**Project Details**

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1. **Introduction**

UK mains drinking water is supplied to premises at a high standard but does contain small numbers of organisms which may be a risk to susceptible hospitalised patients if allowed to multiply within a building’s water system. To date the main organisms thought to pose a risk include Legionella, *Pseudomonas aeruginosa* and atypical mycobacteria. Specific guidance and methods for testing for these organisms in water samples exist. 1, 2 Whilst Cupriavidus *spp* are known to be present in small numbers in incoming mains water the incident at Glasgow Queen Elizabeth University Hospital (QEUH) where *Cupriavidus pauculus* was isolated both from the water system and patient blood cultures is the first report of an outbreak of healthcare acquired *C. pauculus*. 3

Cupriavidus spp belong to a group of organisms known as Opportunistic Premise Plumbing Pathogens (OPPPs). These organisms possess a number of characteristics in common; disinfectant resistance, pipe surface adherence and biofilm formation, growth in amoebae, growth on low organic concentrations and growth at low oxygen levels.4 They are normal inhabitants of incoming mains water (low numbers) and can multiply in premise plumbing to levels capable of producing infections in individuals with predisposing conditions.

Different OPPPs present distinct requirements for sampling, preservation, and analysis creating an impediment to their detection. For many OPPPs there is no recognised method or specific water regulations, for detection in water systems. Water testing methodologies currently exist for Legionella spp, *P. aeruginosa* and atypical mycobacteria.

Little knowledge exists over the occurrence of Cupriavidus spp within healthcare water systems and no guidance or recognised testing methodology currently exists. Little is known about the prevalence

of this organism in healthcare water systems. Detection is further complicated by until recently difficulties in laboratory identification. The advent of MALDI-TOF (mass spectrometry for the rapid and accurate identification of bacteria) has revolutionised the ability to accurately identify many organisms including Cupriavidus spp. Prior to MALDI-TOF biochemically inert organisms including Cupriavidus spp may not have been able to be accurately identified to genus or species level and may have been dismissed as unidentified environmental organisms of dubious clinical significance. So even when isolated from clinical specimens this organism went unnoticed including the link to water systems. Whilst MALDI-TOF is now common in clinical microbiology laboratories this is not the case in many environmental laboratories which test water samples.

1. **Project aims**

**The aims of the project were as follows;**

1. To determine the incidence of Cupriavidus spp in healthcare water systems in Scotland.
2. To determine the incidence of Cupriavidu*s* spp in healthcare water systems outside Scotland to determine whether there are features of Scottish water systems which could account for different ecology
3. Ascertainment of where Cupriavidus spp may be detected in water systems (incoming mains, water tanks, expansion vessels, outlets)
4. The Concentration of Cupriavidus spp present when detected in water., manly outlets in the Glasgow incident had TVCs > 100 cfu/100ml
5. Where Cupriavidus spp is isolated to perform an extended range of antibiotic sensitivities. This might prove useful if Cupriavidusspp is inherently resistant to a broad-spectrum antibiotic which could be incorporated into agar to help make a selective media . Susceptibility testing will also provide information on treatment options.
6. To develop a protocol for detecting Cupriavidus spp in water systems.
7. To detect the presence of other organisms in water samples with particular reference to other OPPPs.
8. **Methods**

Hospitals were approached by the chief investigator to participate based on geographical location to ensure a representative sample of mainland UK

System wide water samples were collected from pre-defined sites across the water system from ten healthcare sites. Participating hospitals were asked to test water storage tanks, expansion vessels and outlets to reach a maximum of 15 samples per hospital.

Water samples were transported to the Glasgow Royal Infirmary (GRI) Microbiology Environmental Laboratory under controlled conditions to minimise specimen deterioration between January-June 2021 in bottles pre supplied by the laboratory.

The Microbiology laboratory at GRI holds ISO 15189 UKAS accreditation for the clinical laboratory and ISO 17025 UKAS accreditation for the environmental laboratory. The ISO 17025 scope of accreditation does not specifically include the testing of water samples for OPPPs such as Cupriavidus sp due to the specialist nature of the testing and the lack of an established methodology, limits of detections and appropriate water regulations.

