Maynard

Elise is an independent consultant to the water and medical devices industries and a former Chair of the Water Management Society (WMSoc).

She is a state-registered microbiologist and a Fellow of WMSoc, IHEEM, RSPH and IBMS. She chairs and presents at numerous international conferences.

Professor Hans-Curt Flemming

Hans-Curt is Emeritis Professor of Aquatic Microbiology at the University of Duisberg-Essen where he was also managing director of the Biofilm Centre. He has researched water microbiology for 40 years, particularly the physical chemistry of biofilm and drinking water hygiene. Hans-Curt has been a president of the International Biodeterioration and Biodegradation Society, is secretary of the IWA Biofilm Specialist Group and a guest professor at the University of Natural Resources and Life Sciences in Vienna.

Share your thoughts with us in the next issue

To keep the conversation on water safety flowing we would really value your reactions to the second issue of Looking Deeper — we would appreciate hearing from you about what you liked, what you feel could be improved on, what topics you want to see discussed. We intend to publish some letters (with your permission) and would also welcome suggestions for contributions from our readers. You can contact us at **editorial@lookingdeeper.co.uk**

In the news...

Natural saboteurs target *Pseudomonas aeruginosa*

Credit: Maarten Ghequire, KU Leuven Centre of Microbial and Plant Genetics, Belgium

LlpA protein antibiotic — colours indicate main domains

Researchers at the Katholieke Universiteit Leuven, Belgium, have now learned how proteins naturally produced by bacteria to kill specific competitors, including *Pseudomonas aeruginosa*, attack and speedily kill other bacteria. They hope such proteins hold potential for new antibiotics.

"The LlpA [bacteriocins] protein has a specific target in the outer wall of the bacterial cells," said postdoctoral researcher Maarten Ghequire, from the KU Leuven Centre of Microbial and Plant Genetics. "That target is a protein as well: the BamA protein, which is involved in maintaining the bacterial cell wall. Without the BamA protein, bacteria cannot survive. LlpA binds to that BamA protein and, by doing so, shuts it down."

"These protein antibiotics are effective as well as very specific in how they operate. They're similar to snipers, whereas traditional antibiotics are more like cluster bombs. Traditional antibiotics are effective against many bacteria but they also kill a lot of harmless organisms.

"Unlike standard antibiotics, LlpA proteins don't even need to get inside the bacteria; they recognise their target and then sabotage it from the outside."

Your office brew may contain more germs than a toilet seat

Drinking tea made by your colleagues at work may turn out to be a health hazard rather than a welcome thirst-quencher.

According to research from Initial Washroom Hygiene, office teabags can carry 17 times more bacteria than found on a toilet seat, with readings of 3,785 against 220 for a toilet seat.

In fact swabs of communal workplace kitchens revealed that 75% of all work surfaces are home to more bacteria than an average feminine sanitary bin.

Half of the kitchens surveyed included extremely high levels of faecal coliforms, with over a quarter of the draining boards tested registering more than four times the level of coliforms considered to be safe. Other kitchen contamination hot spots included handles of kettles, shared fridge-freezers and microwaves.

Initial noted that the lack of hand washing equipment in office kitchens is making surfaces and appliances less hygienic, and could also be making workers more prone to illness. Regular hand-washing with soap and water has been proven to reduce the risk of the spread of pathogens, the company stressed.

Pall Medical/Armitage Shanks Masterclass on water hygiene

Pall Medical and Armitage Shanks co-hosted an educational Masterclass on water hygiene at the Warwick University Conferences Centre on 13 June, 2018.

The speakers included Dr Paz Aranega Bou and Dr Ginny Moore from Public Health England, Dr Mark Garvey from Queen Elizabeth Hospital Birmingham, Dr Christoph Koch from University Hospital Bonn, Dr Michael Weinbren from Kings Mill NHS Foundation Trust and Elise Maynard representing the Water Management Society.

Dr Mark Garvey described the formation of biofilms and a vast array of evidence-based papers regarding the links between various micro-organisms and water sources. This was followed by Elise Maynard who reviewed the regulatory guidance for the UK, which has been made less prescriptive in recent years, the onus being on the creator of the risk, as the owner of that risk. Water Management Groups are being set up to attempt to communicate this better but there are still plenty of examples of contamination noted from site visits at healthcare premises.

Dr Koch described a highly regulated environment in Germany with ordinance, numerous technical and clinical guidelines as well as civil law building regulations. Key differences to the UK include that disinfection is not recommended in German law.

The PHE presentations noted that Carbapenem-resistant *Enterobacteriaceae* (CRE) are endemic in some countries. These tend to be found in biofilms within drains, from where they are able to spread their resistance genes. The design of plumbing components can have an effect on their spread, for example, by minimising splashing or repositioning the drain.

Dr Weinbren closed the day with a very professional and highly visual presentation showing how human factors can have a dramatic effect on the spread of waterborne disease, which need to be taken into account by designers. Dr Weinbren also showed some excellent videos showing water movement, by using fluorescent dye.

