Investigation of *Pseudomonas aeruginosa* on biofilms in water tap assemblies from neonatal units in Northern Ireland

Report Prepared by	Dr Jimmy Walker Miss Anjeet Jhutty Mr Simon Parks Dr Caroline Willis Dr Vicky Copley
Issue Date	29th May 2012
Version Number	2.0 - Final Copy

Report Written By	**Report Authorised By**
Name: Dr Jimmy Walker **Title:** Principal Investigator Biosafety	**Name:** Allan Bennett **Title:** Head of Biosafety

Contents

Acknowledgements 3
Executive summary 4
Background 5
Materials and Methods 7
Transportation and dismantling of taps 7
Microbiological Analysis 7
Microscopy analysis 8
Strain Typing 8
Statistical Analysis 8
Results 11
Microbiological Analysis 11
Association between the aerobic colony count and P. aeruginosa count 13
The effect of tap type on the presence of P. aeruginosa 14
The effect of component type on P. aeruginosa counts 15
The effect of the rosette component on P. aeruginosa counts 16
P. aeruginosa colonisation of flexi-hose and copper pipe 18
Comparison of the effect of component location on the total aerobic and P. aeruginosa colony count 18
Predictive values for P. aeruginosa colonisation of sensor and non-sensor tap components 20
Typing of Pseudomonas aeruginosa strains 21
Microscopy analysis 21
Discussion 26
Microbiological analysis 26
Sensor versus non-sensor taps 27
Presence of P. aeruginosa on individual tap components 27
Typing of P. aeruginosa isolates 28
Microscopy 28
Conclusions 31
Appendix 32
Appendix 1: Contents of carton from the Royal Jubilee Hospital, Belfast 33
Appendix 2: Contents of cartons received from Ulster Hospital 36
Appendix 3: Contents of carton received from Altnagelvin Hospital, Londonderry 40
Appendix 4: Contents of cartons received from Antrim Area Hospital, Antrim and Daisy Hill Hospital Newry 42
Appendix 5: Contents of carton received from RJM, Belfast & Erne Hospital, Enniskillen 46
Appendix 6: Contents of carton received from Craigavon Area Hospital, Craigavon 49
Appendix 7: Glossary of components 51
Appendix 8: Glossary of Rosette Types 56
Appendix 9: Dendogram of isolates from neonatal unit patients with environmental, water and biofilm samples from the tap outlets. 58
References 60

Acknowledgements

The authors would like to acknowledge the contribution of Sara Speight, Tom Pottage, Nick Fletcher, Howard Tolley and Jane Turton (VNTR analysis) as well as Brian Godfrey, George McCracken and Robert Sergeant and their many colleagues from NI who assisted with this study.

Executive summary

A study has been carried out to assess the presence of *Pseudomonas aeruginosa* biofilms on various tap assembly components from neonatal wards in Northern Ireland following four fatal cases of *Pseudomonas aeruginosa* bacteraemia that occurred in neonatal units in Northern Ireland in December 2011 and January 2012.

All taps were replaced from clinical hand wash basins in neonatal units across Northern Ireland, plus a number of other taps which Trusts deem to be at higher risk. A representative sample of these tap assemblies (n=30) and rosettes (n=8) were couriered to HPA Porton Down, Salisbury for investigation. Tap assemblies were dismantled into separate and discrete components (n=494). Each component was assessed for the presence of microbial contamination by enumerating total aerobic colony counts and *Pseudomonas aeruginosa* colony counts using non-selective and selective agars. *P. aeruginosa* isolates recovered from tap components were typed by the variable number tandem repeat (VNTR) technique at HPA Colindale. Selected tap components were also subjected to microscopy to visualise the presence of biofilm using fluorescence and scanning electron microscopy.

There was little correlation (r=0.33) between the aerobic colony count and *P. aeruginosa* presence, indicating that the aerobic colony count could not reliably be used to predict the presence of *P. aeruginosa*.

The highest aerobic colony counts were associated with the integrated mixer and solenoid whilst the highest *P. aeruginosa* counts were recovered from the rosettes and associated components, indicating that *P. aeruginosa* has a preference to colonise different tap location, e.g. the rosette, metal support collar and surrounding tap body.

The analyses of the rosette components and the rosette complexity, rosette type and rosette material indicated that on average a complex rosette (i.e. one with multiple component parts and a higher internal surface area) had a significantly higher expected *P. aeruginosa* count than a simple rosette. Microscopy identified the presence of biofilm on the rosettes and associated components.

Representative isolates recovered from tap assemblies from Belfast (Royal Jubilee Maternity) and Altnagelvin Hospital neonatal units had VNTR profiles that were consistent with the strains that were recovered from the water samples and those that were recovered from the infected patients.

This study has demonstrated a positive association of *P. aeruginosa* with a complex design of rosette in the tap outlet. Further work should determine whether tap outlets used in neonatal units can be redesigned such that complex rosettes are not necessary and manufacturers should investigate the possibility of making the tap outlet removable for decontamination by autoclaving.

Background

Water supplied to plumbing systems is not sterile and the microorganisms in the water phase are capable of forming biofilms. Once microorganisms from the water phase attach to surfaces they will start to form a biofilm. The microorganisms in this biofilm mode of growth are known to be more resistant to disinfectants than free floating water borne microorganisms and hence are more difficult to control (6, 8). All plumbing materials can become contaminated with biofilm which will provide a niche for opportunistic pathogens to survive (1, 17). A number of previous studies have demonstrated that opportunistic pathogens including *Legionella pneumophila* (11, 15), *Stenotrophomonas maltophilia (4, 20)* and *Pseudomonas aeruginosa* (2, 3, 7, 9) can be responsible for nosocomial infections and that water may have been the vehicle of transmission. A number of plumbing materials impact on the microbiological quality of the water (10, 12, 16) such as flexible couplings and have specifically been identified as being susceptible to colonisation (5).

A number of *P. aeruginosa* colonisations and infections occurred in neonatal units in Northern Ireland in late 2011 and early 2012 that resulted in four fatal cases.

As part of the investigations into the outbreak in the neonatal units water samples were taken from water outlets and analysed locally in Northern Ireland for the presence of *P. aeruginosa*. Isolated strains of *P. aeruginosa* were typed using VNTR at the HPA reference laboratory in Colindale and this indicated that that there was a commonality between the strains that had infected the babies and those that were present in the water samples removed from those tap outlets.

As part of the ongoing remediation process all tap outlets and associated pipework were removed (e.g. thermostatic mixer valves, copper tubing, flexible hoses, plastic connectors and rosette) from each neonatal unit in Northern Ireland and replaced with new tap outlets.

A representative sample of the tap assemblies and rosettes were forwarded to the HPA laboratories at Porton Down for subsequent microbiological and microscopic analysis to determine the presence of *P. aeruginosa* in biofilms that may have been present on the plumbing materials associated with the tap assembly components.

This report details the findings of the microbiology and microscopy studies of the different types of taps and their various components.

Materials and Methods

All protocols and procedures were carried out according to HPA quality systems (BSI 9001 and ISO 17025).

Transportation and dismantling of taps

1. Tap assembly units were removed from each of the hospital neonatal units and a representative sample of the taps were couriered to the Food Water and Environmental laboratory at HPA, Porton Down, Salisbury (Appendices 1 - 6) where they were received and signed for.
2. Each tap assembly was then received by the Biosafety Group. Sections of the tap assemblies were then carefully and aseptically dismantled into component parts which were each given unique reference numbers, photographed and recorded. This dismantling process was carried out using manual cutting tools to reduce heat build up that may have reduced the viability of the microorganisms present. Duplicate sections of each component were produced, one of which was sent for microscopy analysis and the other for microbiological analysis (as outlined in HPA Standard Laboratory Procedure number: BIU 27/02).

Microbiological Analysis

Samples for microbiological assessment were placed in 10mls Maximum Recovery Diluent (MRD) together with 10 sterile glass beads (3mm). Each component was scraped using a sterile plastic utensil and the suspension was then agitated by vortexing to aid removal from the biofilm and to re-suspend microorganisms present. Each sample was then plated out onto Plate Count Agar (Oxoid, UK) and *Pseudomonas* Agar (PCN, Oxoid, UK) for the enumeration of Aerobic Colony Count and *P. aeruginosa*, respectively (based on HPA Standard Methods F10 and W6).

Microscopy analysis

Fluorescent microscopy was carried out by staining each surface with propidium iodide and rinsing off the excess stain and viewing each component part using a Nikon microscope (Labophot) with epifluorescence and differential interference contrast) using a 50x water immersion lens (18, 19). The surface of each component was visualised and images captured to represent the presence of biofilm (Imagic Imaging Systems, Germany) (as outlined in Standard Laboratory Procedure number: BIU 28/01).

Where tap components were found to be culture positive for *P. aeruginosa* then a selected number of these biofilm samples were also sent for SEM analysis (based on HPA Standard Operating Procedure : EM/009 and EM/017). The biofilm samples were visualised using a Philips XL30 FEG Scanning Electron Microscope.

Strain Typing

Isolates of *P. aeruginosa* from component biofilm samples known to be associated with a neonatal unit where clinical cases had been identified were retained on nutrient agar slopes (up to a maximum of 5 isolates per tap). The tap biofilm isolates were then sent to the Health Protection Agency Laboratory for HealthCare Associated Infection in Colindale for variable-number tandem-repeat (VNTR) typing as outlined in Turton *et al*. (2009) (14).

Statistical Analysis

Statistical analysis was performed using Stata 12.0 (StataCorp, 2011) (13). Colony counts below the detection limit were assigned an arbitrary value, the midpoint between zero and the detection limit, to allow inclusion in the statistical analysis. Components with <19 CFU of *P. aeruginosa* were thus set to 10 colony forming units (CFU) and components with an aerobic colony count <200 CFU were set to 100 CFU. If counts below the detection limit are in fact true zeroes then this approach

will result in understatement of tap attribute effects and is consequently quite conservative.

Tap components were assigned to one of eight categories based upon their location within a tap (see Appendix 7 for image glossary of selected components). These categories were:

1. Connectors
2. Isolation valve
3. Intergrated mixer and solenoid
4. Mixer
5. Rosette
6. Solenoid
7. Tap body
8. Water in sample bag

Subcategories were also created to identify brand of tap; simple roses and complicated roses; plastic roses and metal roses; rosette components; and the type of pipe between the solenoid and tap (copper or flexi-hose).

Total aerobic colony counts and *P. aeruginosa* counts follow an approximately log-normal distribution and were transformed to the log scale prior to modelling to facilitate analysis with linear models. Bacterial colony counts were found to be correlated within taps and hospitals and linear mixed effects models were therefore used to examine the relationship between counts and various tap attributes. In the mixed effects framework tap and hospital were modelled as random effects, and the tap attributes were modelled as fixed effects. A logistic regression model was also used to examine the probability that at least one component within a tap recorded a detectable level of *P. aeruginosa*.

Four sets of models were built in total.

1. A logistic regression model to examine the association between brand of tap and the probability that at least one tap component had detectable *P. aeruginosa.*
2. A mixed effects regression model to examine the association between tap component category and aerobic colony count.
3. Various single variable mixed effects regression models to examine the association between *P. aeruginosa* count and the various tap component categories (e.g. location; simple rose vs. complicated rose etc.).
4. A mixed effects multiple regression to examine the association between *P. aeruginosa* count and both component location and brand of tap (considered jointly in the same model).

Results

In total, 30 tap assemblies were received for analysis. Three of the taps were received with a newly replaced *in situ* rosette as well as the original rosette (separate). Five additional rosettes were also received that had been removed and separated from their tap assemblies - these tap assemblies were not sent to the HPA for analysis. Each tap assembly was broken down into a series of different sections and a total of 494 individual component or swab samples were generated from the taps and rosettes. Out of the 30 taps that were received, 23 (73%) of these were sensor taps (all of a single brand) and 4 of the 8 additional complex rosettes were also from the same brand of sensor taps.

Microbiological Analysis

P. aeruginosa was detected on 14% of components (range of counts from 20 cfu to 2.2 x 10^7 cfu) and from a number of different tap assemblies from different neonatal units including Belfast (RJM/T7, RJM/T8, RJM/T9, RJM/T26, RJM/T27), Ulster (ULS/T1, ULS/T2, ULS/T3, ULS/T7, ULS/T9, ULS/T10, ULS/T15), Altnagelvin (ALT/T3A, ALT/T6), Enniskillen (ERN/T1) and Antrim (ANT/T3).

Tap types, both sensor operated and non-sensor operated, shared common component parts in specific locations. For the purpose of analysis, taps were categorised by location categories: tap body, connectors, isolation valve, integrated mixer and solenoid, mixer, rosette, solenoid and water in tap sample bag (see Appendix 7 for an image glossary of selected components).

To provide a detailed analysis, main groups were also categorised into component parts, for example, the complex rosette component found in sensor taps was further categorised into four sub-components: the centrepiece, collar, filter and washer. Due to the highly varied designs of rosettes examined, each rosette was categorised (image classification key described in Appendix 8), and details on the complexity and material were also factored into analysis (Table 1). Pipework used to connect the tap and solenoid valve was sub-divided into copper pipe or flexi-hose (Table 1).

Characteristic	All component		Components with *P. aeruginosa* CFU above detection limit		
	N	**%**	**N**	**%**	**Median Pseudomonas CFU (IQR)**
Tap component location					
Tap body	126	26	18	26	6,340 (22,580)
Connectors	31	6	0	0	-
Isolation valve	30	6	0	0	-
Integrated Mixer and solenoid	38	8	1	1	60 (0)
Mixer	98	20	2	3	20 (0)
Rosette	97	20	41	60	52,033 (816,820)
Solenoid	54	11	5	7	520 (23,380)
Water in tap sample bag	8	2	1	1	700,000 (0)
Tap Type					
Sensor Tap	395	80	63	90	23,400 (579,220)
Non-Sensor Tap	97	20	7	10	1,440 (15,540)
Rosette components sensor taps only					
Centrepiece	25	31	13	32	180,000 (898,200)
Collar	26	33	12	29	71,970 (1,308,020)
Filter	12	15	8	20	39,431 (307,280)
Washer	17	21	8	20	17,802 (43,300)
Simple rose					
No	69	78	37	88	52,033 (895,600)
Yes	20	22	5	12	60,606 (58,806)
Rose type category*					
A Neoperl complex	70	72	37	90	52,033 (895,600)
B Neoperl simple	10	10	4	10	31,203 (59,316)
C Armitage Shanks	1	1	0	0	-
D U/K	2	2	0	0	-
E Plastic and metal filter Rose	4	4	0	0	-
F Metal Star Rose	10	10	0	0	-
Metal rose					
No	27	77	13	100	180,000 (898,200)
Yes	8	23	0	0	-
Connection between tap and solenoid					
Copper pipe	22	48	1	13	520 (0)
Flexi-hose	24	52	7	88	16,200 (573,720)

*Appendix 8

Table 1. Summary statistics for tap component attributes indicating number in the sample and median *P. aeruginosa* count for components with counts above detection limit.

***Association between the aerobic colony count and P. aeruginosa* count**

The relationship between the aerobic colony count and the presence of *P. aeruginosa* count (Figure 1) for all 494 components was analysed. The results demonstrated that many of the components that have a high aerobic colony count have no detectable *P. aeruginosa.* The correlation between the two sets of counts is weak (r=0.33).