100ml of water was filtered onto;

1)Chromogenic Coliform Agar (E&O) incubated for 24hr at 36®C

2)Tryptone Soya Agar (TSA, OXOID) incubated for 48hr at 36® C

3)Pseudomonas isolation agar (E&O) incubated for 48hr at 36®C

4) CARBAPENEMASE Agar (Colorex mSuperCARBA E&O) incubated for 24hr at 36®C

5)CHROMID CARBASMART (Biomerieux) incubated at 24hr for 36 ®C

At 24 hrs Chromogenic coliform plates were read and any coliforms identified. Carbapenem plates were read at 24hrs. TSA and Pseudomonas plates were read together at 48 hr. Gram negative organisms testing oxidase positive were sub-cultured for purity and further identified using MALDITOF. Any Gram negative that did not identify was retested by MALDITOF before being sent for identification by VITEK2. If the organism failed to ID it was reported as an unidentified Gram-negative rod. Isolates that identified as *Cupriavidus* spp by either MALDITOF or VITEK2 were also sent to a UKHSA reference laboratory for confirmation and a second independent laboratory for 16S PCR.

All Cupriavidus isolates were set up for antimicrobial susceptibility disc testing using the Gram negative antibiotic sets using an antibiotic disc methodology with a 0.5 Macfarlane inoculum. The zone sizes of the antibiotic discs were recorded for each isolate for; amoxycillin, aztreonam, coamoxiclav, ciprofloxacin, gentamicin, meropenem, tazocin, temocillin. A cotrimoxazole, meropenem and minocycline MIC was also determined for each Cupriavidus isolate.

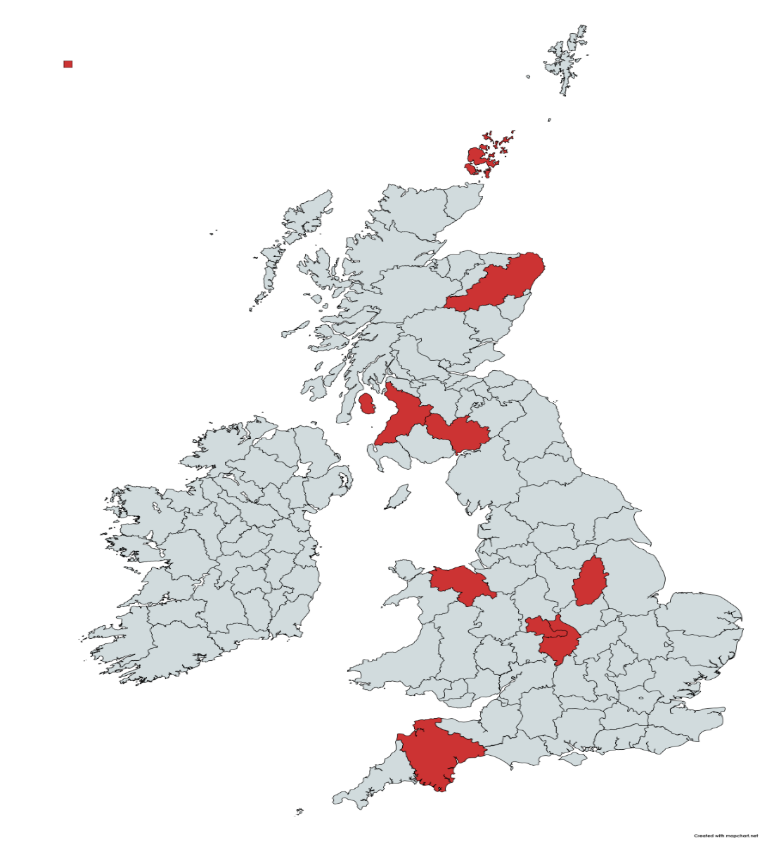
Total viable counts of waters were carried out using a pour plate method utilising Yeast extract agar. 1 ml of water was pipetted onto four Petri dishes. Within 20 minutes of the 1ml being dispensed 15-20ml of molten agar was added to the Petri dish within 20 minutes of the 1ml being dispensed. Following mixing the agar was allowed to set at room temperature, plates were inverted and Incubated plates at 22®C and 37®C .Total viable counts (TVCs) were reported for each outlet expressed as TVC/ml. If below 100 colony forming units (cfu) the results were quantified.