A wider discussion on the topics presented in the Masterclass will be featured in the next issue of Looking Deeper.

Dates for diaries...

IHEEM 2018 Regional Conference & Exhibition
30–31/08/2018 Cardiff
www.iheem.org.uk/events

11th Infection Prevention Society Conference
30/09–02/10/2018 Glasgow
www.ips.uk.net/conference/

IHEEM Annual Conference
9–10/10/2018 Manchester
www.iheem.org.uk/Healthcare-Estates

WMSoc Designing Out Too
14/11/2018 London
www.wmsoc.org.uk/events

WWEM 2018
21–22/11/2018 Telford
www.wwem.uk.com

11th Healthcare Infection Society Conference
26–28/11/2018 Liverpool
www.his.org.uk/education-events/his-2018

Water Safety Forum — report from the first ‘round table’ panel

The first ‘round table’ discussion from the Water Safety Forum (WSF) was held at the Water Management Society offices in Tamworth on 10 May with a panel of unbiased experts covering clinical infection control, microbiological risk assessment, clinical microbiology, facilities management and plumbing engineering (see overleaf).

One goal of the WSF is to improve insight into the current key issues and concerns of users around sanitaryware and fittings in the healthcare environment — to consider what part manufacturers can play in product interfaces, ultimately leading to production of better solutions for water hygiene in hospitals and other healthcare settings.

The May event focused on taps and showers, with particular consideration for the highest-risk (augmented care) areas, for example, intensive care units (ICUs), burns units and neonatal/paediatric facilities — and sparked a wide-ranging and intense discussion by panel members.

Views exchanged included: requirements of paediatric facilities versus those for adult augmented care, risk assessment, different outlet types, splashing as a cause of transmission of waterborne pathogens, staff training on outlet use, product testing, the use of thermostatic mixing taps (TMTs) versus manual taps and commissioning.

A key theme that emerged was the impact of human behaviour — specifically in terms of how outlet use can contribute to contamination issues and how this might be addressed through targeted water management strategies and better understanding of waterborne infections by healthcare personnel.

This report is intended as a snapshot of the discussion — but since the conversation was so broad, we have split it into two parts, with Part 2 to be featured in the next issue of Looking Deeper.

Neonatal and paediatrics — special requirements

Under a risk-based approach to water management, considerations for infection control in neonatal and paediatric facilities may be different from those in adult augmented care units. Beds in paediatric ICUs and some units might be considered as ‘augmented care’, because hospitalised children tend to have complex health needs that require high intensity and high dependency care. Young patients often need lengthy hospital stays, sometimes up to two years, and the scenario in terms of cumulative risk can be very different from adult facilities. In particular, wash hand basin (WHB) contamination on paediatric wards tends to be high: entire families, including very young siblings, visit young patients. It is important to actively engage visiting youngsters in hand hygiene but hard to prevent this happening in an ‘untrained’ way — making WHBs more prone to contamination. In addition, many patients are in nappies and hands are washed in WHBs after nappy changes.

The panel discussed how water management strategies might be targeted specifically for neonatal and paediatric scenarios. While keeping hot water temperatures above 55°C and cold water below 20°C in order to combat *Legionella* risks is the primary control method for water management in most healthcare environments, in paediatric settings there is a risk of scalding where children are accessing WHBs.

Conversely, the risk of *Legionella* infections are not as widely studied in children, though there have been increasing cases in the literature in recent years from both nosocomial acquisition and via home water births.

Pseudomonas and *Legionella*

The panel noted that patients (in neonatal, paediatric and adult care) are potentially more likely to transmit pathogens to WHBs, sinks and drains than the other way round and that *Pseudomonas aeruginosa* infections in patients can also occur due to direct transfer from other patients. *Pseudomonas* infections are more likely to be prevented by good WHB and tap outlet design, with good hygiene potentially more important than temperature and other systemic control methods.

However, systemic high water temperatures should be maintained for *Legionella* control of all public buildings. It was noted that *Legionella* is more endemic than many people realise because it is being under-diagnosed; some investigations of locations where positive cases of *Legionella* have occurred have revealed contamination at >80% of outlets within hospitals, due to the complex relationship of *Legionella* with biofilms and the difficulty of accurately detecting it with conventional sampling and testing methods.

Scalding risk assessments

The panel emphasised the importance of scalding risk assessments (RAs) instead of a ‘blanket approach’ to installation of thermostatic mixing valves (TMVs), that blend hot and cold water to provide constant warm water temperatures. They noted that a ‘scalding’ is not about momentary exposure to hot water, but is an injury — and many factors can influence whether an individual who was exposed to hot water might sustain a scalding injury. However, it was also noted that the key functionality of TMVs is to deliver a constant, comfortable hot water stream to encourage thorough hand washing.