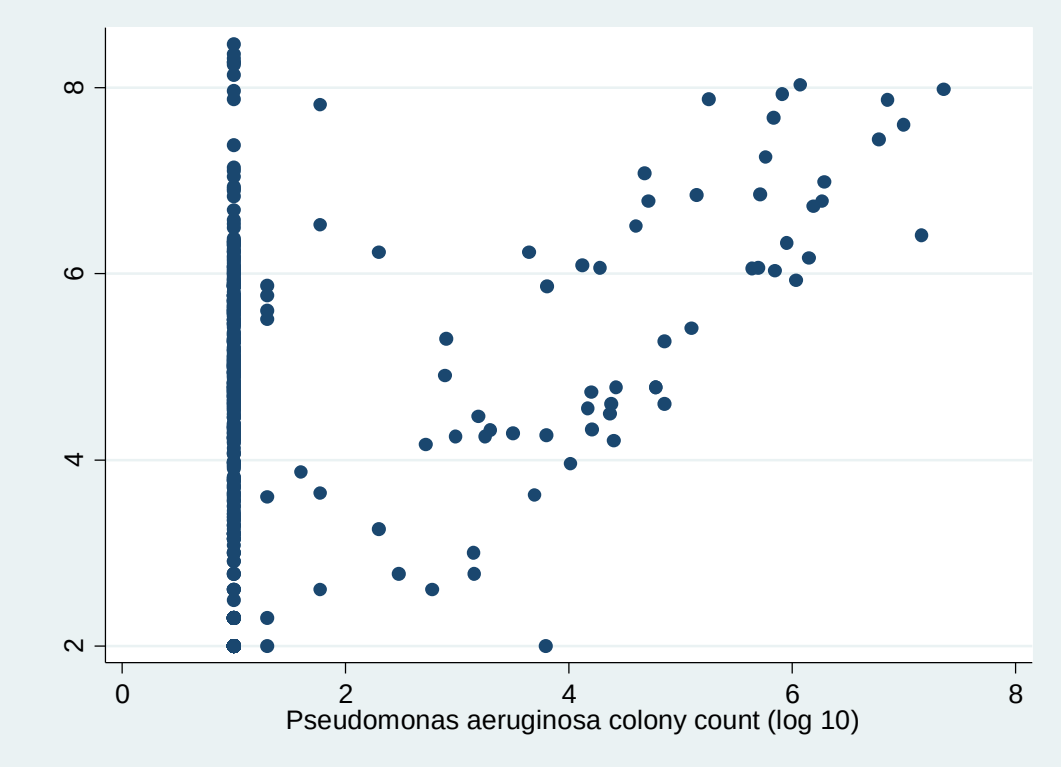


Figure 1. The relationship between Pseudomonas aeruginosa count (CFU per component) and aerobic colony count (CFU per component).

The effect of tap type on the presence of P. aeruginosa

The results indicated that sensor taps had a significantly greater probability (65%) of having at least one component positive for *P. aeruginosa* compared to non-sensor taps (17%) (Table 2). *P. aeruginosa* detection from non-sensor tap components (median count: 1,440 CFU) was less likely and was also associated with a lower median count of *P. aeruginosa* than that from sensor tap components (23,400 CFU) (Table 2). However, the significant differences between sensor and non-sensor taps were found to be attributable to the type of rosettes fitted (Table 3 and 4) with only the sensor taps having been fitted with complex rosettes.

Characteristic	Odds ratio compared to referent (95% CI)	*p* value	Probability of *P. aeruginosa* above detection limit (95% CI)
Tap Type			
Sensor tap	Referent		0.65 (0.44-0.82)
Non sensor tap	0.11 (0.02-0.61)	0.012	0.17 (0.04-0.48)

Table 2. Probability that different tap types will be colonised (above detection limits) with *P. aeruginosa* (n=35).

The effect of component type on P. aeruginosa counts

When the interactions of both tap type and specific components are considered, the rosette component of sensor operated taps is significantly associated with *P. aeruginosa* colonisation (Table 3). The mean *P. aeruginosa* count found in the non-sensor tap rosettes was 2% of the mean count found in the rosettes from sensor taps (95% CI 0.3%.-14.1%, p<0.01). This difference is highly significant and not observed with other components (Table 3).

When comparing *P. aeruginosa* counts for sensor operated taps only, the rosette category is significantly higher than any other components (coefficient of 12.44, 95% CI 6.07-25.50) (Table 3).

Characteristic	Ratio of geometric mean *P. aeruginosa* colony count for category to referent (95% CI)	p value
Tap component location (Sensor taps only)		
Tap body	referent	
Connectors	0.47 (0.18-1.22)	0.121
Isolation valve	0.11 (0.04-0.30)	<0.001
Integrated mixer and solenoid	0.46 (0.18-1.13)	0.091
Mixer	0.15 (0.08-0.31)	<0.001
Rosette	12.44 (6.07-25.50)	<0.001
Solenoid	0.24 (0.11-0.52)	<0.001
Tap bag	0.64 (0.11-3.79)	0.625
Tap Type		
Sensor tap	referent	
Non-sensor tap	0.35 (0.06-2.03)	0.240
Tap Type & location interaction		
Not sensor & tap body	referent	
Not sensor & Isolation valve	8.29 (0.36-192.16)	0.187
Not sensor & Rosette	0.06 (0.01-0.29)	<0.001
Not sensor & Water in sample bag	1.41 (0.05-43.96)	0.844

Table 3. *P. aeruginosa* colony count for specific components in sensor and non-sensor taps

The effect of the rosette component on P. aeruginosa counts

Although rosette components represent 20% of all components, they make up 60% of all components with detectable levels of *P. aeruginosa* (Table 1).

The analyses of the rosette component and the rosette complexity, rosette type and rosette material (Table 4) indicate that, on average, a complex rosette has a higher expected *P. aeruginosa* count than a simple rosette: 1549 CFU and 3 CFU, respectively.

The rosette categories C to F (Table 1) did not have a significant association with *P. aeruginosa* colony counts.

Individual rosette components, e.g. washer, filter, collar and centrepiece from sensor taps, all had similar *P. aeruginosa* counts and were not significantly different from one another (Table 4).

Characteristic	Ratio of geometric mean *P. aeruginosa* colony count for category to referent (95% CI)	p value	Expected *P. aeruginosa* colony count (95% CI) assuming tap and hospital effect=0
Rosette components sensor taps only (n=80)			
Centrepiece	referent		495 (41-6051)
Collar	0.69 (0.12-3.85)	0.668	339 (29-4002)
Filter	0.99 (0.11-9.29)	0.992	489 (27-8795)
Washer	0.51 (0.07-3.65)	0.500	251 (17-3713)
Simple rose (n=89)			
No	referent		1549 (147-16380)
Yes	0.00 (0.00-0.01)	<0.001	3 (0-32)
Rose type category* (n=97)			
A Neoperl complex	referent		588 (47-7316)
B Neoperl simple	0.00 (0.00-0.00)	<0.001	0 (0-9)
C Armitage Shanks	0.02 (0.00-137.99)	0.375	10 (0-56650)
D U/K	0.02 (0.00-20.44)	0.260	10 (0-7570)
E Plastic and Metal Filter Rose	0.02 (0.00-69.51)	0.337	10 (0-27660)
F Metal star rose	0.02 (0.00-4.17)	0.147	10 (0-1331)
Metal rose (n=35)			
No	referent		360 (30-4298)
Yes	0.04 (0.00-3.51)	0.159	15 (0-710)

* Appendix 8

Table 4. *P. aeruginosa* colony count for sensor and non sensor taps and the rosette component

P. aeruginosa colonisation of flexi-hose and copper pipe

The flexi-hose components were associated with approximately 3 times more *P. aeruginosa* than copper pipe (coefficient 2.96, 95% CI 0.91-9.58) but this effect was not statistically significant (p=0.07), perhaps due to the low numbers with detectable counts (Table 5) and the low sample numbers (Table 1).

Characteristic	Ratio of geometric mean *P. aeruginosa* colony count for category to referent (95% CI)	p value	Expected *P. aeruginosa* colony count (95% CI) assuming tap and hospital effect=0
Connection between tap and solenoid (n=46)			
Copper pipe	referent		20 (6-72)
Flexi-hose	2.96 (0.91-9.58)	0.071	60 (16-219)

Table 5. *P. aeruginosa* colony count for different tap to solenoid connectors

Comparison of the effect of component location on the total aerobic and P. aeruginosa colony count

The total aerobic colony counts were significantly higher for the integrated mixer and solenoid than other components and were associated with 71 times the counts of the tap body (coefficient of 71.09, 95% CI 18.77-269) (Table 6).

However, when the same regression model was run with *P. aeruginosa* counts, (Table 7), it was the rosette which was associated with higher *P. aeruginosa* counts than all other components, i.e. up to 7.81 times the *P. aeruginosa* count of the tap body (coefficient of 7.81, 95% CI, 4.11-14.84). Both of these effects are highly statistically significant (p<0.01).

Characteristic	Ratio of geometric mean ACC for category to referent (95% CI)	p value
Tap component location		
Tap body	referent	
Connectors	0.62 (0.15-2.53)	0.504
Isolation valve	0.24 (0.06-0.92)	0.038
Integrated mixer and solenoid	71.09 (18.77-269.25)	<0.001
Mixer	7.00 (2.56-19.12)	<0.001
Rosette	4.01 (1.59-10.16)	0.003
Solenoid	8.96 (2.92-27.52)	<0.001
Tap bag	2.61 (0.26-25.69)	0.411

Table 6. Total aerobic colony count for various tap component locations for both sensor and non-sensor tap.

Characteristic	Ratio of geometric mean *P. aeruginosa* colony count for category to referent (95% CI)	p value	Expected *P. aeruginosa* colony count (95% CI) assuming tap and hospital effect=0
Tap component location (n=482)			
Tap body	referent		25 (7-84)
Connectors	0.40 (0.15-1.03)	0.057	10 (2-41)
Isolation valve	0.10 (0.04-0.26)	<0.001	3 (1-11)
Integrated mixer and solenoid	0.39 (0.16-0.95)	0.039	10 (2-39)
Mixer	0.12 (0.06-0.25)	<0.001	3 (1-11)
Rosette	7.81 (4.11-14.84)	<0.001	194 (57-662)
Solenoid	0.19 (0.09-0.41)	<0.001	5 (1-18)
Tap bag	0.59 (0.13-2.77)	0.506	15 (2-96)

Table 7. *P. aeruginosa* colony count for various tap component locations for both sensor and non-sensor tap.

Predictive values for P. aeruginosa colonisation of sensor and non-sensor tap components

Table 8 provides an illustration of the expected *P. aeruginosa* counts for sensor and non-sensor taps. Sensor taps and non-sensor taps have four components in common. There was no significant difference (95% CI) between the expected counts for the tap body, isolation valve and water from tap sample bag between the sensor and non-sensor taps.

However, the expected *P. aeruginosa* colony count is quite different in the case of the rosette, with the sensor tap having an expected CFU of 596 (95% CI, range 175-2033) whereas the non-sensor tap has an expected CFU of 13 (95% CI, range 3-61). The lower range of the sensor tap, 175 CFU, (95% Cl) is also above the upper range of the 61 CFU for the non-sensor tap (95% Cl).

	Expected CFU Sensor tap (95% CI)	Expected *P. aeruginosa* CFU Non sensor tap (95% CI)
Tap body	48 (14-163)	17 (4-66)
Connectors	22 (5-94)	-
Isolation valve	5 (1-22)	16 (1-396)
Integrated mixer and solenoid	22 (5-89)	-
Mixer	7 (2-26)	-
Rosette	596 (175-2033)	13 (3-61)
Solenoid	11 (3-42)	-
Water from tap sample bag	31 (5-200)	15 (1-364)

Table 8. Expected *P. aeruginosa* colony counts for sensor and non-sensor taps and component location categories

Typing of Pseudomonas aeruginosa strains

P. aeruginosa isolates recovered from plumbing materials from the Royal Jubilee Hospital and Altnagelvin Hospital were sent to the Laboratory of HealthCare Associated Infection for identification using VNTR. Strains from Ulster, Enniskillen and Antrim Hospitals were not forwarded for VNTR typing as no patients were infected.

P. aeruginosa biofilm isolates recovered from taps received from the Royal Jubilee Hospital (RJM/T7, RJM/T8 and RJM/T9) produced VNTR profiles which were consistent with the same strain found in patients and water from this hospital (the 'Belfast' strain; VNTR profile 12,5,1,5,2,2,8,2,9).

Isolates from biofilms of one of the tap received from Altnagelvin Hospital (ALT/T3A) also had a VNTR profile that was consistent with Strain 4 (8,2,5,3,4,2,7,2,10), which was also isolated from Patient 7. Furthermore, the profile obtained from isolates from tap ALT/T6 (8,3,4,5,2,3,5,2,10) was consistent with tap water isolates from the same hospital.

Microscopy analysis

Both fluorescent microscopy and scanning electron transmission microscopy detected the presence of microbial biofilms on a number of different components (Figures 2 - 9). Confluent biofilms were observed on sections of the plastic rosettes (internal, external curvatures, flat horizontal areas, mesh grids and on the prongs) as well as on the internal and external areas of the metallic collars surrounding the rosettes. Biofilms were also observed on the organic flexible hoses but not on the cross linked polypropylene (PEX) surfaces (however, there were very limited numbers of PEX tubing samples).