1. **Results section**

Water samples were received from ten hospital sites. Of the ten sites sampled all sent pre-flush samples from outlets. Eight out of ten were able to send storage tank samples. Two hospitals sent samples from outlets only. Only three hospitals were able to sample expansion vessels. 157 samples were tested in total

*Geographic results*

The ten sites sampled are depicted on the map below and are a representative sample of mainland UK. Four of ten hospitals sampled had *Cupriavidus* spp isolated from water samples. Of the five positive results three were *C. pauculus* with one sample each testing positive for *C. gilardii and C metalladurans*

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*Quantification of Cupriavidus spp results and location within the water system*

The chart below displays the number of samples by location. The majority of samples were from outlets (120/157, 76%) and therefore the periphery of the water system.

The chart below depicts the number and percentage of outlets testing positive in each hospital and the number and percentage of positive samples with organism counts of > 100cfu/100ml. The percentage positivity ranged from 26.7%-95% and the percentage of positive samples with counts > 100 cfu/100ml ranged from 37.5%-100%.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Hospital** | **No of samples testing positive** | **Percentage of samples testing positive** | **No of positive samples with counts > 100 cfu/100 ml** | **Percentage of positive samples >100 cfu/100ml** |
| A | 9/15 | 60% | 7/9 | 77.8% |
| B | 11/15 | 73% | 6/11 | 54.5% |
| C | 8/16 | 50% | 3/8 | 37.5% |
| D | 11/16 | 68.7% | 9/11 | 81.8% |
| E | 8/15 | 53% | 8/8 | 100% |
| F | 19/20 | 95% | 10/19 | 52.6% |
| G | 4/15 | 26.7% | 2/4 | 50% |
| H | 13/15 | 86.7% | 12/13 | 92% |
| I | 11/15 | 73% | 6/11 | 54.5% |

Of the four hospitals isolating *Cupriavidus* spp the organism was present in a total of five samples. Apart from one expansion vessel the results were obtained from the outlets. No samples from mains supply or storage tanks isolated *Cupriavidus* spp. Three of the five positive samples had counts of *Cupriavidus* spp > 100 cfu/100ml. All Cupriavidus isolates grew on TSA and three grew on Pseudomonas agar plates. None grew on either of the Carbapenamase agars.

Four isolates failed to identify on MALDITOF as *Cupriavidus* spp but were identified as such using VITEK2. However, these isolates were not confirmed as *Cupriavidus* spp by the UKHSA reference laboratory and were subsequently identified as *Xenophilus aerolatus* using 16S PCR (Micropathology Ltd). The five isolates our laboratory identified as *Cupriavidus* spp were confirmed as such by both UKHSA and a second independent private laboratory.

A total of seven samples were obtained from expansion vessels with only one of these testing positives for *C. pauculus* as reported above. Two others tested positive for *Blastomonas ursincola* and *Sphingomonas paucimobilis* both at counts > 100 cfu/100ml.

*Susceptibility testing*

The table depicts Cupriavidus isolates, minocycline, meropenem and co-trimoxazole MICs and disc testing results. There are no interpretative criteria for Cupriavidus species and previous publications have utilised EUCAST criteria for Pseudomonas spp or CLSI criteria for non *Enterobacteriaceae* where EUCAST breakpoints don’t exist. With regards susceptibility to Meropenem four of five isolates had MIC > 32 mg/L and would be classed as fully resistant as per EUCAST interpretative criteria for *Pseudomonas* spp be classed as fully resistant as per EUCAST interpretative criteria for Pseudomonas. Of the isolates tested for cotrimoxazole all five were sensitive applying EUCAST breakpoints. All isolates were Minocycline sensitive applying CLSI interpretative criteria (<=4 mg/L)