However, many staff do not feel qualified to carry out scalding RAs, the panel said, and consequently tend to be ‘risk averse’ because the HTM 04-01 mentions that scalding is a “Never Event”. The caveat is that this is in relation to full body immersion, such as baths and showers and should not be applied to all water outlets, for example, where there is no patient usage/access.

Some older designs of TMVs can add too much complexity to water systems, some panel members said, and suggested that retrospective scalding RAs could lead to simplified control systems — sometimes a manual mixer tap is a better solution, although all taps need to be able to be dismantled and easily cleaned.

RAs for *P.aeruginosa* are multifactorial and need to consider not only patients’ susceptibility to infection with *P.aeruginosa*, but also need to take into account sink and tap designs, cleaning regimes, cleaners’ competence, nursing practices and all uses of water that comes into contact with patients and their environment. The panel felt that an overall Water Hygiene RA would be preferable to separate RAs for *P. aeruginosa*, *Legionella* and so on., but recognised the governance requirements for *Legionella*.

Wash hand basins — reducing splashing risks

It was stressed that there are likely to be many more transmission events taking place than are currently identified. There was a lively discussion on the risks related to placement of WHBs and failures in the way they are used. The panel agreed that there is often an under-appreciation of the dangers associated with WHBs because ward staff tend to consider these outlets as 'safe' and are unaware of the dangers from splashing — that water droplets can travel up to two metres from the source and may be carrying potential microbial pathogens.

The panel identified the primary cause of splashing as a combination of the act of hand washing, pressure in the system and flow rate. They acknowledged the significance of design in this process — even someone adept at hand washing, with water delivered under high pressure, will generate more splashing than under a low pressure stream. The panel recognised that work needs to be done to establish how water systems can be designed to minimise splashing and how to set an appropriate flow rate for the appropriate point of use device for each water outlet.

The following considerations were made, to be based on individual risk assessment:

- Decide whether hand wash stations are really needed in every room, for example where clean equipment is stored
- WHBs should not be placed next to surfaces used for other activities — remove surfaces next to sinks, or
- Follow the example of Germany where a Perspex® sheet is often placed on either side of WHBs to contain splashing
- Equipment should never be stored next to WHBs or sinks
- Disposal of patient fluids should always be carried out in a dirty utility area/sluice
- Tap levers are often turned on and off by hand on outlets that should be elbow-operated, thereby introducing contamination — sensor-operated outlets could be safer than elbow-operated outlets, provided that the water flow is not in prolonged contact with components which could promote growth.

Staff training

There is a lack of communication and education regarding the risk from waterborne pathogens and some clinical staff may not fully understand the dynamics of cross-infection — for example, why patient fluids should not be disposed of in WHBs and therefore why different dedicated sinks and sluices are needed, or how contamination can be spread to the outside of a point-of-use (POU) filter.

In turn, without this knowledge, clinical staff cannot pass on information to their colleagues. Training and education is needed but this requires organisation — yet is almost impossible to fit more training into already over-stretched work schedules. Some of the microbiological information is complex and unfamiliar concepts, such as aseptic technique, may be hard to retain.

Even with increased staff awareness, as discussed above in the context of paediatric facilities, more than half of individuals accessing ward WHBs will be patients and visitors who cannot be trained!

It is also important that cleaners are instructed properly but there is often a high turnover of cleaning staff — so constant on-going training and awareness is needed.

The panel noted that:

- More automation could be part of the solution because sensor operated taps are easy for anyone to use and would reduce the risk from cross infection. Another advantage is that automated taps could be pre-set to flush during daytime hours to keep water flowing and prevent stagnation. However, there is still a learning curve for users to recognise the sensor, so tap and shower outlets could still be touched during hand washing
- Outlets need to be designed to be easy to clean and disinfect
- Installation of tap spouts above WHBs that can be changed and that are easy to clean could reduce cross contamination
- The most visually obvious WHBs are those that are most used and because they are highly visible to staff and patients, individuals using those sinks tend to spend longer washing their hands — resulting in a lower transmission incidence in the other WHBs on a unit[1]
- WHBs could be labelled in some way to aid correct hand washing. For example, sinks could be colour-coded to educate people to distinguish different sink functions — e.g. between those for disposal and those for hand washing.

References

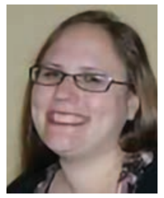
1. Cloutman-Green, E. *et al*, "The important role of sink location in handwashing compliance and microbial sink contamination", *Am J Infect Control* **42**: 554–5, 2014.

A second forum is in the planning stages. We have heard from several people wishing to be involved — they will be contacted as soon as the next date is finalised.

Water Safety Forum panel

Elise Maynard — Chair

Independent consultant to the water and medical devices industries — a state-registered microbiologist and former Chair of the Water Management Society.