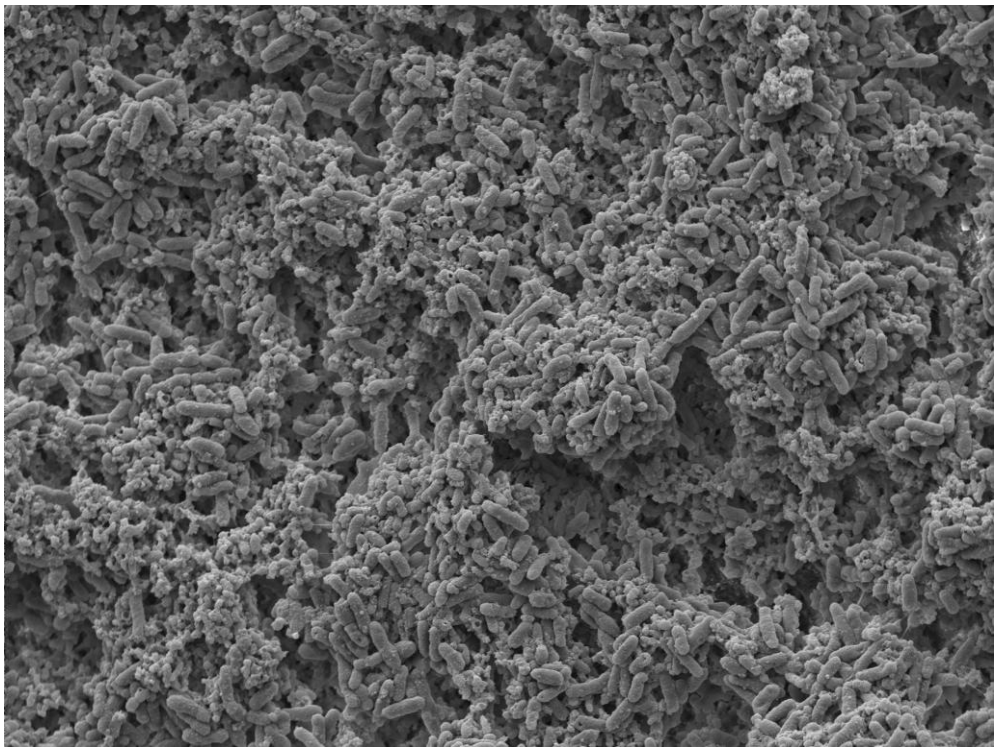


Figure 2. SEM image of biofilm on plastic complex rosette (Sample No: 372284)

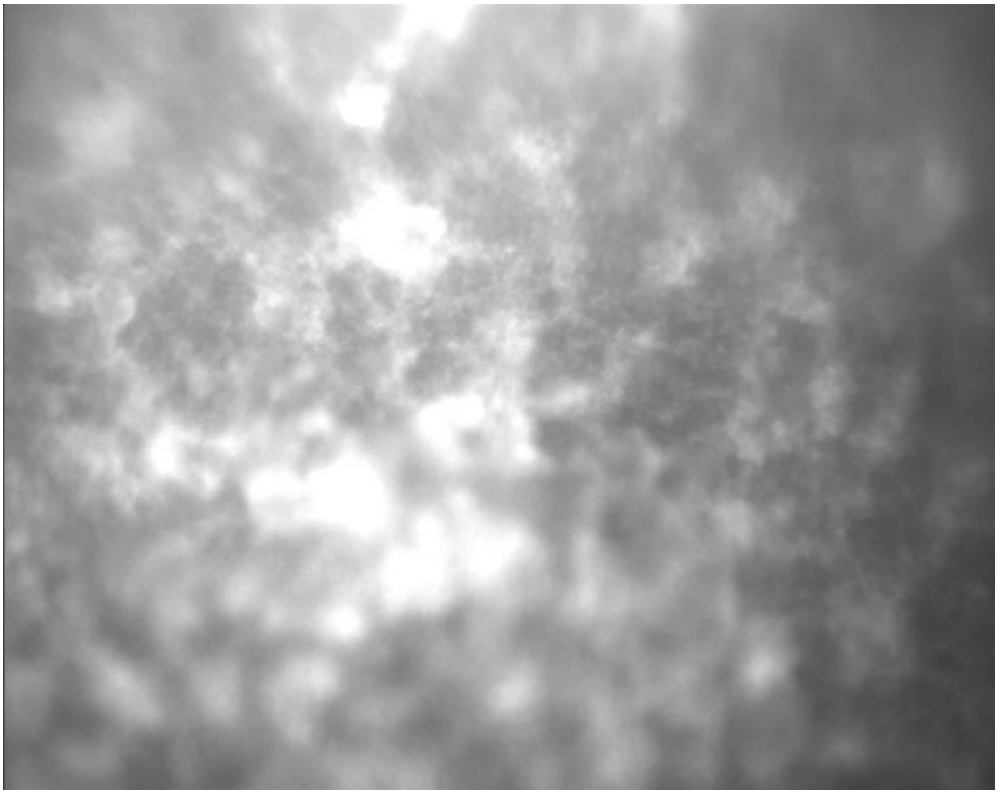


Figure 3. Fluorescent Light Microscopy image of Biofilm on plastic complex rosette (Sample No: 372395)

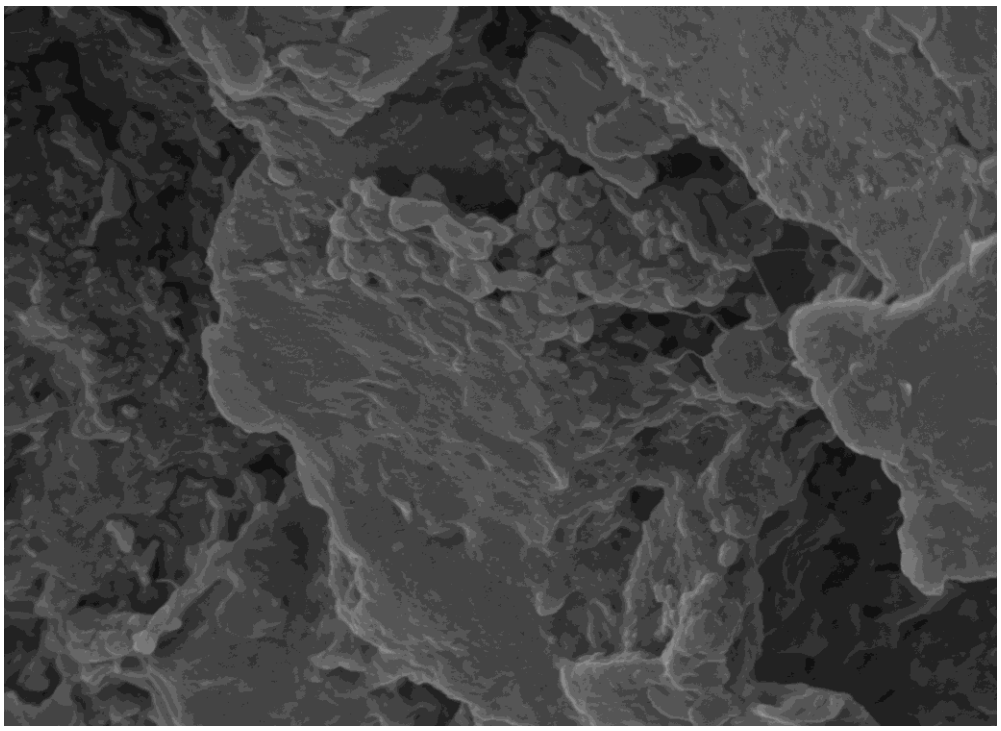


Figure 4. SEM image of biofilm on wire mesh washer (Sample No: 372367)

Figure 5. Fluorescent Light Microscopy image of biofilm on wire mesh washer (Sample No: 372307)

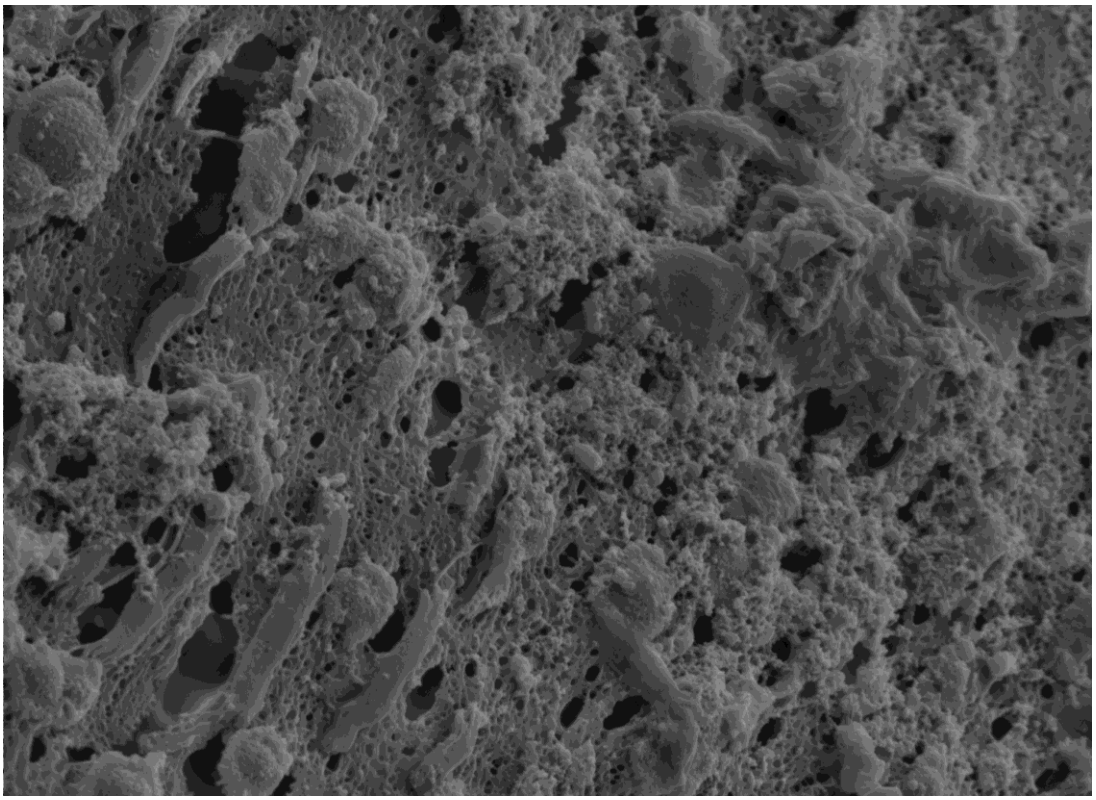


Figure 6. SEM image of biofilm on plastic rosette filter (Sample No: 372383)

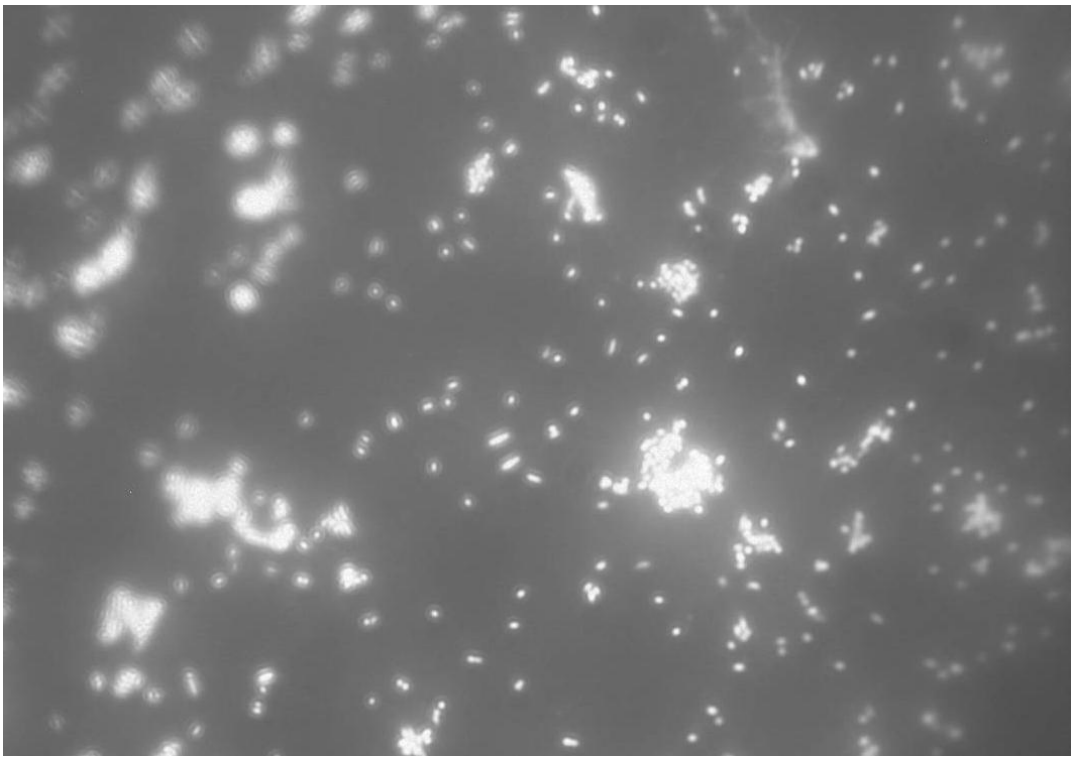


Figure 7. Fluorescent Light Microscopy image of biofilm on plastic rosette filter (Sample No: 375089)

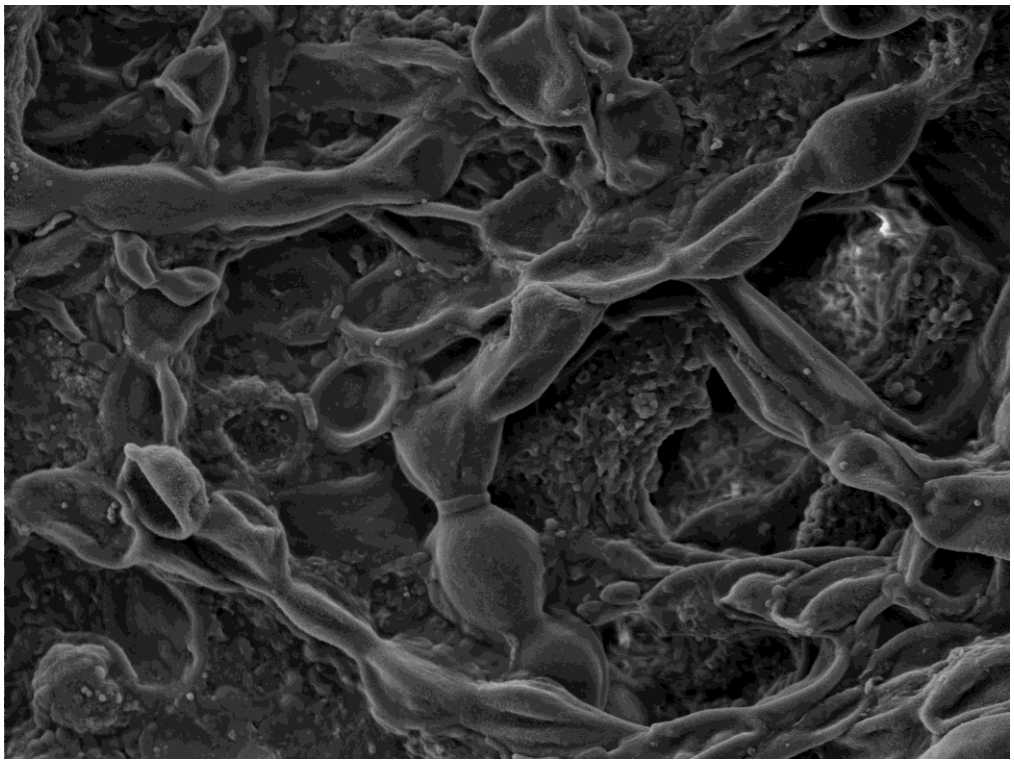


Figure 8. SEM image of biofilm on metal rosette collar (Sample No: 372381)

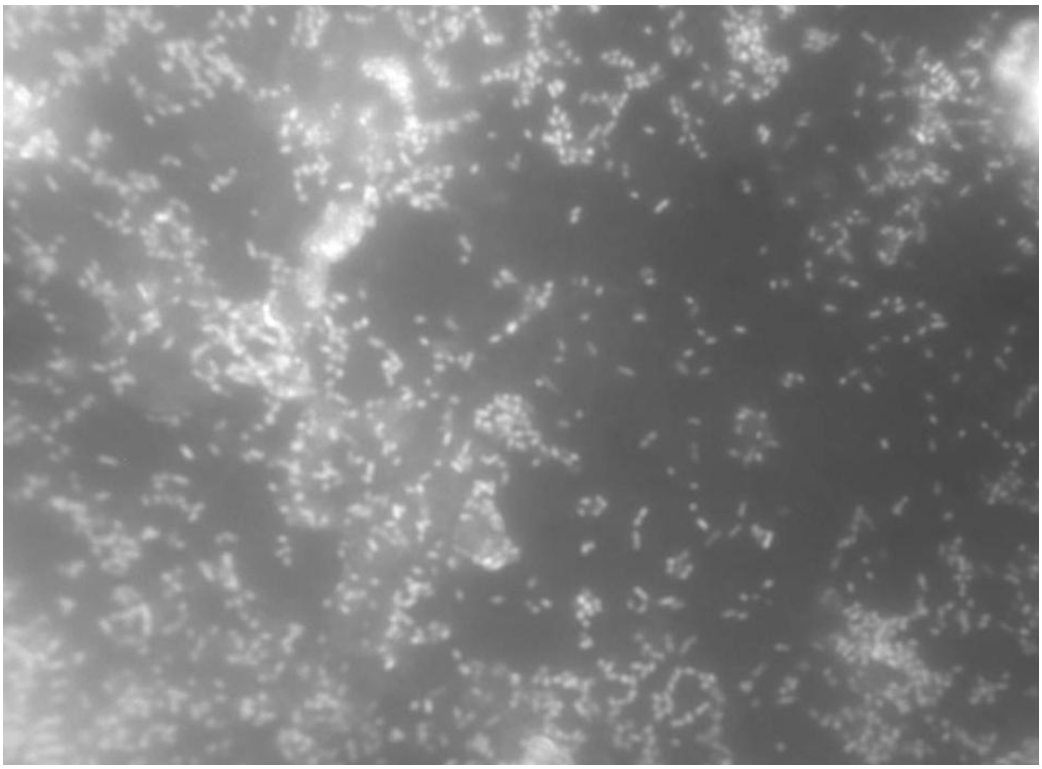


Figure 9. Fluorescent Light Microscopy image of biofilm on metal rosette collar (Sample No: 372388)

Discussion

Following an outbreak of *P. aeruginosa* in neonatal units in Northern Ireland, this study set out to assess presence of *P. aeruginosa* and overall total aerobic bacterial colony counts from a range of tap assemblies that had been removed from these units.