\*CLSI criteria

\*\* No zone interpretative criteria

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| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Minocycline MIC\*  S ≤4 mg/L | Meropenem MIC  S≤2 mg/L | Cotrimoxazole MIC \*  S≤2 mg/L | Meropenem  Disc  S ≥24mm  R < 18mm | Ciprofloxacin  Disc  S ≥50 mm  R < 26mm | Aztreonam  Disc  S≥50mm  R<18 | Tazocin  Disc  S≥50  R<18 | Gentamicin  Disc \*\* | Coamoxiclav  Disc \*\* | Amoxycillin  Disc \*\* | Temocillin  Disc \*\* |
| Isolate A | 0.19 | >32 | 0.5 | 0mm | 32mm | 17mm | 33mm | 0mm | 0mm | 0mm | 12mm |
| Isolate B | 0.125 | 0.38 | 0.38 | 34mm | 34mm | 12mm | 38mm | 22mm | 38mm | 32mm | 0mm |
| Isolate C | 0.19 | >32 | 0.38 | 0mm | 33mm | 0mm | 30mm | 0mm | 10mm | 0mm | 0mm |
| Isolate D | 0.19 | >32 | 0.5 | 0mm | 33mm | 0mm | 29mm | 0mm | 12mm | 0mm | 0mm |
| Isolate E | 0.25 | >32 | 0.38 | 0mm | 32mm | 0mm | 35mm | 0mm | 13mm | 0mm | 0mm |

*Presence of other OPPPs*

The table below depicts the range of other OPPPs isolated from the water tested. As expected, all hospitals had evidence of other OPPPS to varying degrees.

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Hospital | A | B | C | D | E | F | G | H | I | J |
| *Acidovorax delafieldii* |  |  |  |  |  |  |  |  |  |  |
| *Acidovorax temperans* |  |  |  |  |  |  |  |  |  |  |
| *Acinetobacter baumanii* |  |  |  |  |  |  |  |  |  |  |
| *Acinetobacter beijerinckii* |  |  |  |  |  |  |  |  |  |  |
| *Acinetobacter haemolyticus* |  |  |  |  |  |  |  |  |  |  |
| *Acinetobacter junii* |  |  |  |  |  |  |  |  |  |  |
| *Blastomonas ursincola* |  |  |  |  |  |  |  |  |  |  |
| *Brevundimonas diminuta/vesicularis* |  |  |  |  |  |  |  |  |  |  |
| *Chryseobacterium gleum* |  |  |  |  |  |  |  |  |  |  |
| *Chryseobacterium indologenes* |  |  |  |  |  |  |  |  |  |  |
| *Comomonas testeroni* |  |  |  |  |  |  |  |  |  |  |
| *Cupriavidus gilardii* |  |  |  |  |  |  |  |  |  |  |
| *Cupriavidus pauculus* |  |  |  |  |  |  |  |  |  |  |
| *Delftia acidovorans* |  |  |  |  |  |  |  |  |  |  |
| *Elizabethkingia meningoseptica* |  |  |  |  |  |  |  |  |  |  |
| *Herbaspirillium huttiense* |  |  |  |  |  |  |  |  |  |  |
| *Myroides spp* |  |  |  |  |  |  |  |  |  |  |
| *Pseudomonas aeruginosa* |  |  |  |  |  |  |  |  |  |  |
| *Pseudomonas anguilliseptica* |  |  |  |  |  |  |  |  |  |  |
| *Pseudomonas fluorescens* |  |  |  |  |  |  |  |  |  |  |
| *Pseudoxanthamonas mexicana* |  |  |  |  |  |  |  |  |  |  |
| *Pseudomonas oleovorans* |  |  |  |  |  |  |  |  |  |  |
| *Pseudomonas putida* |  |  |  |  |  |  |  |  |  |  |
| *Roseomonas gilardii* |  |  |  |  |  |  |  |  |  |  |
| *Roseomonas mucosa* |  |  |  |  |  |  |  |  |  |  |
| *Serratia marcescens* |  |  |  |  |  |  |  |  |  |  |
| *Sphingomonas multivorum* |  |  |  |  |  |  |  |  |  |  |
| *Sphingomonas paucimobilis* |  |  |  |  |  |  |  |  |  |  |
| *Sphingomonas thalophilium* |  |  |  |  |  |  |  |  |  |  |
| *Stenotrophomonas maltophilia* |  |  |  |  |  |  |  |  |  |  |
| Unidentified Gram negative |  |  |  |  |  |  |  |  |  |  |
| Xenophilus aerolatus |  |  |  |  |  |  |  |  |  |  |

The chart below displays the number of water samples positive for each organism. *B ursincola* was the most commonly isolated Gram negative followed by Acidovorax spp, Sphingomonas spp and Brevunidimonas spp.