Elaine Cloutman-Green

Principle Clinical Scientist in Infection Prevention and Control and NIHR Clinical Lecturer, Infection Prevention and Control at the NHS Great Ormond Street Hospital for Children.

George McCracken

Director of Estates, Risk and Environment at the NHS Belfast Health and Social Care Trust.

Dr Mike Weinbren

Consultant Clinical Microbiologist and Director of Infection Control, Kings Mill Hospital, Sherwood Forest Hospitals NHS Foundation Trust, and Chair of the Hospital Infection Society working party on water.

Mike Quest

Independent water hygiene and safety authorised engineer, NHS Authorising Engineer and trainer, including NHS training at Eastwood Park, Gloucestershire.

Mark Griffiths

Mechanical engineer at the Chesterfield Royal Hospital NHS Foundation Trust, responsible for water maintenance, ventilation and chemical gases.

Dr Paul McDermott

Independent advisor in biological risk and former Health and Safety Executive specialist inspector on *Legionella* risk control. Involved in development of HSE strategies on *Legionella*.

Do 'antimicrobial' outlets provide lower *Pseudomonas* persistence in hospital tap water?

Does Pseudomonas aeruginosa transferred to tap outlets from contaminated cleaning cloths lead to contamination of tap water? And how do antimicrobial/antibiofilm tap outlets affect this process to keep patients safer?

A paper published in the Journal of Hospital Infection last year concluded that contaminated cleaning cloths do indeed have the potential to transfer *P. aeruginosa* and that antimicrobial outlet fittings (OFs) may offer some protection from delivery of continually contaminated water.[1]

Water outlets are recognised as a potential reservoir for the growth of biofilm that may harbour potential pathogens such as *Pseudomonas aeruginosa* and infections with *P. aeruginosa* have been linked with contaminated hospital taps. The most notorious incident is the case of 25 babies admitted to neonatal intensive care units in Northern Ireland (NI) between November 2011 and January 2012, of whom three died. This case was the prime stimulus for the development of the HTM 04-01 guidelines on control of *Pseudomonas aeruginosa* in augmented care units, which was updated in 2016.[2]

OFs may become contaminated from the water supply or through 'retrograde contamination'. This might occur as a result of human behaviour, including via splashback from a contaminated waste trap or surface, inappropriate dispersal of patient fluids or during cleaning.

Scientists from Public Health England's Biosafety, Air and Water Microbiology Group at Porton Down* and the Centre of Biological Sciences at the University of Southampton investigated retrograde contamination, specifically via cleaning cloths, of conventional OFs and those of a simplified design and/or incorporating 'antimicrobial' materials such as copper and silver or 'antibiofouling' plastics and looked at whether this contamination persists and/or leads to contamination of tap water.

The anti-microbial effect of copper and silver ions is well known, with silver utilised as long ago as Ancient Greece and Rome, where silver vessels were used to keep drinking water clean, and is still used today to line water tanks on spacecraft.

This is the first study to investigate OF contamination due to contamination of *P. aeruginosa* with a cleaning cloth — previous studies have all focused on post-incident investigation or contamination of a system.

Methods

Four types of OF were used in the study: one conventional OF A multi-layered plastic design and three designed to reduce risk of microbial colonisation. OF B was a simple design with a single-bore copper interior lining, OF C was similar but constructed from a plastic said to reduce impurity and bacterial adherence, while OF D had a conventional flow-straightening device made from silver-impregnated plastic, with a copper alloy sheath.

The researchers inoculated swatches of microfibre cleaning cloths with 10 mL of a bacterial test suspension. These were weighed and wrung, re-weighed after inoculation and then were used to wipe the accessible surfaces of the OFs, which were then inserted into the taps of an experimental water distribution system (EWDS) and left in place for 15 mins, one, four, eight, 12 or 24 hours.

After each time period, OFs were removed from the taps, cleaned with a sterile solution and vortexed with sterile glass beads. The resulting suspension was serially diluted, plated out and cultured to assess survival of *P.aeruginosa* on the OFs. Each experiment was repeated four times.

To detect *P. aeruginosa* in water dispensed from contaminated OFs, OFs contaminated as above were inserted into the EWDS and each tap was flushed either once or several times over a 20 minute period, to simulate an infrequently or frequently used tap respectively, and water samples were collected. Each tap assembly contained a thermostatic mixing valve to maintain water temperature at 43°C. Chlorine levels were between 0.03 and 0.22 mg/L.