A number of the tap assemblies (n=30) and rosettes (n=8) were removed and stored at room temperature in Northern Ireland prior to being couriered to HPA Porton. Taps from the neonatal unit in Belfast had been exposed to a deep clean using vaporised hydrogen peroxide prior to removal and replacement (verbal communication from George McCracken). Whilst this may have reduced counts on the outside collar of the rosette, the recovery of *P. aeruginosa* demonstrates that biofilms are not only persistent but also resistant to such deep cleaning processes and raises issues about the efficacy of such processes.

The study set out to assess the microbiological content of various tap components n=494), visualise the presence of biofilm, and type *P. aeruginosa* isolates recovered to determine if they were the same as those isolated from the water samples and clinical samples.

Microbiological analysis

Tap assemblies from Belfast, Ulster, Altnagelvin, Enniskillen and Antrim neonatal units were found to be positive for *P. aeruginosa*. Overall, 14% of all components were positive for *P. aeruginosa*. Some of the components were heavily colonised with *P. aeruginosa*, with up to 2.2 x 10^7 cfu per sample being recovered.

Aerobic colony counts

Aerobic micro-organisms were recovered from 78% of components analysed (range 200 – 2.9 x 10^8 CFU per sample). It is often recommended that the aerobic colony count is used as an indicator of the microbiological status in a particular water

system over time. However, it is clear from the results that there was little correlation between the aerobic colony count and the colony count of *P. aeruginosa*. Hence, from this study, it is recommended that the aerobic colony count cannot reliably be used to predict the presence or quantity of *P. aeruginosa.*

The integrated mixer and solenoid had on average the highest total aerobic bacterial colony count. However, the rosette and its components had on average the highest *P. aeruginosa* counts. These effects are highly significant, indicating that *P. aeruginosa* is predominantly located in different parts of the tap in comparison to other aerobic microorganisms.

Sensor versus non-sensor taps

Sensor taps are commonly used in healthcare facilities to allow the tap to be used without touching any of the components, thus reducing the risk of hand contact transmission of healthcare associated infections (HCAI).

In this study, sensor taps (all of a single brand) accounted for 79% of the tap assemblies that were received for analysis and the results indicated that these sensor taps had a greater probability for being colonised with *P. aeruginosa* and also had a higher *P. aeruginosa* count than the non-sensor taps analysed.

Presence of P. aeruginosa on individual tap components

The rosette and its associated components were significantly colonised ($P<0.05$) with *P. aeruginosa* in comparison to other components. Whilst rosette components represented 20% of all components, they represented 60% of all components with detectable levels of *P. aeruginosa.* Different types of rosettes were analysed including plastic and metal rosettes as well as simple and complicated designs (Appendix 8). Analysis indicated that it was the plastic complex rosettes (only found in the sensor tap brand received) that had significantly higher counts of *P. aeruginosa*. These results make it difficult to determine whether it is the design of the rosette or another attribute associated with a sensor tap that causes the increase

in *P. aeruginosa* growth, e.g. water flow rates through the tap. As part of the analysis, each rosette was sectioned into a different number of individual components, each of which was subsequently analysed for the presence of *P. aeruginosa,* and there was no significant difference between the different components including washer, filter, collar and centrepiece from sensor taps.

Where the rosettes were found to be positive, the metal collar and swabs taken from inside the tap body were also found to be positive. This would indicate that just replacing the rosette component would still leave behind a biofilm composed of *P. aeruginosa* that would contaminate the water when the tap is flowing again.

There was no significant difference between the *P. aeruginosa* counts from flexible tubes versus copper pipes. However, the sample size was rather small and further work would be advised.

Typing of P. aeruginosa isolates

Representative isolates recovered from tap assemblies from Belfast (Royal Jubilee Maternity) and Altnagelvin hospital neonatal units were analysed by the VNTR method and found to be consistent with strains recovered from the water samples and from the infected patients (strains 2 and 4). One other strain matched only to the water isolates.

This typing data links the strains recovered from the biofilms on the tap surface, the water from the tap and clinical samples isolated from the patients.

Microscopy

The presence of biofilms was extensively found on a range of rosette and tap components using fluorescence microscopy and SEM. Whilst the SEM often presented the surfaces being covered in thick layer of material in which it was difficult to observe microorganisms, the fluorescent microscopy presented this as a thick biofilm layer in which the microorganisms were clearly enmeshed.

This study indicated that *P. aeruginosa* heavily colonised specific components in a tap. i.e. the complex rosettes.

As the complex rosette and its associated components become contaminated, the biofilm will shed micro-organisms into the water phase. When that tap is operated, the water containing the waterborne *P. aeruginosa* will be flushed out during hand washing.

As the tap outlets are supplied with water from a thermostatic mixer valve (TMV) at approximately 42°C then there will be no thermal control of the microorganisms downstream of the TMV. As such, each time that the tap is operated, the biofilm on the rosette components in the tap body will be replenished with freshly oxygenated water, containing a supply of nutrients that will encourage further biofilm growth on the rosette. The rosette itself will present a large surface area to volume ratio and will also retain water in dead spaces thereby creating a moist stagnant environment (between plastic rosette and metal collar, Appendix 7) in which the biofilm will grow.

The biofilm is intrinsically attached to the rosette components and tap body due to the complex design and retention of water. The more simple rosettes or those that were made of metal were associated with less *P. aeruginosa* growth. Decontamination of the biofilm is inherently difficult. The removal and replacement of the rosette and metal collar will still leave behind a *P. aeruginosa* biofilm on the tap body. Therefore, this may explain why replacing the tap body is one of the ways of controlling the presence of *P. aeruginosa* in water samples from such outlets.

However, as the results have shown, even where the tap body has been replaced there may still be contamination of other components such as the flexible tubes and thermostatic mixer valve. These contaminated components would also lead to biofilm being sloughed off that would result in sporadic *P. aeruginosa* counts in water samples and would, within a few weeks, also result in the contamination of the new tap body and rosette.

Whilst the rosette components were contaminated with the same *P. aeruginosa* isolates that were also recovered from patients, it is not possible to determine from these data whether the taps were the source of patient contamination, or whether an external factor resulted in the contamination of both patients and taps.

The results indicated that it was the more complex rosettes that were heavily contaminated. However, these complex rosettes were only found in sensor taps (all of which were a single brand); therefore, it is not possible to determine from these results whether it is the design of the rosette or another attribute associated with the sensor taps that causes the increase in *P. aeruginosa* growth e.g. flow rates. Rosettes are present in the tap body to produce a straightened flow that is conducive for good hand washing. In an attempt to reduce the potential colonisation of the tap outlet it was recommended that the rosettes be removed from some taps in the neonatal units in Northern Ireland. However, this led to splashing in the area around the taps resulting in health and safety issues with wet floors. Moreover, increased splashing may also lead to a potential contamination risk.

This study indicates that further work is required on the use of rosettes in tap bodies. If simpler rosettes that prevent biofilm build-up cannot be designed and used in sensor taps, then tap bodies without rosettes should be developed.

As the tap outlet and the rosette area have been implicated as the main area in the tap where *P. aeruginosa* accumulates then it would be prudent for manufacturers to redesign the tap outlet to be removable to enable decontamination or sterilisation, preferably using autoclaving.

Conclusions

1. Tap assemblies from Belfast, Ulster, Altnagelvin, Enniskillen and Antrim neonatal units were found to be positive for the presence of *P. aeruginosa.*

2. There was little correlation between the total aerobic bacterial colony count and the presence of *P. aeruginosa;* hence, high aerobic colony counts cannot be used to reliably predict the presence of opportunistic pathogens.

3. When individual tap components were analysed, the rosette and associated components were found to be significantly more colonised with *P. aeruginosa* in sensor taps. However, since no other components showed this difference between tap types, it is likely to be the design of the rosette component, rather than the tap type, that encourages *P. aeruginosa* growth.

4. Typing of strains from tap biofilms from Belfast (Royal Jubilee Maternity) and Altnagelvin indicated that isolates were the same strains as those recovered from the water samples in those neonatal units and from the clinical patient isolates. However, it cannot be determined from these data whether the tap was the source of the patient colonisation, or whether external contamination of the tap resulted in the biofilm growth.

5. Further research is required into improved tap designs and improved methods of tap decontamination.

Appendix

Appendix 1: Contents of carton from the Royal Jubilee Hospital, Belfast

Contents of Carton 1 from the Royal Jubilee Hospital, Belfast

CARTON	PHA Reference for the Tap	Sub Assembly	Description
Carton 1	RJM/T6/1	RJM/T6/1/A	Tap Assembly
		RJM/T6/1/B	Solenoid and Blender Valve
		RJM/T6/1/C	Right Angle Bend Connector & Straight Connector – Short Section
		RJM/T6/1/D	Straight Connector – Long Section
	RJM/T7/1	RJM/T7/1/A	Tap Assembly
		RJM/T7/1/B	Solenoid and Blender Valve
		RJM/T7/1/C	Right Angle Bend Connector
		RJM/T7/1/D	Straight Connector – Short Section
		RJM/T7/1/E	Straight Connector – Long Section
	RJM/T8/1	RJM/T8/1/A	Tap Assembly
		RJM/T8/1/B	Solenoid and Blender Valve
		RJM/T8/1/C	Right Angle Bend Connector & Straight Connector – Short Section
		RJM/T8/1/D	Straight Connector – Long Section
	RJM/T9/1	RJM/T9/1/A	Tap Assembly
		RJM/T9/1/B	Solenoid
		RJM/T9/1/C	Blender Valve
		RJM/T9/1/D	Right Angle Bend Connector
		RJM/T9/1/E	Straight Connector – Short Section
		RJM/T9/1/F	Straight Connector – Long Section
	RJM/T10/1	RJM/T10/1/A	Tap Assembly
		RJM/T10/1/B	Solenoid and Blender Valve
		RJM/T10/1/C	Right Angle Bend Connector
		RJM/T10/1/D	Straight Connector – Short Section
		RJM/T10/1/E	Straight Connector – Long Section
	RJM/T11/1	RJM/T11/1/A	Tap Assembly
		RJM/T11/1/B	Solenoid and Blender Valve
		RJM/T11/1/C	Right Angle Bend Connector & Straight Connector – Short Section
		RJM/T11/1/D	Straight Connector – Long Section
	RJM/T10/1	RJM/T10/1	Filter for Tap RJMT10/1/A

Royal Jubilee Hospital, Belfast

On the 3rd of February, 6 complete sensor tap assemblies were received by HPA Porton. Tap assemblies were identified as Dart Valley model sensor taps (Figure 1.1). These taps contained rosette type A roses (Appendix 9).

Taps were supplied with associated solenoid, thermal mixer (labelled blender valve on the contents of carton sheet) and copper pipework. The solenoid and simple thermal mixer were integrated into one component piece (Figure 1.2 and 1.3).

These tap assemblies were broken down into approximately 20 individual components and analysed (as outlined in BIU 27/02).

On the 24th of February a further 3 complete sensor taps were received by HPA Porton (Appendix 7) and analysis was refined to the tap body and flow straightener.

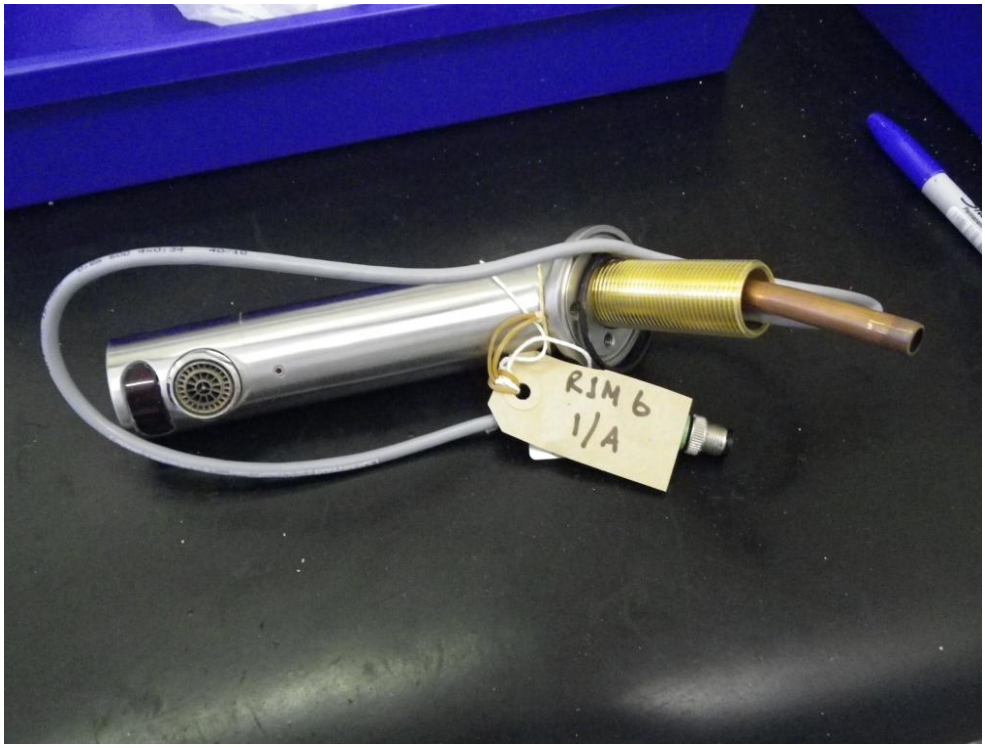


Figure 1.1. Tap assembly with plastic flow straightener in the tap spout nozzle and copper piping from The Royal Jubilee Hospital, Belfast.