**Discussion**

Hospital water systems are not sterile and will contain micro-organisms which are of minimal consequence to the majority of patients, provided the water systems are maintained and used correctly. That hospital water systems are not sterile supported by our findings in that all hospitals had evidence of opportunistic premise plumbing pathogens. It is important that levels of micro-organisms are kept safe and that water systems do not promote biofilm formation and proliferation of organisms. Testing and additional precautions are implemented to prevent infections in patients who are immunosuppressed and more susceptible to waterborne infections. Patient groups most at risk are those with haematological malignancies, transplant patients, those nursed in intensive care and burns units and patients with indwelling long lines.

Hospital outbreaks of water borne pathogens are well described. Causative pathogens include Legionella spp, Pseudomonas spp., and other Gram negative bacteria such as *S. maltophilia*, atypical mycobacteria and fungi.

Cupriavidus spp are ubiquitous in the environment, and the organism is found in soil, water and plants.5 Cupriavidus was formerly described as Ralstonia a species of proteobacteria. It is an aerobic Gram-negative motile bacterium which is catalase and oxidase positive. Typical colonies are round, smooth and non-pigmented.5 It is nutritionally versatile and can tolerate harsh environments having been found in the international space station potable water supply during pre-consumption testing. 6

*C. pauculus* is a rare clinical pathogen, published case reports describe sepsis, abscesses, tenosynovitis, osteomyelitis, pneumonia and peritonitis. 7 Contaminated water sources such as hydro pools, ECMO and bottled water have been reported. One study reported a pseudo-outbreak involving 27 patient samples which tested positive for *C. pauculus* due to contaminated swabs being rinsed in tap water in an outpatient clinic.5 In a new build Glasgow hospital, cases of bacteraemia in paediatric haemato-oncology patients with *C. pauculus* and other Gram-negative organisms were associated with a contaminated water supply.3 98 outlets were tested from the haemato-oncology unit with 75 of these testing positive for *C. pauculus* (76.5%). 37 of the 75 had counts in excess of 100 cfu/100ml (49%). 3 Contamination was systemic with outlets testing positive in wards throughout the children’s and adult hospitals. The organism was detected further back within the water system with pre and post filtration storage tanks, risers and expansion vessels also testing positive for Cupriavidus and other OPPPs. 3 No Cupriavidus was detected in mains water samples. Investigation revealed a number of risk factors in the hospital water system and construction commissioning process which predisposed to biofilm formation and widespread systemic contamination. 3,8

This study wished to ascertain whether Cupriavidus is typically found in hospital water or whether the problem was in some way unique to Glasgow or Scotland. Our results indicate that the organism is present in hospital water systems elsewhere in Scotland and in hospitals elsewhere in the UK. The percentage of positive results and levels in the water were significantly less than those documented in the Glasgow incident with only five of a total of 159 (3%) samples from ten sites testing positive for the organism. When present the organism was predominantly detected at the periphery of the water system. All hospitals tested had evidence of other OPPPs present in the water. Some of the more common OPPPs isolated were Acidovorax spp, *B.ursincola*, Brevundimonas spp and Sphingomonas spp. We could find no published reports of clinical infection due to *B ursincola* or Acidovorax spp. Brevunidmonas spp are rare opportunistic pathogens but have been linked to bacteraemia, liver abscess, peritoneal dialysis associated peritonitis, septic arthritis, endocarditis and keratitis. 9 Similarly Sphingomonas spp are rare causes of infection but have been linked to prosthetic valve endocarditis, spondylodiscitis and bacteraemia 10-12 Pseudomonas *aeruginosa* and *Stenotrophomonas maltophilia* , both significant clinical pathogens were also detected. Some hospitals had other organisms isolated that have the potential to be clinically significant e.g., *Elizabethkingia miricola, Roseomonas mucosa, Serratia marcescens* and Acinetobacter spp.