Numbers of water samples delivered via five consecutive 30s flushes through contaminated outlet fittings (OFs) that contained *P. aeruginosa* at levels above (>10 cfu/100 mL) or below (1–9 cfu/100 mL) (augmented care hospital alert limit)

OF type	P. aeruginosa (cfu per 100 mL)	Frequency of tap use — flushes				
		1	2	3	4	5
OF-A	>10	12	11	8	8	6
	1-9	0	1	2	1	2
OF-B	>10	12	0	0	0	0
	1-9	0	0	0	0	0
OF-C	>10	12	2	1	0	0
	1-9	0	0	0	1	0
OF-D	>10	12	11	2	0	0
	1-9	0	1	4	4	2

Results

• Contamination

Pseudomonas was found to have been transferred to all the OFs, with significantly fewer transferred to OF C:

OF A: 5.7×10^{5} cfu
OF B: 1.9×10^{6} cfu
OF C: 1.4×10^{5} cfu
OF D: 2.9×10^{6} cfu

• Survival of *P. aeruginosa*

Significant $\log_{10}$ reductions were observed for all OF types by 15 minutes. No significant reductions were observed between four and eight hours, with no significant difference in *P. aeruginosa* viability on antimicrobial OFs compared to the conventional OF after 24 hours.

• Water contamination

OF A: All water samples collected from taps fitted with OF A immediately after contamination were positive above the augmented care alert limit of > 10 cfu/100mL, as was water collected during all subsequent flushes. After the fifth flush, 50% of samples were still contaminated with *P. aeruginosa*.

OF B: All water delivered after the second flush was below detection limits.

OF C: *P. aeruginosa* was detected at the alert limit in one and two samples collected after the second and third flushes, although the majority of the samples were below detection by the fourth flush.

OF D: Alert levels of *P. aeruginosa* were found in water delivered until the fourth flush and in contrast to OF B and C, the bacterium was detected in 17% of samples by the fifth flush.

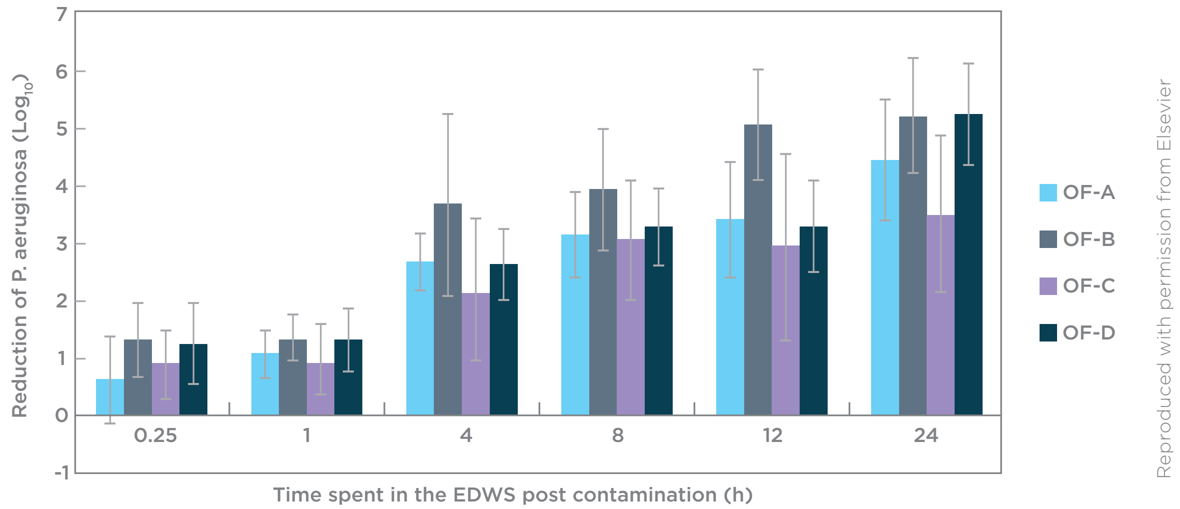
Conclusions

The study demonstrated that OFs may become contaminated when wiped with a contaminated cloth and that this contamination may persist. The anti-microbial OFs did not significantly reduce microbial contamination over 24 hours, but *P. aeruginosa* levels in tap water from affected taps did decrease with increased tap usage. Over a series of flushes the 'antimicrobial/ antibiofilm' OFs reduced contamination more efficiently than conventional OFs. .

Significantly fewer bacteria were transferred to OF-C when wiped with a contaminated microfibre cloth compared to the other OFs.

The authors advised that reliance on 'antimicrobial' material, including those incorporated into an OF, is an "insufficient control measure". They reminded of the importance of flushing and frequent tap use to reduce water system stagnation and that "factors contributing to retrograde contamination.... be investigated and addressed."

Reduction ($\log_{10}$) in cloth-transferred *P. aeruginosa* on outlet fitting types [mean (N 1/4 12) ± standard deviation]. (EWDS: experimental water distribution system).



*The views expressed in this research paper are those of the authors only and not of Public Health England.