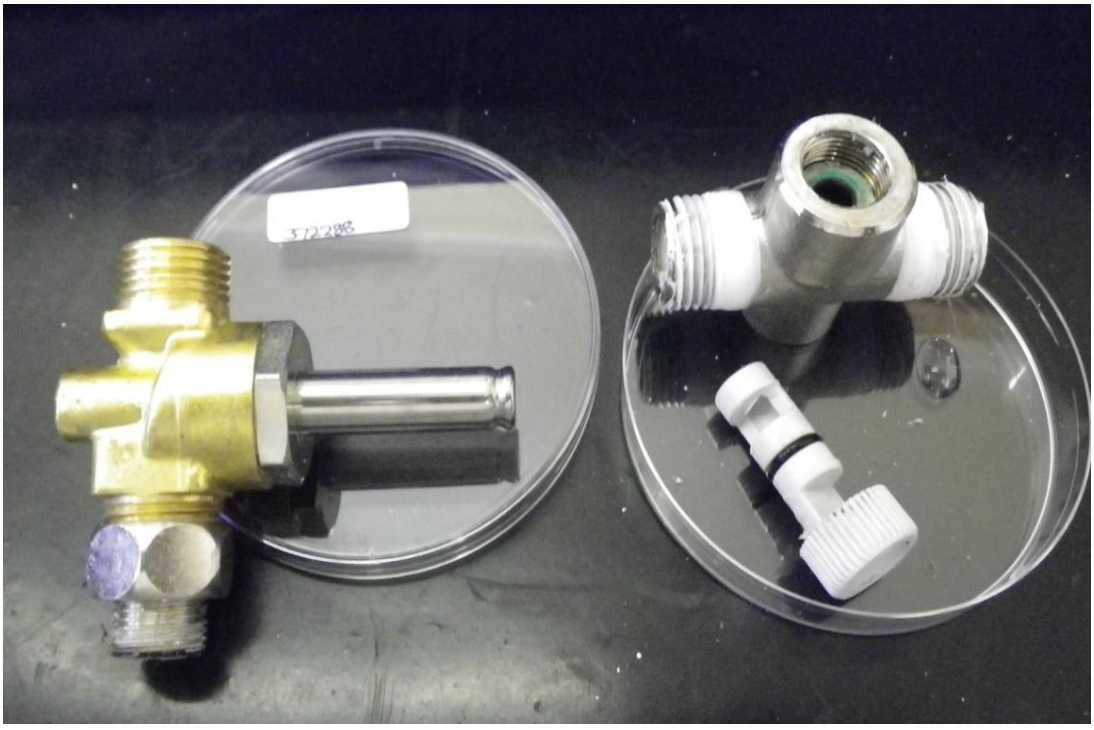


Figure 1.2 and 1.3. Integrated solenoid and blending valve from The Royal Jubilee Hospital, Belfast.

Appendix 2: Contents of cartons received from Ulster Hospital

Contents of Carton 2 from the Ulster Hospital, Dundonald

CARTON	PHA Reference for the Tap	Trust Ref – Found on inner bags	Description
2	ULS/T1	03/02/132 Basin 7	Tap assembly – TMV, Solenoid, Flexible connector, Tap and Rose
	ULS/T2	03/02/133 Basin 8	Tap assembly – TMV, Solenoid, Flexible connector, Tap and Rose
	ULS/T3	03/02/134 Basin 9	Tap assembly – TMV, Solenoid, Flexible connector, Tap and Rose
	ULS/T10	03/02/154 Basin 15	Tap assembly – TMV, Solenoid, Flexible connector, Tap and Rose
	The following tap roses were replaced 27/01/2012 packaged separately		
	ULS/T1	03/02/132 Basin 7	Original Rose
	ULS/T2	03/02/133 Basin 8	Original Rose
	ULS/T3	03/02/134 Basin 9	Original Rose

Contents of Carton 3 from the Ulster Hospital, Dundonald

CARTON	PHA Reference for the Tap	Trust Ref – Found on inner bags	Description
3	ULS/T13	03/02/129 Basin 4	Tap assembly – TMV, Solenoid, Flexible connector, Tap and Rose
	ULS/T15	03/02/130 Basin 5	Tap assembly – TMV, Solenoid, Flexible connector, Tap and Rose
	ULS/T14	03/02/131 Basin 6	Tap assembly – TMV, Solenoid, Flexible connector, Tap and Rose
	ULS/T7	03/02/150 Basin 13	Tap assembly – TMV, Solenoid, Flexible connector, Tap and Rose
	The following tap roses were replaced 27/01/2012 packaged separately		
	ULS/T7	03/02/150 Basin 13	Original Rose
	ULS/T9	03/02/152 Basin 14	Original Rose

Ulster Hospital, Dundonald

On the 9th of February, 8 complete tap assemblies with the original rosettes (Basins 7, 8, 9, 15, 4, 5, 6 and 13) and 4 additional rosettes from Basins 13, 14, 8, 9 were received by HPA Porton. These taps contained rosette type A and B roses (Appendix 9).

Tap assemblies were identified as the same Dart Valley model sensor taps as those used by Belfast (Figure 2.1).

Unlike The Royal Jubilee Hospital, however, the associated solenoid and thermal mixer valve were not integrated and were of a different manufacture. This thermal mixer contained isolation valves on each side of a large central mixing chamber (Figure 2.2).

Ethylene Propylene Diene Monomer flexi-hoses were used to connect the tap unit to the solenoid unit instead of copper pipe (Figure 2.3). Two makes of EPDM inner hose were used, black and clear.

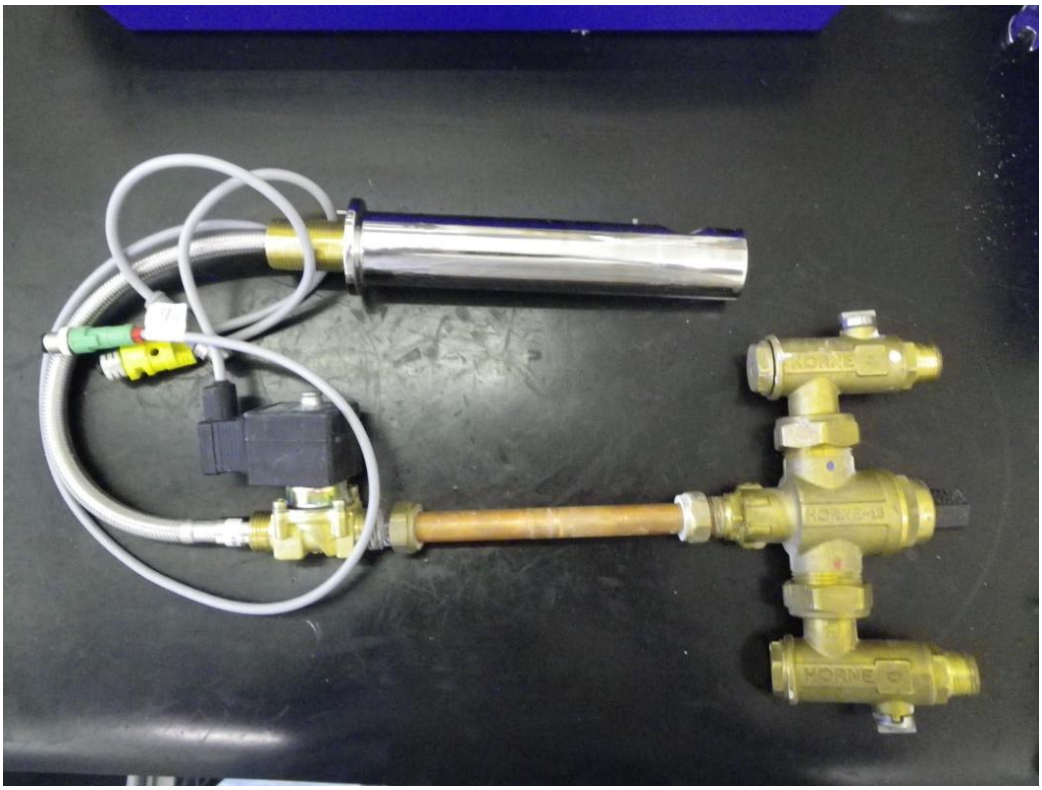


Figure 2.1. Tap assembly and components with flexible hoses from Ulster Hospital, Dundonald.

Figure 2.2. Solenoid and thermal mixing valve from Ulster Hospital, Dundonald.

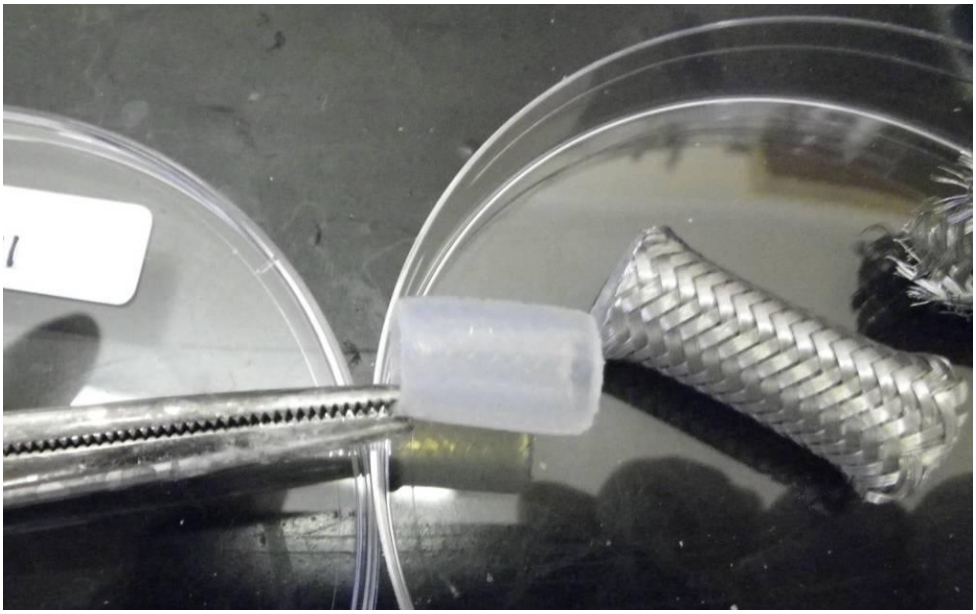


Figure 2.3. Flexi tubing connection tap to solenoid from Ulster Hospital, Dundonald

Appendix 3: Contents of carton received from Altnagelvin Hospital, Londonderry

Contents of Carton 4 from the Altnagelvin Hospital, Londonderry

CARTON	PHA Reference for the Tap	Type	Description
4	ALT/T3	Dart Valley WM - sensor	Tap Head & diffuser, TMV, Solenoid and flexible connector
	ALT/T6	Dart Valley WM - sensor	Tap Head & diffuser, TMV, Solenoid and flexible connector
	ALT/T10	Dart Valley WM - sensor	Tap Head & diffuser, TMV, Solenoid and flexible connector
	ALT/T12	Dart Valley WM - sensor	Tap Head & diffuser, TMV, Solenoid and flexible connector

Altnagelvin Hospital, Londonderry

On the 24th of February four sensor tap assemblies (manufactured by Dart Valley) were received by HPA Porton (Figure 3.1). These taps contained rosette type A roses (Appendix 9).

Like the tap assemblies received from Ulster Hospital, these taps also used black EPDM flexi-hoses to connect the tap unit to the solenoid unit instead.

Microbiological and microscopy analysis was refined to the tap head, flow straightener and flexi tubing.

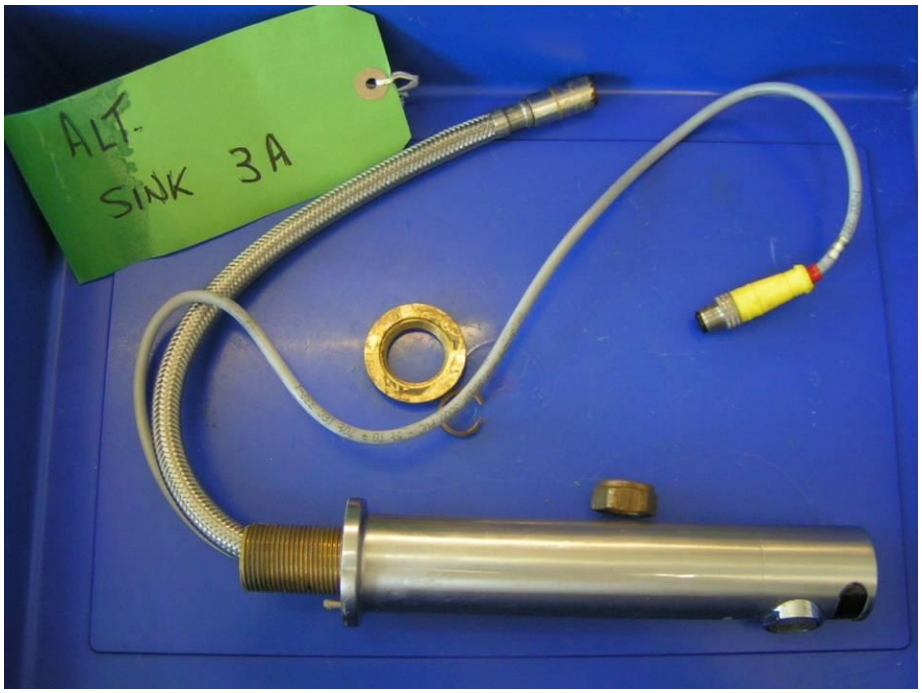


Figure 3.1. Tap from Altnagelvin Hospital, Londonderry

Appendix 4: Contents of cartons received from Antrim Area Hospital, Antrim

Contents of Carton 5 from the Antrim Area Hospital, Antrim

CARTON	PHA Reference for the Tap	Type	Description
5	ANT/T3	U/K	Mixer Assembly - twin lever
	ANT/T4	Armitage Shanks	Mixer Assembly - twin lever
	ANT/T9	U/K	Mixer Assembly - single lever with two flexible pipes attached

Contents of Carton 6 from the Antrim Area Hospital, Antrim & Daisy Hill Hospital, Newry

CARTON	PHA Reference for the Tap	Type	Description
6	ANT/T5	U/K	Mixer Assembly - twin lever
	ANT/T6	U/K	Mixer Assembly - twin lever
	DHH/T8	Dart Valley - Sensor	Tap Head & Solenoid

Antrim Area Hospital, Antrim

On the 24th of February, 2012, five tap assemblies were received by HPA Porton. Four of these were manufactured by U/K and one of these was manufactured by Armitage Shanks.

Of the taps manufactured by U/K one was a monoblock design and contained flexi tubing (Figure 4.1) and type E rose (Appendix 8).

The other three U/K manufactured taps followed a traditional swan neck and lever design (Figure 4.2). These taps contained rosette type D roses (Appendix 8).

The one tap manufactured by Armitage shanks was a monoblock design (Figure 4.3 and contained rosette type C rose (Appendix 8).

Figure 4.1. Taps manufactured by U/K from Antrim Area Hospital, Antrim

Figures 4.2 & 4.3. Taps manufactured by Armitage Shanks from Antrim Area Hospital, Antrim

Daisy Hill Hospital, Newry

On the 24th of February, 2012, one tap assembly was received by HPA Porton. The tap assembly was only accompanied by the solenoid unit and did not contain other components such as the rosette centre piece (Figure 4.4).