Clinical isolates of *C. pauculus* remain rare in the UK. Data obtained from Public Health England (PHE) showed 14 cases of bacteraemia in a six year period (2015-2020) with no evidence of clustering. During the same time frame there were 12 cases of bacteraemia reported in Scotland which included the three cases linked to the Glasgow outbreak. Outbreak cases aside the incidence would appear to be higher in Scotland. It is not clear whether this is a true increased incidence or due to case ascertainment i.e. is it possible labs were detecting the organism but dismissing Cupriavidus spp as an environmental contaminant resulting in under reporting.

Another aim of the study was to develop a protocol for testing for Cupriavidus in water samples and develop a selective media to aid laboratories with detection. Based on the variable antibiotic susceptibility testing results it was not felt possible to develop a selective media. Cupriavidus was noted to grow well on TSA with three isolates also growing on Pseudomonas agar. No growth was detected on SuperCARBA or CHROMID CARBASMART agar. Cupriavidus on Pseudomonas agar was a buff colony, and slowly oxidase positive. Further identification was undertaken using MALDI-TOF . Some isolates failed identification on the MALDI-TOF but were identifiable on VITEK2. However, these four isolates were not confirmed as such by the reference laboratory and subsequently identified as another Gram negative organism, *Xenophilus aerolatus* using 16S PCR . *X. aerolatus* is a very rare Gram negative organism first identified from air samples in South Korea . 13 We could find only one published case report involving a child on peritoneal dialysis with peritonitis as a result of the organism, which was identified by 16S rRNA sequencing.13 We could find no literature describing the isolation of *X.aerolatus* from water samples, this is possibly due to its absence from the databases of laboratory identification systems and the need for 16S PCR for its identification.

Based on our experience with this *Cupriavidus* spp during the Glasgow outbreak and this study we would recommend TSA agar to labs wishing to isolate *Cupriavidus* spp in water systems. Plates yielding oxidase positive colonies failing to identify on day 1 should be re-incubated and re-tested at 48 hours. We would caution against utilising VITEK 2 for initial identification or confirmation of suspected Cupriavidus isolates failing to identify on MALDITOF and would recommend 16S PCR as the confirmatory step in lab testing algorithms.

Antibiotic susceptibility testing results were variable for the Cupriavidus water isolates tested. No interpretative criteria exist for *Cupriavidus* spp so interpretative criteria for *P. aeruginosa* were used in line with other publications. 14 No interpretative criteria for *P. aeruginosa* exist for amoxycillin and temocillin as they are inherently resistant to these antibiotics. Similarly, all of the Cupriavidus isolates were resistant to aztreonam and amoxycillin. Fluoroquinolones and tazocin demonstrated some activity. The results of testing showed that 4 of 5 isolates were resistant to Meropenem with MIC≥ 32. The optimal therapy for clinical infections due to Cupriavidus is yet to be determined. As there are no agreed interpretative criteria for disc testing MIC testing applying Pseudomonas interpretative criteria is essential to inform treatment choice. All five isolates tested were sensitive to cotrimoxazole and minocycline. Our findings are similar to Massip et al who undertook susceptibility testing on 34 clinical isolates of *Cupriavidus* spp.14 They deduced that meropenem and aminoglycosides were unreliable but that minocycline and cefepime exhibited the best in vitro activity. 62% of the isolates they tested were susceptible to cotrimoxazole. They reported discrepancies between Imipenem and Meropenem susceptibilities postulating this may be due to over expression of efflux pumps, similar to *P. aeruginosa*.14

Similar to *P. aeruginosa*, Cupriavidus spp were detected predominantly in the periphery of the water system. Expansion vessels were highlighted as a source of contamination in the Glasgow incident where it was noted that the wrong type had been installed i.e., bladder instead of flow through which reduce stagnation. 3 They were also found to be made of a material conducive to biofilm formation. In this study we received limited samples from expansion vessels due to problems accessing them and only one of seven sampled tested positive for *C. pauculus*. Two other expansion vessels grew *B ursincola and S paucimobilis*. All three expansion vessels testing positive had organism counts of > 100 cfu/100ml.