References

1. Hutchins C.F. *et al*, "Contamination of hospital tap water: the survival and persistence of *Pseudomonas aeruginosa* on conventional and 'antimicrobial' outlet fittings", *J Hosp Infection*, (2017), http://dx.doi.org/10.1016/j.jhin.2017.06.005
2. www.gov.uk/government/uploads/system/uploads/attachment_data/file/140105/Health_Technical_Memorandum_04-01_Addendum.pdf

Numbers, materials and methods

- Test suspension: cryopreserved *P. aeruginosa* subcultured on Tryptone Soya Agar suspended in 100 mL of Oxoid nutrient broth, incubated at 37°C for 16 hrs, with shaking at 120 rpm
- Cleaning cloths: microfibre from Arco, Hull, UK, cut into 10 cm² swatches
- Neutralisers: sterile thiosulphate Ringer solution (Oxoid) or Dey-Engley neutralizing broth (Sigma)
- 100 μL aliquots plated onto CN agar and incubated at 37°C for 24–48 hours
- Remaining suspension filtered through Pall 0.2 μm membrane and cultured on CN agar 37°C for 24–48 hours
- Flushing water samples collected: 500 mL in 20 mg/L sodium thiosulphate
- Presence of *P. aeruginosa* determined by filtering 100 mL of each sample and culturing as above
- Data analysis: GraphPad Prism (version 7 for Windows).

Credit — PHE, Porton Down

Porton Down experimental water distribution system (EWDS)

The implications of 'dormant' or ('VBNC') bacteria in persistent bacterial contamination

Susan Pearson talks to biofilms expert Professor Hans-Curt Flemming about how the extraordinary ability of bacteria to survive under extreme conditions may pose a problem for water systems in healthcare facilities.

Emeritus Professor Hans-Curt Flemming is the former Director of the Biofilm Centre in the Faculty of Chemistry at the University of Duisberg-Essen in Germany.

Professor Flemming discusses how cases of persistent bacterial contamination may be due to the survival behaviour of bacteria themselves — although the significance of this phenomenon in infection control is still not fully understood.

How does standard water monitoring sometimes fail to reflect bacterial load?

The risk management approach to control of potentially dangerous waterborne pathogens at the heart of guidance such as HSG274 and HTM 04-01 has gone a long way towards reducing the incidence of infections with organisms such as *Legionella pneumophila* and *Pseudomonas aeruginosa* — yet the tried and tested microbiological monitoring of water on which this control is based may not fully reflect the true picture of bacterial contamination of water.

The traditional 'gold standard' for assessing water samples, in use for over 30 years and currently a legal requirement, is an agar plate culture method that quantifies bacterial growth following inoculation of water samples on culture plates. Results are expressed by counting colony forming units (CFUs), with each colony considered to be equivalent to a single live bacterial cell in a sample. However, this method does not count the many cells that can be seen on a culture plate under magnification that have not multiplied into colonies; because they have not grown, the conventional view is that these are 'dead' or 'non-viable' cells.

Bacterial metabolism has two modes: growth, when cells increase in numbers and weight, and a maintenance metabolism, when growth is shut down in response to stress but the bacteria remain dormant and do not grow on the media that are normally used to verify their presence. In this 'viable but non-culturable' (VBNC) state, the organisms still continue to repair their membranes, renew their enzymes or shut down their metabolism. This stress response may take place due to conditions such as exposure to biocides, toxic metals, antibiotics, desiccation, radiation, nutrient depletion or unsuitable temperatures.

The non-colony forming cells that can be seen on a culture plate under the microscope may be dead, but they also might be cells that are not in a growth phase but retain the potential for resuscitation.

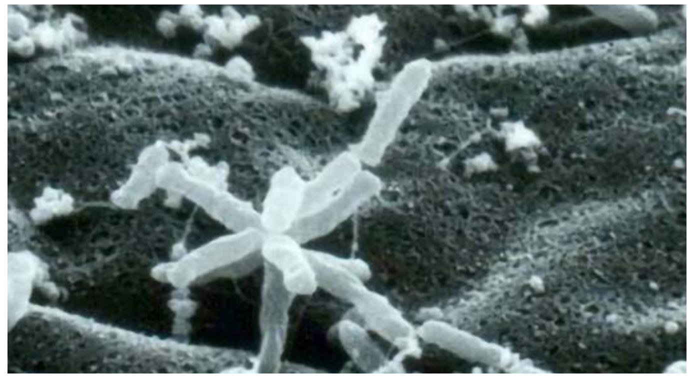
In recent years, development of more sensitive real-time molecular techniques, such as quantitative polymerase chain reaction (qPCR) methods provide fast quantification of bacterial DNA, but many do not distinguish between DNA from live cells and dead or VBNC cells. However, a 'toolbox' of methods, for example, *in situ* fluorescent hybridisation (FISH) for detection of ribosomal RNA, is available to assess 'life signs' in non-growing bacteria.

This VBNC bacterial state occurs particularly frequently in biofilms and has been demonstrated for many waterborne pathogens.[1,2] The VBNC state is important because these cells can be resuscitated and are able to regain their virulence.

Significantly, the human body might provide conditions conducive for resuscitation, which would be particularly dangerous for high risk groups such as the immuno-compromised, diabetics and chemotherapy and post-operative patients.