Figure 4.4. Tap from Daisy Hill Hospital, Newry

Appendix 5: Contents of carton received from RJM, Belfast & Erne Hospital, Enniskillen

Contents of Carton 7 from the RJM, Belfast & Erne Hospital, Enniskillen

CARTON	PHA Reference for the Tap	Type	Description
7	RJM/T26	Dart Valley - Sensor	Tap Head, Blender Valve, Solenoid & associate pipe work
	RJM/T27	Dart Valley - Sensor	Tap Head, Blender Valve, Solenoid & associate pipe work
	RJM/T28	Dart Valley - Sensor	Tap Head, Blender Valve, Solenoid & associate pipe work
	ERN/T1		Mixer Assembly - twin lever

Erne Hospital, Enniskillen

On the 24th of February, 2012, one swan neck and lever style tap assembly (Figure 5.1), five rosettes (Figure 5.2) and one thermal mixer (Figure 5.3) were received by HPA Porton.

The five rosettes were categorised as type F rosettes (Appendix 8)

Figure 5.1. Tap from Erne Hospital, Enniskillen

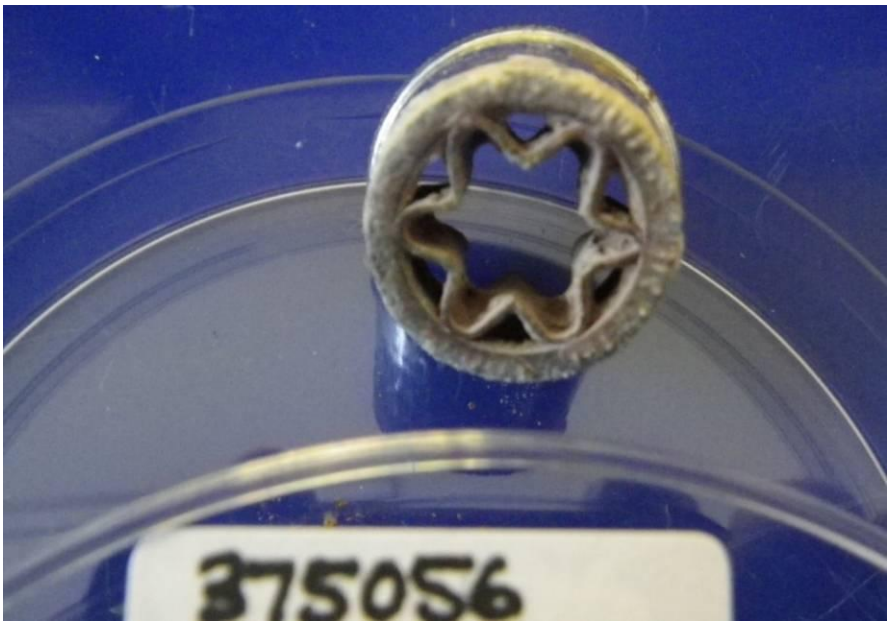


Figure 5.2. Flow straighten piece from Erne Hospital, Enniskillen

Figure 5.3. Thermal mixer from Erne Hospital, Enniskillen

Appendix 6: Contents of carton received from Craigavon Area Hospital, Craigavon

Contents of Carton 8 from the Craigavon

CARTON	PHA Reference for the Tap	Type	Description
8	Basin S Tap 30	U/K	Twin Lever Mixer Tap
	Basin T Tap 32	U/K	Twin Lever Mixer Tap

Craigavon Area Hospital, Craigavon

On the 17th of February two swan neck and lever style tap assemblies were received by HPA Porton (Figure 6.1). The tap unit was broken down into 12 samples to be examined by microbiology and also microscopy.

The tap outlets contained a washer, screw groves and had traces of tape or glue, suggesting the presence of a flow straightener which had not been supplied for analysis (Figure 6.2).

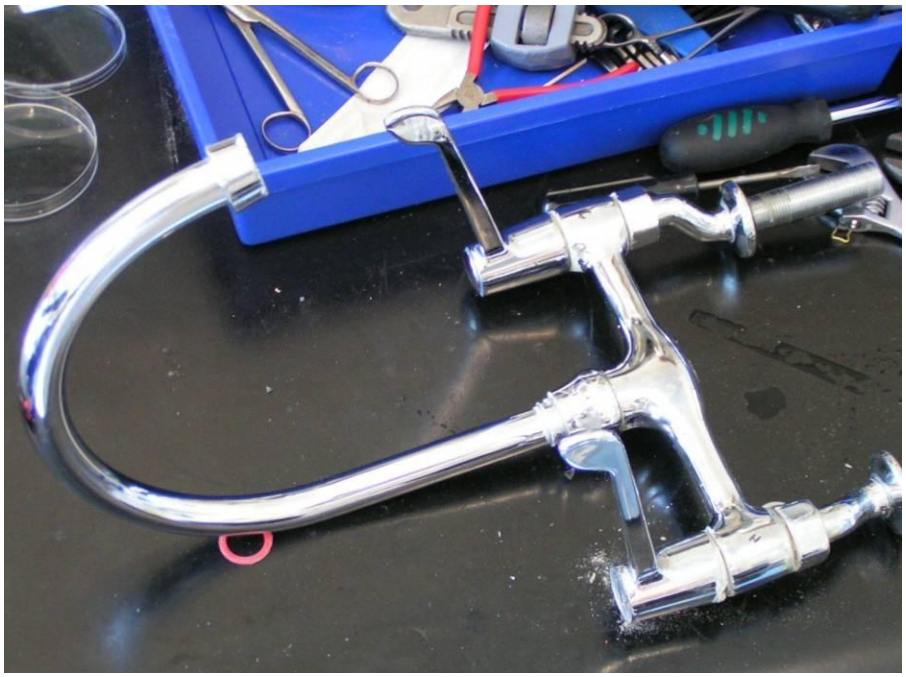


Figure 6.1. Tap from Craigavon Area Hospital, Craigavon

Figure 6.2. Image of tap outlet from Craigavon Area Hospital, Craigavon

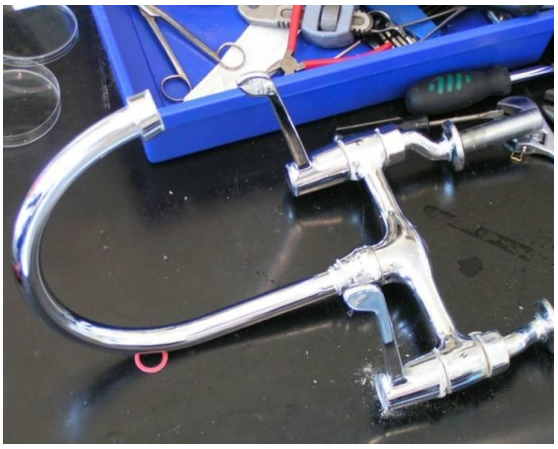
Appendix 7: Glossary of components

Types of Tap

Sensor Tap – These taps use built in infrared sensors to control the solenoid valve, which then releases the water at a standard heat and flow rate.

Sensor

Mono bloc mixer taps – Mono bloc mixer taps work by adjustment of a single lever which dually controls both cold and hot water flow. This then flows into a central mixing chamber before reaching the tap outlet. Two mono bloc mixer taps units were supplied by Northern Ireland

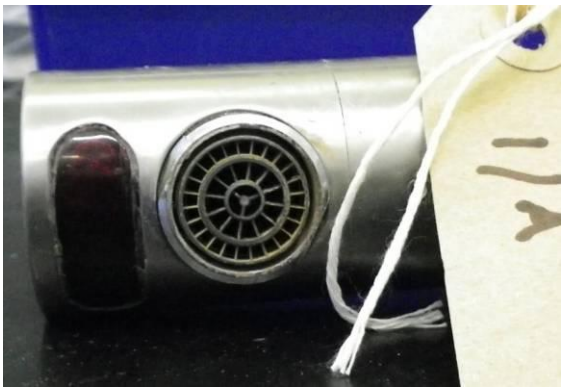


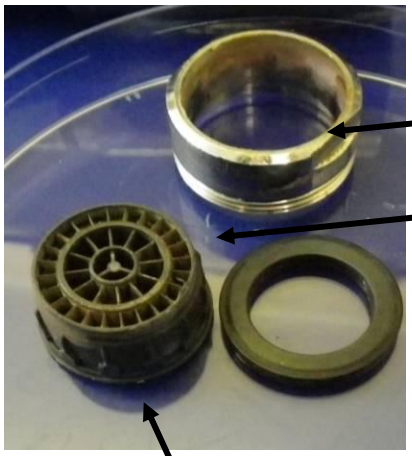
Traditional bridge mixer tap - These taps control flow and temperature through manually adjusting corresponding levers. This action changes the position of internal tap valve mechanisms (which act in place of the solenoid) and the hot and cold water are mixed in the “bridge” or in tap outlet.

There were 6 traditional bridge mixer taps with twin lever design and swan neck style tap units supplied by Northern Ireland.

Rosette components

Rosette / Flow straightener or aerator – A single component which sits at the end of the tap outlet. This is a complex structure comprising of many surfaces, see pictures below.

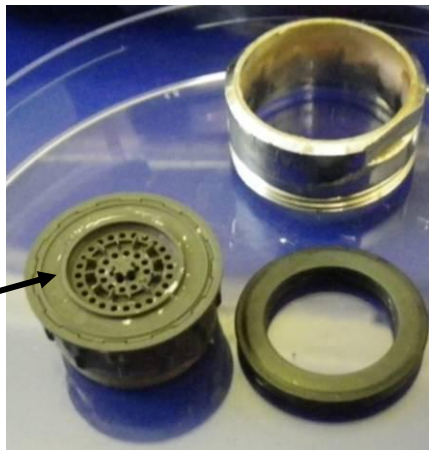




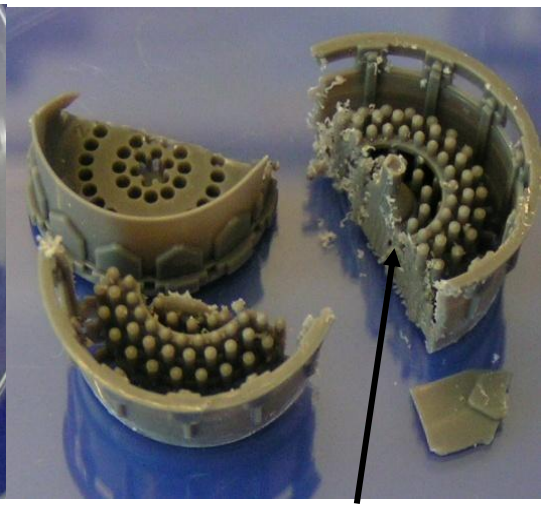
Metal collar

Outward face

Inward face



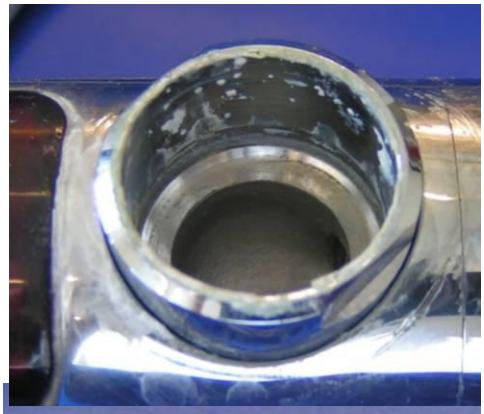
Outer ridged surface



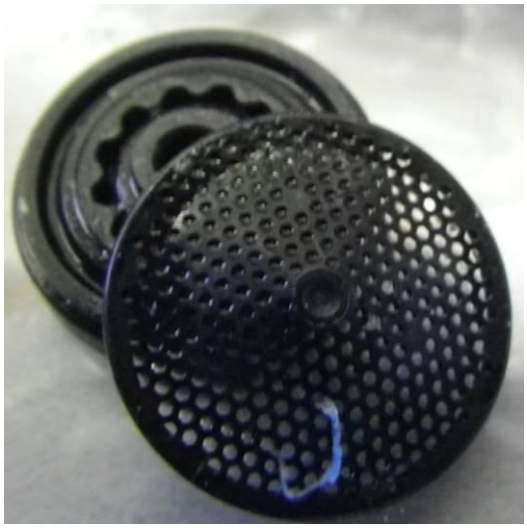
Inner ridged surface

Plastic villi shapes which sit behind outward face

Rosette / Flow straightener metal collar – Screws into tap body and holds the Rosette piece in place



Rosette / Flow straightener washer – This sits on the inward face and is moulded into the rosette component and around the filter piece.



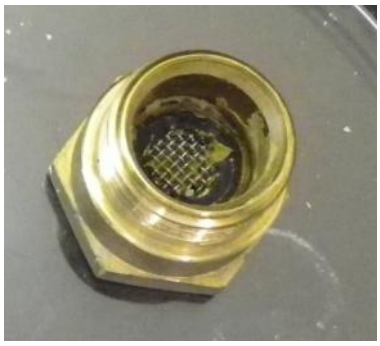
Rosette / Flow straightener filter – This sits on the inward face and is moulded into the rosette component.

Solenoid valve - controls flow rate or starts and stops water flow controlled at a consistent flow rate. These can be automated by infrared sensor technology or manual.

Both thermal mixer units and blender valves were supplied by Northern Ireland and were of varying complexity and design.

Bender valve - Controls the temperature of the water by presetting the temperature by varying input of cold and hot supply feeds.

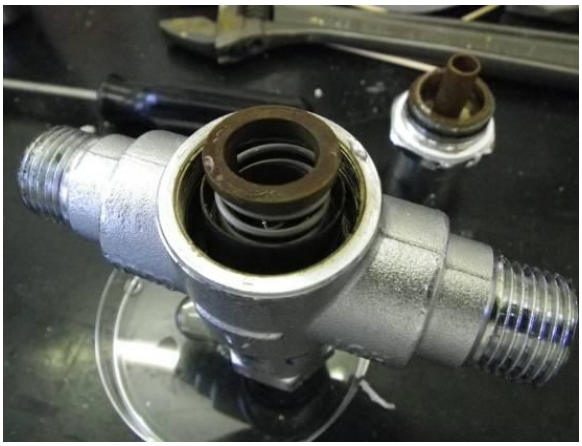
The valves supplied by The Royal Jubilee Hospital, Belfast contained washers with coarse wire mesh inbuilt to cold and hot water pipe connectors to filter large debris before entering mixer. This mixer also had one way valves placed at the hot and cold inlet to prevent backflow. This was a simplistic design and a central plastic temperature control valve controlled the cold and hot water feeds.



Thermostatic mixer valves - The thermal mixers supplied by Ulster Hospital, Dundonald were much larger and more complex. Cold and hot water supplies were fed into chambers lined with a fine wire mesh. These chambers housed individual isolation valves to cut off each feed.

These hot and cold chambers then feed into a central chamber with a complex temperature control valve comprising of plastic and metal components.

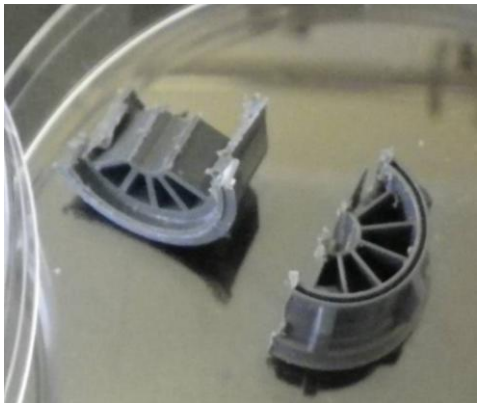
The thermal mixer taken from Erne Hospital, Enniskillen also contained one way valves at the hot and cold inlet to prevent backflow. The temperature control valve was a complex metal component fixed inside the main chamber.