Currently in Scotland water testing is recommended for Legionella as per the HSE approved code of practice and for Pseudomonas in augmented care units (at least six monthly). 1, 2 A separate aide memoire published in 2019 alerts infection control teams to other waterborne pathogens, some of which featured in the Glasgow incident. 15 This document states that if there is an indication of an association with water or water-related equipment, consideration should be given to conducting environmental sampling. Given that *Cupriavidus spp* is not associated with endogenous carriage and published literature to date has only established water as a source there is a strong argument for testing water when a single patient case is detected without waiting for further linked cases. Indeed, in the Glasgow incident the three cases were separated by several months. This likely reflects that specific host factors are associated with the development of infection i.e., severe immunosuppression, presence of invasive devices.

Three isolates of Cupriavidus spp grew on selective Pseudomonas Isolation Agar (containing cephalothin, fucidin and cetrimide) so it is possible it may be detected by labs routinely sampling for Pseudomonas. If Cupriavidus spp is detected on routine water sampling (due to its ability to grow on Pseudomonas isolation agar) it would be important to risk assess the implications for both patients and the water system with implementation of control measures to protect vulnerable patient groups e.g., haemato-oncology. Similar strategies with regards to testing after a single case of invasive infection and implementing measures for high-risk groups on detection could also be applied to other rare non-exogenous pathogens with established links to water e.g., *Brevundimonas spp*, *Sphingomonas spp*, *Delftia acidovorans, Comomonas testeroni, Elizabethkingia sp.* Detection of water contamination with Cupriavidus spp in Glasgow came about following the detection of high TVCs on routine testing of outlets in an aseptic pharmacy( >10cfu/ml at 37®C and /or >100 cfu/ml at 22®C). 3 The organisms were identified following negative results for the usual water quality indicators (Legionella, Coliforms, E coli, Pseudomonas). Consideration should be given to full identification of TVCs in high-risk units given the risk to immunosuppressed patient groups from certain OPPPs. Similarly drains should not be overlooked as a source of these OPPPS. 3

*Limitations of this study*

The main limitation of this study was that only a small number of UK hospitals submitted water for testing. Despite this we were able to address the study aims, finding evidence of Cupriavidus sppin hospital water systems elsewhere in Scotland and the UK. Access to sampling of expansion vessels and storage tanks was difficult for some centres and therefore outlets were predominantly sampled. As a result our finding that Cupriavidus is found mainly in the periphery of the system may be subject to sampling bias. There are limitations in our susceptibility testing as this was undertaken to investigate the possibility of developing a selective media for Cupriavidus isolation as opposed to determining the most suitable therapeutic agents for patients. However, there are some interesting observations which we have chosen to report.

1. **Recommendations**

1) Clinical isolates of Cupriavidus from BSI are rare in the UK and this study has shown that Cupriavidus spp are present in some UK hospital water systems. We would therefore recommend Cupriavidus spp are classed as alert organisms in infection control policy and water testing in the event of a patient related bacteraemia due to this organism.

2) Consideration should also be given to water testing following bacteraemias due to other rare and unusual water borne pathogens such as Delftia acidovorans, Sphingomonas spp, Brevunidomonas spp, Comamonas spp and Elizabethkingia spp

3) Laboratory testing for rare and unusual water pathogens is underdeveloped and non standardised. Further work is required to determine the optimum methodology for recovery of these organisms from water systems. Work is also required on the formulation of standard microbiological investigations/recommended methodology for environmental labs to follow.

4) On the basis of our susceptibility testing and the available literature on the subject suitable therapeutic agents for patient infections include minocycline and septrin. It should be noted that isolates are commonly resistant to meropenem which is the usual escalation from tazocin for neutropenic sepsis. Meropenem should be avoided where Cupriavdus spp has been isolated until sensitivities are available.

5) Data on antibiotic susceptibility testing is not robust enough for Cupriavidus spp to enable the creation of a selective media to isolate this organism for water samples and a combination of TSA and Pseudomonas agar is recommended.

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