For those involved in infection control and management of healthcare water systems, the big questions are: how 'dead' are these bacteria? Can they be resuscitated? And if so, can they regain their virulence? Is the presence of potential pathogens in water samples being missed? Particularly important is that in recurring contaminations, the cause may be due to resuscitated cells seeded from biofilms in the water system, rather than from the invasion of new cells.

Credit — Prof HC Flemming, Biofilm Centre, Universitat Duisberg Essen



Non-colony forming (CFU) bacteria on culture plate — are they alive or dead?

How 'dead' is dead?

In research into the apparent ability of water containing copper ions, in concentrations common to drinking water, to 'kill' *P. aeruginosa* in culture, CFU numbers decreased as copper concentration increased — yet overall cell numbers on culture plates remained the same. The research at Duisburg-Essen also established that loss of culturability in planktonic (free-floating bacteria) is dependent on the concentration of copper ions, originating from copper components in the system. However, the total (observed) cell numbers remain the same, with all cells retaining their membrane integrity, ribosomal RNA (an indicator for protein production) and constant ATP levels.

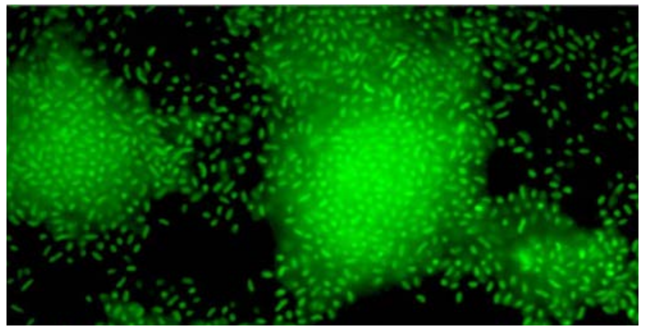
In further research into the ability of bacteria to remain infective (cytotoxic) during the VBNC state or following resuscitation, a copper chelator was used to remove copper from the bacterial cells. It was found that total cell numbers remained the same, but over time, increasing numbers of these cells became culturable, indicating resuscitation of the bacterial cells. In the presence of copper, it was found that *P. aeruginosa* in the VBNC state does not kill bronchial epithelial cells, which are known target cells for infection with the organism, but once resuscitated, the formerly copper-treated cells killed the epithelial cells within nine hours, exactly the same time frame as the wild type which was not exposed to copper.[3,4]

There appears to be a resuscitation window for copper exposure; when *Pseudomonas* cells are exposed with copper for longer periods of time, e.g. weeks, they cannot be resuscitated with this chelator,[5] and some strains of *Pseudomonas* move into the VBNC status much sooner than others, ⇨

suggesting there is no cut-and-dried pattern. However, resuscitation is a complex phenomenon and resuscitation with the copper chelator is only one of the routes back to viability and virulence.

Silver also has well established antimicrobial properties. Further research has looked at the efficacy of silver and silver nitrate ions against planktonic cells and biofilm using both culture and non-culture methods.

In liquid media, both planktonic growth and biofilm formation were found to be inhibited. The researchers found that loss of culturability was always higher in planktonic cells than the attached biofilms, but that re-suspended biofilm cells became more susceptible to silver ions than to attached biofilms. This suggests that bacteria within biofilms are much more tolerant to silver than are free-floating bacteria.[6]



Credit – Prof J.S. Webb, University of Southampton

GFP-labelled *P. aeruginosa* biofilm on a glass surface

Are VBNC Legionella infective?

It has been shown that a high percentage of *Legionella* bacteria in water systems are not culturable, but the potential infectivity of these VBNC legionellae is unclear. Recent research has shown that VBNC *Legionella* strains, including the main human pathogen, *L. pneumophila* serogroup 1, can directly infect different types of human macrophages and amoebae even after one year of starvation in ultrapure water, although under these conditions, the efficacy of the infection is reduced.[7] However, it still remains unclear how VBNC cells can attach to cells, express virulence factors and induce the infection process.

Conclusion

Cell death can only be confirmed by suitable culture-independent methods and the ability of cells to resuscitate remains the most compelling parameter for assessing relevance to human health. There are still many open questions about what conditions induce the VBNC state. It remains unclear how to rate health risks associated in detection of VBNC organisms in drinking water systems and, most of all, how to make the VBNC state irreversible, as most bacteria appear inactivated and not virulent while in that state.

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Recommended reading

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Latest Research

J Hosp Infect.

2018 Mar 24. pii: S0195-6701(18)30174-9. doi: 10.1016/j.jhin.2018.03.023. [Epub ahead of print]

The Hand Wash Station, friend and fiend? Weinbren MJ.