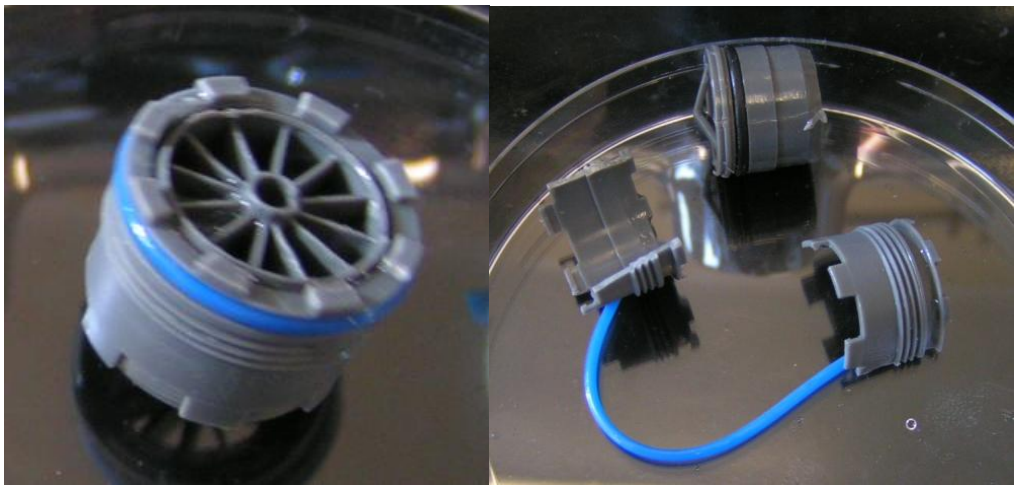
Appendix 8: Glossary of Rosette Types

Neoperl Complex - A

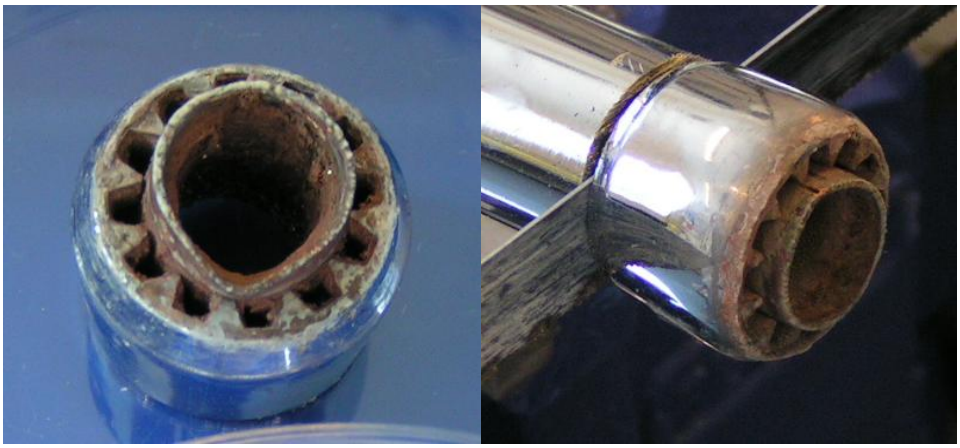
Neoperl Simple - B



Armitage Shanks (ANT - T4) – C

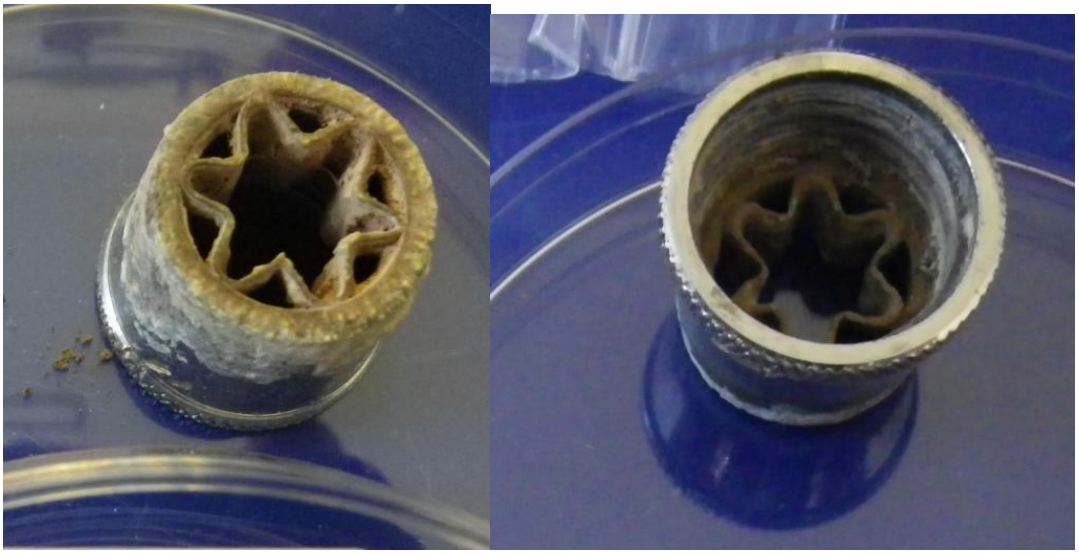


U/K (ANT - T3 / T5 / T6) – D



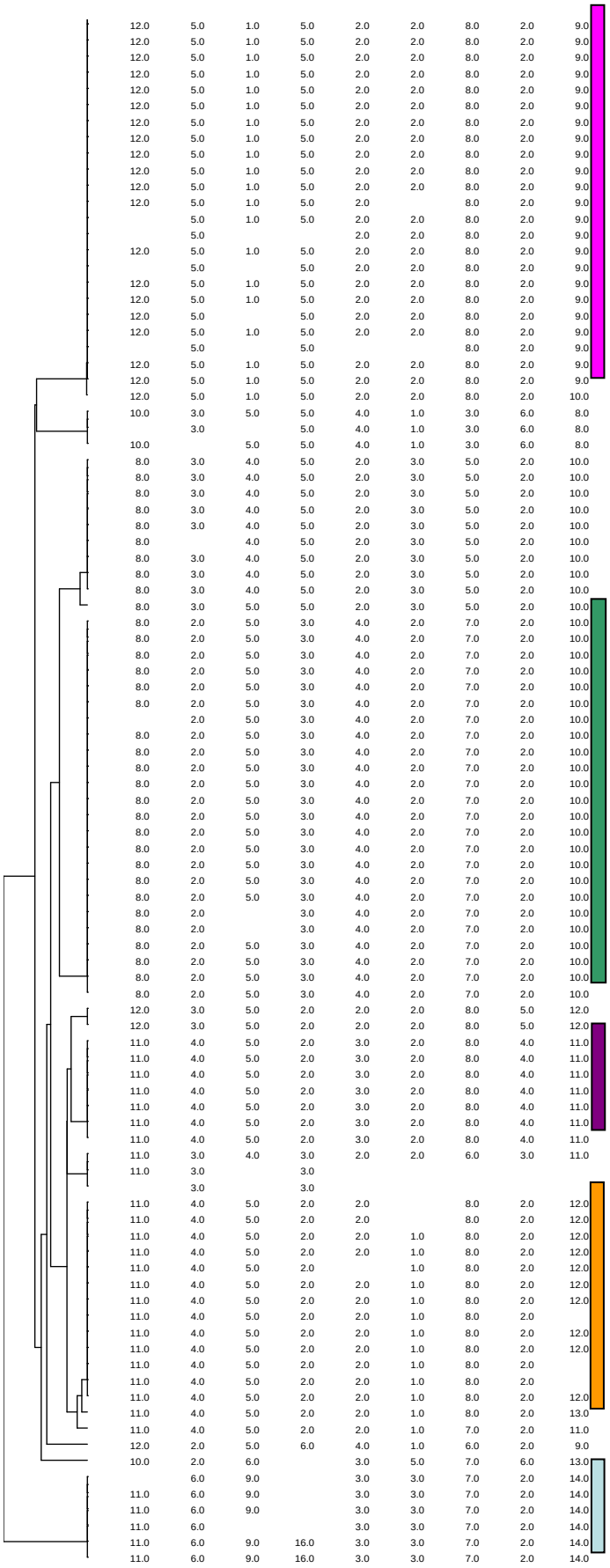
Plastic and Metal Filter Rose (ANT -T9) – E

Metal Star Rose (ERN T1-T5) - F



Appendix 9: Dendogram of isolates from neonatal unit patients with environmental, water and biofilm samples from the tap outlets.

Pseudomonas_VNTR														
172	211	213	214	217	222	207	209	61						
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H115000384	RGRO01	Patient 8	A11011433	NEONATAL ICU	
12.0	5.0		5.0	2.0	2.0	8.0	2.0	9.0	H120240282	RGRO01	Patient 9	A12100437	NEONATAL ICU	
12.0	5.0		5.0	2.0	2.0	8.0	2.0	9.0	H120360347	RGRO01	Patient 11	A12100984	ICU NEONATAL	
12.0	5.0		5.0	2.0	2.0	8.0	2.0	9.0	H120360348	RGRO01	Patient 10	A12101319	ICU NEONATAL	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420198	RGRO01	Patient 13	A12000645	NICU	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420199	RGRO01	Patient 14	A12000664	NICU	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420200	RGRO01	Patient 16	A12602277	NICU	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420201	RGRO01	Patient 15	A12602286	NICU	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420202	RGRO01	Patient 11	A12100984	NICU	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420203	RGRO01		CML-12-00227		
12.0	5.0	1.0	5.0						H120420204	RGRO01		CML-12-00227		
12.0	5.0	1.0	5.0						H120420205	RGRO01		CML-12-00227		
12.0	5.0	1.0	5.0						H120420206	RGRO01		CML-12-00227		
12.0	5.0		5.0						H120420207	RGRO01		CML-12-00227		
12.0	5.0		5.0	2.0	2.0	8.0	2.0	9.0	H120420208	RGRO01		CML-12-00228		
12.0	5.0		5.0						H120420209	RGRO01		CML-12-00228		
12.0	5.0	1.0	5.0						H120420210	RGRO01		CML-12-00228		
12.0	5.0	1.0	5.0						H120420211	RGRO01		CML-12-00228		
12.0	5.0	1.0	5.0						H120420212	RGRO01		CML-12-00228		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420213	RGRO01		CML-12-00230		
12.0	5.0	1.0	5.0						H120420214	RGRO01		CML-12-00230		
12.0	5.0	1.0	5.0						H120420215	RGRO01		CML-12-00230		
12.0	5.0	1.0	5.0						H120420216	RGRO01		CLM-12-00230		
12.0	5.0	1.0	5.0						H120420218	RGRO01		CML-12-00230		
12.0	5.0		5.0	2.0	2.0	8.0	2.0	9.0	H120420220	RGRO01		CML-12-00220		
12.0	5.0	1.0	5.0						H120420222	RGRO01		CML-12-00220		
12.0	5.0	1.0	5.0						H120420224	RGRO01		CML-12-00220		
12.0	5.0	1.0	5.0						H120420226	RGRO01		CML-12-00220		
12.0	5.0	1.0	5.0						H120420228	RGRO01		CML-12-00220		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420243	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120420244	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120420245	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120420246	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120420247	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420248	RGRO01		CML-12-00221		
12.0	5.0	1.0	5.0						H120420249	RGRO01		CML-12-00221		
12.0	5.0	1.0	5.0						H120420250	RGRO01		CML-12-00221		
12.0	5.0	1.0	5.0						H120420251	RGRO01		CML-12-00221		
12.0	5.0	1.0	5.0						H120420252	RGRO01		CML-12-00221		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420253	RGRO01		CML-12-00222		
12.0	5.0		5.0						H120420254	RGRO01		CML-12-00222		
12.0	5.0		5.0						H120420255	RGRO01		CML-12-00222		
12.0	5.0	1.0	5.0						H120420256	RGRO01		CML-12-00222		
12.0	5.0	1.0	5.0						H120420257	RGRO01		CML-12-00222		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420258	RGRO01		CML-12-00232		
12.0	5.0	1.0	5.0						H120420259	RGRO01		CML-12-00232		
12.0	5.0	1.0	5.0						H120420260	RGRO01		CML-12-00232		
12.0	5.0	1.0	5.0						H120420261	RGRO01		CML-12-00232		
12.0	5.0	1.0	5.0						H120420262	RGRO01		CML-12-00232		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420263	RGRO01		CML-12-00241		
12.0	5.0	1.0	5.0						H120420264	RGRO01		CML-12-00241		
12.0	5.0	1.0	5.0						H120420265	RGRO01		CML-12-00241		
12.0	5.0	1.0	5.0						H120420266	RGRO01		CML-12-00241		
12.0	5.0	1.0	5.0						H120420267	RGRO01		CML-12-00241		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420268	RGRO01		CML-12-00244		
12.0	5.0	1.0	5.0						H120420269	RGRO01		CML-12-0244		
12.0	5.0	1.0	5.0						H120420270	RGRO01		CML-12-00244		
12.0	5.0	1.0	5.0						H120420271	RGRO01		CML-12-00244		
12.0	5.0	1.0	5.0						H120420272	RGRO01		CML-12-00244		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420273	RGRO01		CML-12-00242		
12.0	5.0	1.0	5.0						H120420274	RGRO01		CML-12-00242		
12.0	5.0	1.0	5.0						H120420275	RGRO01		CML-12-00242		
12.0	5.0	1.0	5.0						H120420276	RGRO01		CML-12-00242		
12.0	5.0	1.0	5.0						H120420277	RGRO01		CML-12-00242		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120420278	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120420279	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120420280	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120420281	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120420282	RGRO01		CML-12-00243		
12.0	5.0	1.0	5.0						H120440147	RGRO01		CML-12-00292		
12.0	5.0	1.0	5.0						H120440148	RGRO01		CML-12-00292		
12.0	5.0	1.0	5.0						H120440149	RGRO01		CML-12-00292		
	5.0	1.0	5.0						H120440150	RGRO01		CML-12-00292		
12.0	5.0	1.0	5.0						H120440151	RGRO01		CML-12-00292		
12.0	5.0	1.0	5.0						H120440152	RGRO01		CML-12-00294		
12.0	5.0	1.0	5.0						H120440153	RGRO01		CML-12-00294		
12.0	5.0	1.0	5.0						H120440154	RGRO01		CML-12-00294		
12.0	5.0	1.0	5.0						H120440155	RGRO01		CML-12-00294		
12.0	5.0	1.0	5.0						H120440156	RGRO01		CML-12-00294		
12.0	5.0	1.0	5.0						H120440157	RGRO01		CML-12-00295		
12.0	5.0	1.0	5.0						H120440158	RGRO01		CML-12-00295		
12.0	5.0	1.0	5.0						H120440159	RGRO01		CML-12-00295		
12.0	5.0	1.0	5.0						H120440160	RGRO01		CML-12-00295		
12.0	5.0	1.0	5.0						H120440161	RGRO01		CML-12-00295		
12.0	5.0	1.0	5.0						H120440162	RGRO01		CML-12-00296		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120440163	RGRO01		CML-12-00296		
12.0	5.0	1.0	5.0	2.0		8.0	2.0	9.0	H120440164	RGRO01		CML-12-00296		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120440165	RGRO01		CML-12-00296		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120440166	RGRO01		CML-12-00296		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120440167	RGRO01		CML-12-00297		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120440168	RGRO01	Patient 21	6237		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120440169	RGRO01	Patient 21	6237		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120440173	RGRO01	Patient 24	6213		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120440174	RGRO01	Patient 24	6213		
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120480296	RGRO01	Patient 26	A12000535	PICU	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	H120480300	RGRO01	Patient 18	A12000993	NICU	



12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0		H120440173	RGRO01	Patient 24	6213	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0		H120440174	RGRO01	Patient 24	6213	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0		H120480296	RGRO01	Patient 26	A12000535	PICU
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0		H120480300	RGRO01	Patient 18	A12000993	NICU
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0		H120480301	RGRO01	Patient 17	A12000951	NICU
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0		H120480304	RGRO01		0112-3389	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0		H120620655	CRAI10	Patient 27	6243	NNU
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0		H120620656	CRAI10	Patient 27	6243	NNU
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980358	SOUTP1	RJH/5/1/A	372242A	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980359	SOUTP1	RJH/5/1/A	372242B	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980360	SOUTP1	RJH/5/1/A	372242D	
12.0	5.0	1.0	5.0	2.0		8.0	2.0	9.0	●	H120980361	SOUTP1	RJH/5/1/A	372240B	
	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980362	SOUTP1	RJH/5/1/A	372240C	
	5.0			2.0	2.0	8.0	2.0	9.0	●	H120980363	SOUTP1	RJH/6/1/A	372261A	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980364	SOUTP1	RJH/6/1/A	372261B	
	5.0		5.0	2.0	2.0	8.0	2.0	9.0	●	H120980365	SOUTP1	RJH/6/1/A	372263A	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980366	SOUTP1	RJH/6/1/A	372264A	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980367	SOUTP1	RJH/6/1/A	372265C	
12.0	5.0		5.0	2.0	2.0	8.0	2.0	9.0	●	H120980368	SOUTP1	RJH/7/1/A	372283B	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980369	SOUTP1	RJH/7/1/A	372283C	
	5.0		5.0			8.0	2.0	9.0	●	H120980370	SOUTP1	RJH/7/1/A	372286A	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980371	SOUTP1	RJH/7/1/A	372286B	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	9.0	●	H120980372	SOUTP1	RJH/7/1/A	372286D	
12.0	5.0	1.0	5.0	2.0	2.0	8.0	2.0	10.0		H120440762	ANTR00	Patient 19	2001123044	NEONATAL
10.0	3.0	5.0	5.0	4.0	1.0	3.0	6.0	8.0		H120480303	RGRO01		0112-3386	
	3.0		5.0	4.0	1.0	3.0	6.0	8.0		H120520558	ANTR00	Patient 20	2401126167	NICU
10.0		5.0	5.0	4.0	1.0	3.0	6.0	8.0	●	H121140409	SOUTP1	375084-ALT/T3A		
8.0	3.0	4.0	5.0	2.0	3.0	5.0	2.0	10.0		H120580613	ALTN00	TAP	CML-12-00390-3	
8.0	3.0	4.0	5.0	2.0	3.0	5.0	2.0	10.0		H120580623	ALTN00	TAP	CML-12-00406-1	
8.0	3.0	4.0	5.0	2.0	3.0	5.0	2.0	10.0		H120580624	ALTN00	TAP	CML-12-00407-1	
8.0	3.0	4.0	5.0	2.0	3.0	5.0	2.0	10.0		H120580627	ALTN00	TAP	CML-12-00390-4	
8.0	3.0	4.0	5.0	2.0	3.0	5.0	2.0	10.0	●	H121140413	SOUTP1	375065-ALT/T6		
8.0		4.0	5.0	2.0	3.0	5.0	2.0	10.0	●	H121140414	SOUTP1	375066-ALT/T6		
8.0	3.0	4.0	5.0	2.0	3.0	5.0	2.0	10.0	●	H121140415	SOUTP1	375067-ALT/T6		
8.0	3.0	4.0	5.0	2.0	3.0	5.0	2.0	10.0	●	H121140416	SOUTP1	375069-ALT/T6		
8.0	3.0	4.0	5.0	2.0	3.0	5.0	2.0	10.0	●	H121140417	SOUTP1	375070-ALT/T6		
8.0	3.0	5.0	5.0	2.0	3.0	5.0	2.0	10.0		H120480298	RGRO01	Patient PICU2	A12000540	PICU
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120300455	ALTN00	Patient 7	6619 100112	NNICU
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120380419	ALTN00	SINK BESIDE BATH IN SCBU	6044 120112	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120380427	ALTN00	TAP OF SINK BY DOOR OF SCBU	6057 120112	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120380428	ALTN00	BASIN OF SINK BY DOOR OF SCBU	6058 120112	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120520554	ALTN00	SINK,INSIDE VALVE OF TAP	6803 260112	NNICU
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120520555	ALTN00	SINK,"O" RING FROM INSIDE TAP	6804 260112	NNICU
	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120520556	ALTN00	TAP,FRONT OF SCBU	6811 240112 TAP	NNICU
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120520557	ALTN00	SINK,FRONT OF SCBU	6811240112 SINK	NNICU
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120580611	ALTN00	TAP	CML-12-00384-2	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120580612	ALTN00	TAP	CML-12-00385-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120580616	ALTN00	TAP	CML-12-00402-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120580617	ALTN00	TAP	CML-12-00403-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120580618	ALTN00	TAP	CML-12-00398-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120580620	ALTN00	TAP	CML-12-00399-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120580621	ALTN00	TAP	CML-12-00386-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120580622	ALTN00	TAP	CML-12-00387-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120780621	ALTN00		CML-12-01244-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120780622	ALTN00		CML-12-01245-1	
8.0	2.0		3.0	4.0	2.0	7.0	2.0	10.0		H120780623	ALTN00		CML-12-0246-1	
8.0	2.0		3.0	4.0	2.0	7.0	2.0	10.0		H120780624	ALTN00		CML-12-01248-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0		H120780625	ALTN00		CML-12-01249-1	
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0	●	H121140405	SOUTP1	575080-ALT/T3A		
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0	●	H121140406	SOUTP1	375081-ALT/T3A		
8.0	2.0	5.0	3.0	4.0	2.0	7.0	2.0	10.0	●	H121140408	SOUTP1	375083-ALT/T3A		
12.0	3.0	5.0	2.0	2.0	2.0	8.0	5.0	12.0		H120480297	RGRO01	Patient PICU1	A12000538	PICU
12.0	3.0	5.0	2.0	2.0	2.0	8.0	5.0	12.0		H120480299	RGRO01	Patient PICU1	A12000610	PICU
11.0	4.0	5.0	2.0	3.0	2.0	8.0	4.0	11.0		H115000385	RGRO01	Patient 1	A11011463	NEONATAL ICU
11.0	4.0	5.0	2.0	3.0	2.0	8.0	4.0	11.0		H115080324	ALTN00	Patient 1	8008 301111	49
11.0	4.0	5.0	2.0	3.0	2.0	8.0	4.0	11.0		H120300457	ALTN00	Patient 6	6623 100112	NEOC
11.0	4.0	5.0	2.0	3.0	2.0	8.0	4.0	11.0		H120380425	ALTN00	BASIN OF SINK AT FRONT OF ICU	6056 120112	
11.0	4.0	5.0	2.0	3.0	2.0	8.0	4.0	11.0		H120720130	RGRO01	Patient 1	A12050820	NICU
11.0	4.0	5.0	2.0	3.0	2.0	8.0	4.0	11.0		H120720131	RGRO01	Patient 1	A1212948	NICU
11.0	4.0	5.0	2.0	3.0	2.0	8.0	4.0	11.0		H120720132	RGRO01	Patient 1	A12102949	NICU
11.0	3.0	4.0	3.0	2.0	2.0	6.0	3.0	11.0	●	H120980373	SOUTP1	RJH9/1/B	372367A	
11.0	3.0		3.0						●	H120980374	SOUTP1	RJH9/1/B	3722367B	
	3.0		3.0						●	H120980380	SOUTP1	RJH9/1/B	372367C	
11.0	4.0	5.0	2.0	2.0		8.0	2.0	12.0		H115080325	ALTN00	Patient 3	8007 071211	49
11.0	4.0	5.0	2.0	2.0		8.0	2.0	12.0		H115080326	ALTN00	Patient 4	8018 101211	49
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0	12.0		H115100630	ALTN00	Patient 4	6004-12-12-11	49 NNICU
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0	12.0		H115100631	ALTN00	FAUCET IN ICU ROOM	6049131211	49 NNICU
11.0	4.0	5.0	2.0		1.0	8.0	2.0	12.0		H115100632	ALTN00	Patient 3	6038091211	49
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0	12.0		H115100633	ALTN00	Patient 3	6001-10-1211	49
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0	12.0		H115100634	ALTN00	SINK AT BACK OF ICU ROOM	6032-121211	NICU
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0			H120300456	ALTN00	Patient 4	6622 100112	NNICU
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0	12.0		H120380423	ALTN00	TAP OF SINK AT FRONT OF HDU	6049 120112	
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0	12.0		H120580614	ALTN00	TAP	CML-12-00391-1	
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0			H120580625	ALTN00	TAP	CML-12-00390-1	
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0			H120580626	ALTN00	TAP	CML-12-00390-2	
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0	12.0		H120620659	ALTN00	Patient 4	6805	NNICU
11.0	4.0	5.0	2.0	2.0	1.0	8.0	2.0	13.0		H120580628	ALTN00	TAP	CML-12-00391-3	
11.0	4.0	5.0	2.0	2.0	1.0	7.0	2.0	11.0		H120380421	ALTN00	SINK AT BACK OF SCBU	6046 120112	
12.0	2.0	5.0	6.0	4.0	1.0	6.0	2.0	9.0		H121060451	ALTN00		3002020312	ICU
10.0	2.0	6.0		3.0	5.0	7.0	6.0	13.0		H120900129	RGRO01		A12002577	SCBU
	6.0	9.0		3.0	3.0	7.0	2.0	14.0		H120440170	RGRO01	Patient 22	6216	
11.0	6.0	9.0		3.0	3.0	7.0	2.0	14.0		H120440171	RGRO01	Patient 23	6224	
11.0	6.0	9.0		3.0	3.0	7.0	2.0	14.0		H120440172	RGRO01	Patient 23	6224	
11.0	6.0			3.0	3.0	7.0	2.0	14.0		H120520545	RGRO01	Patient 25	3840119685	NICU
11.0	6.0	9.0	16.0	3.0	3.0	7.0	2.0	14.0		H120620657	CRAI10	Patient 23	6215/DARK	NNU
11.0	6.0	9.0	16.0	3.0	3.0	7.0	2.0	14.0		H120620658	CRAI10	Patient 23	6215	NNU

References

1. Anaissie, E. J., S. R. Penzak, and M. C. Dignani. 2002. The hospital water supply as a source of nosocomial infections: a plea for action. Arch Intern Med 162:1483-92.
2. Aumeran, C., C. Paillard, F. Robin, J. Kanold, O. Baud, R. Bonnet, B. Souweine, and O. Traore. 2007. *Pseudomonas aeruginosa* and *Pseudomonas putida* outbreak associated with contaminated water outlets in an oncohaematology paediatric unit. J Hosp Infect 65:47-53.
3. Bert, F., E. Maubec, B. Bruneau, P. Berry, and N. Lambert-Zechovsky. 1998. Multi-resistant *Pseudomonas aeruginosa* outbreak associated with contaminated tap water in a neurosurgery intensive care unit. J Hosp Infect 39:53-62.
4. Cervia, J. S., G. A. Ortolano, and F. P. Canonica. 2008. Hospital tap water as a source of *Stenotrophomonas maltophilia* infection. Clin Infect Dis 46:1485-7.
5. Department of Health. 2010. Estates and Facilities Alert: Equipment: Flexible water Supply Hoses: http://www.hydrop.com/Art/5-5-10%20Flexible%20Hoses%20DH%20(2010)%2003[1].pdf
6. Donlan, R. M., and J. W. Costerton. 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clinical and Microbiological Reviews 15:167-93.
7. Ferroni, A., L. Nguyen, B. Pron, G. Quesne, M. C. Brusset, and P. Berche. 1998. Outbreak of nosocomial urinary tract infections due to *Pseudomonas aeruginosa* in a paediatric surgical unit associated with tap-water contamination. J Hosp Infect 39:301-7.
8. Gilbert, P., and D. Allison. 2000. Biofilms and their resistance towards antimicrobial agents, p. 125-143. *In* H. N. Newman and M. Wislon (ed.), Dental plaque revisited: oral biofilms in health and disease. Bioline, Cardiff.
9. Halabi, M., M. Wiesholzer-Pittl, J. Schoberl, and H. Mittermayer. 2001. Non-touch fittings in hospitals: a possible source of *Pseudomonas aeruginosa* and *Legionella* spp. J Hosp Infect 49:117-21.
10. Lehtola, M. J., I. T. Miettinen, T. Lampola, A. Hirvonen, T. Vartiainen, and P. J. Martikainen. 2005. Pipeline materials modify the effectiveness of disinfectants in drinking water distribution systems. Water Res 39:1962-71.
11. Lepine, L. A., D. B. Jernigan, J. C. Butler, J. M. Pruckler, R. F. Benson, G. Kim, J. L. Hadler, M. L. Cartter, and B. S. Fields. 1998. A recurrent outbreak of nosocomial legionnaires' disease detected by urinary antigen testing: evidence for long-term colonization of a hospital plumbing system. Infect Control Hosp Epidemiol 19:905-10.
12. Moritz, M. M., H. C. Flemming, and J. Wingender. 2010. Integration of Pseudomonas aeruginosa and Legionella pneumophila in drinking water biofilms grown on domestic plumbing materials. Int J Hyg Environ Health.
13. StataCorp. 2011. Stata Statistical Software: Release 12. College Station, T. S. L.
14. Turton, J. F., S. E. Turton, L. Yearwood, S. Yarde, M. E. Kaufmann, and T. L. Pitt. 2010. Evaluation of a nine-locus variable-number tandem-repeat scheme for typing of *Pseudomonas aeruginosa*. Clin Microbiol Infect 16:1111–1116.
15. Visca, P., P. Goldoni, P. C. Luck, J. H. Helbig, L. Cattani, G. Giltri, S. Bramati, and M. Castellani Pastoris. 1999. Multiple types of *Legionella pneumophila* serogroup 6 in a hospital heated-water system associated with sporadic infections. J Clin Microbiol 37:2189-96.
16. Waines, P. L., R. Moate, A. J. Moody, M. Allen, and G. Bradley. 2011. The effect of material choice on biofilm formation in a model warm water distribution system. Biofouling 27:1161-74.
17. Walker, J. T., C. W. Mackerness, J. Rogers, and C. W. Keevil. 1995. Biofilm - A haven for Waterborne pathogens., p. 196-204. *In* H. M. Lappin-Scott and J. W. Costerton (ed.), Microbial Biofilms. Cambridge University Press, London.

18. Walker, J. T., J. Verran, R. D. Boyd, and S. Percival. 2001. Microscopy methods to investigate structure of potable water biofilms. Methods in Enzymology 337:243-55.

19. Walker, J. T., D. Wagner, W. Fischer, and C. W. Keevil. 1994. Rapid detection of biofilm on corroded copper pipes. Biofouling 8:55-63.

20. Weber, D. J., W. A. Rutala, C. N. Blanchet, M. Jordan, and M. F. Gergen. 1999. Faucet aerators: A source of patient colonization with *Stenotrophomonas maltophilia*. Am J Infect Control 27:59-63.