Hand washing is a key barrier to cross infection performed at a hand wash station (HWS), an interface between water and drainage systems. Widespread and often uncritical placement/design and use of HWSs is not without attendant risks. Recognition of the associated hazards went unheeded for over 45 years despite warnings in the literature until the neonatal outbreak of *Pseudomonas* in Belfast in 2012 forced change. Minimising risk requires a holistic approach beyond the mere testing of water from the outlet of a HWS for the presence of *Pseudomonas aeruginosa* or other pathogens. Literature reports of outbreaks linked to HWS outside of neonatal units are overrepresented by multi-resistant organisms, and increasingly so by carbapenemase producing organisms. Evidence suggests a large proportion of waterborne transmissions go undetected. Much could be done to improve current design use and placement of HWSs and is critically assessed in this article.

Chemosphere.

2018 Mar 22;203:368-380. doi: 10.1016/j.chemosphere.2018.03.143. [Epub ahead of print]

Impact of biofilm formation and detachment on the transmission of bacterial antibiotic resistance in drinking water distribution systems. Zhang J, Li W, Chen J, Qi W, Wang F, Zhou Y.

There is growing awareness of the antibiotic-resistance crisis and its implications for public health among clinicians, researchers, politicians and the public. Bacterial antibiotic resistance transition and the role of biofilms in a drinking water distribution system (DWDS) was studied. Several different antibiotic resistant bacteria (ARB) with resistance to tetracycline, sulfamethoxazole, clindamycin and norfloxacin were tracked for one year in a DWDS. The results indicated that the amount of ARB increased in tap water, presumably due to biofilm detachment. The effect of biofilm detachment on the transmission of antibiotic resistance from biofilms to tap water was explored by using a bacterial annular reactor. The percentage of ARB of inlet water, outlet water and biofilms ranged from 0.26% to 9.85%, 1.08%–16.29%, and 0.52%–29.97%, respectively in a chlorinated system, and from 0.23% to 9.89%, 0.84%–16.84%, and 0.35%–17.77%, respectively, in a chloraminated system. The relative abundances of antibiotic resistance *Acinetobacter, Sphingomonas* and *Bradyrhizobium* were higher in outlet water than in inlet water, as determined by high-throughput sequencing. The amount of ARB percentage varied with the concentration of viable but non-culturable (VBNC) cells ($r = 0.21$, $n = 160$, $P < 0.05$) in biofilm, suggesting a higher antibiotic resistance mutation rate in VBNC cells. Our results suggest that biofilm detachment was promoted by disinfectant and affected the overall bacterial antibiotic resistance of microbes in tap water.

Trends Microbiol.

2018 Jul: 26(7):637-638. doi: 10.1016/j.tim.2018.04.006. Epub 2018 May 10.

Stenotrophomonas maltophilia. An, SQ and Berg G.

Description of the key regulated traits of *Stenotrophomonas maltophilia*, important for beneficial plant interactions, and also its increasing incidence as a nosocomial and community-acquired infection. *Stenotrophomonas maltophilia* is a cosmopolitan and ubiquitous bacterium found in a range of environmental habitats, including extreme ones, although in nature it is mainly associated with plants. *S. maltophilia* fulfils important ecosystem functions in the sulphur and nitrogen cycles, in degradation of complex compounds and pollutants, and in promotion of plant growth and health. *Stenotrophomonas* can also colonize extreme man-made niches in hospitals, space shuttles, and clean rooms. *S. maltophilia* has emerged as a global opportunistic human pathogen, which does not usually infect healthy hosts but is associated with high morbidity and mortality in severely immunocompromised and debilitated individuals. *S. maltophilia* can also be recovered from polymicrobial infections, most notably from the respiratory tract of cystic fibrosis patients. Close relatives of *S. maltophilia*, for example, *S. rhizophila*, provide a harmless alternative for biotechnological applications without human health risks.

How does bowel cancer develop with a low oxygen supply?

Armitage Shanks is supporting Nottingham University PhD student Hannah Bolland through its 200 year-old charity initiative **Armitage Thanks** to study the behaviour of bowel cancer when it develops in areas of low oxygen.

Hannah Bolland

The behaviour of this cancer under low oxygen conditions is a significant factor in the aggressiveness of bowel cancer and the patient's prognosis as cancer becomes harder to treat when it has a low oxygen supply. A key reason for this is that the blood vessels in the tumour form in a distorted way that makes it difficult for chemotherapy to reach all the necessary areas. About one third of bowel cancers have this characteristic and there are currently no treatments specific to low oxygen bowel cancer.

Under the supervision of Professor Alan McIntyre, Hannah, who is now in year two, has identified a single protein that is key to enabling bowel cancer cells to survive in low oxygen conditions. She has also tested this in 3D structures called Spheroids. This protein is now a viable target for the development of therapies. Over the next year Hannah will move on to look at what mechanisms the protein uses to enable the cancer cells to survive.

If current progress continues, the work will enable us to find out whether a patient's cancer has low oxygen characteristics and therefore ensure that they receive early and appropriate treatment. It will also translate into the development of new therapies that can treat these cancers in a way that can't be done with current chemotherapy.