Domestic shower hose biofilms contain fungal species capable of causing opportunistic infection

John Moat, Athanasios Rizoulis, Graeme Fox and Mathew Upton

ABSTRACT

The domestic environment can be a source of pathogenic bacteria. We show here that domestic shower hoses may harbour potentially pathogenic bacteria and fungi. Well-developed biofilms were physically removed from the internal surface of shower hoses collected in four locations in England and Scotland. Amplicon pyrosequencing of 16S and 18S rRNA targets revealed the presence of common aquatic and environmental bacteria, including members of the Actinobacteria, Alphaproteobacteria, Bacteroidetes and non-tuberculous Mycobacteria. These bacteria are associated with infections in immunocompromised hosts and are widely reported in shower systems and as causes of water-acquired infection. More importantly, this study represents the first detailed analysis of fungal populations in shower systems and revealed the presence of sequences related to *Exophiala mesophila*, *Fusarium fujikuroi* and *Malassezia restricta*. These organisms can be associated with the environment and healthy skin, but also with infection in compromised and immuno-competent hosts and occurrence of dandruff. Domestic showering may result in exposure to aerosols of bacteria and fungi that are potentially pathogenic and toxigenic. It may be prudent to limit development of these biofilms by the use of disinfectants, or regular replacement of hoses, where immuno-compromised persons are present.

Key words | *Exophiala*, *Fusarium*, *Malassezia*, shower hose biofilm

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INTRODUCTION

Water supplies in domestic environments have been the focus of studies into transmission of opportunistic infections with environmental organisms, usually in people who have a compromised immune system. It is known that environmental organisms, including *Legionella* can become established in shower hoses (Tobin *et al.* 1980; Bauer *et al.* 2008; Whiley *et al.* 2015), subsequently forming aerosols and being inhaled leading to infection (Pedro-Botet *et al.* 2002). A number of studies, using culture-based and culture-independent methods, have attempted to characterise the organisms present in these aerosols and the surrounding environment, demonstrating the presence of potentially pathogenic biofilm forming Gram-negative bacteria in hospital water systems (Decker & Palmore 2013). Culture-independent methods have been used to demonstrate the presence of potentially pathogenic bacteria on domestic shower curtains (Kelley *et al.* 2004) and showerheads (Feazel *et al.* 2009). Likewise, recent molecular and culture-based work has demonstrated the presence of non-tuberculous Mycobacteria (NTM) in domestic water (Thomson *et al.* 2013a, 2013b) and demonstrated infection acquired from aerosols of this water (Falkinham *et al.* 2008; Thomson *et al.* 2013a, 2013b). O'Brien *et al.* (2000) have suggested that our increased tendency to shower has contributed to a rise in NTM disease.

In contrast to the significant body of knowledge surrounding the bacterial components of domestic water system biofilms, there is currently a relatively limited understanding of the fungal members of these communities and none of the previous studies of shower systems have included detailed analysis of fungal species.

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We have used culture-independent methods to investigate the bacterial and fungal diversity of biofilms recovered from domestic shower hoses, revealing the presence of several genera or species that represent a risk to immuno-compromised hosts.

MATERIALS AND METHODS

Shower hose samples

A total of four hose samples were examined: one hose was recovered from a hotel in Northwest England (NW; latitude 53.414 °N, longitude −2.124 °W) and a further three from domestic settings in Scotland (Fife; 56.135 °N, −3.376 °W) and in the Southeast of England (SE1; 51.167 °N, 1.289 °W & SE2; 51.424 °N, 0.560 °W). The SE1 hose was recovered from a shower that had been installed less than a month before removal of the hose. Other hoses had been in place for an undetermined, but extended time. All samples were obtained with the full consent of the property owners, without restriction.

All wet hoses were filled with tap water at source, their ends being sealed with sterile screw caps, before being transported to Manchester at ambient temperature. On arrival, hoses were stored at 4 °C and were analysed within 48 hours.

Extraction of total DNA from shower hose biofilms

Hoses were surface sterilised with ethanol, aseptically sectioned into 7 cm lengths and the biofilm physically removed using a sterile scraper and recovered into sterile phosphate buffered saline. Biofilm material was recovered from the sections of hose closest to each end of the hose. Samples were mechanically disrupted by vigorous mixing for 2 minutes in the presence of 2 g of sterile coarse sand and 3 mm diameter glass beads. Total DNA was recovered using the MoBio Soil extraction kit (Cambio Ltd, UK) and stored at −20 °C.

Amplicon pyrosequencing for characterization of bacterial and fungal biofilm communities

Polymerase chain reaction (PCR) of the V1-V2 hypervariable region of the bacterial 16S rRNA gene was performed using tagged fusion universal bacterial primers 27F (Lane 1991) and 338R (Hamady *et al.* 2008), synthesised by IDTdna (Integrated DNA Technologies, Belgium). The fusion forward primer (5′ CCATCTCATCCCTGCGTGTCTCCGAC*TCAG*NNNNNNNNNNAGAGTTTGATGMTGGCTCAG 3′) contained the 454 Life Sciences 'Lib-L Primer A', a 4 base 'key' sequence (*TCAG*), a unique ten-base barcode 'MID' sequence for each sample (N), and bacterial primer 27F (underlined). The reverse fusion primer (5′ CCTATCCCCTGTGTGCCTTGGCAGTC*TCAG*TGCTGCCTCCCGTAGGAGT 3′) contained the 454 Life Sciences 'Lib-L Primer B', a 4 base 'key' sequence (*TCAG*), and bacterial primer 338R (underlined). PCR of the fungal 18S rRNA small subunit was carried out using tagged forward fusion primer FU18S1 (5′-GGAAACTCACCAGGTCCAGA-3′) and reverse fusion primer Nu-SSU-1536 (5′-ATTGCAATGCYCTATCCCCA-3′) (Gangneux *et al.* 2011).

The PCR amplification was performed in 50 μl volume reactions using 0.5 μl (2.5 units) FastStart High Fidelity DNA polymerase (Roche Diagnostics GmbH, Mannheim, Germany), 1.8 mM $MgCl_2$, 200 μM of each dNTP, 0.4 μM of each forward and reverse fusion primers. The PCR conditions included an initial denaturing step at 95 °C for 2 min, followed by 35 cycles of 95 °C for 30 sec, 55 °C for 30 sec, 72 °C for 45 sec, and a final elongation step at 72 °C for 5 min.

The 16S and 18S rRNA gene fragments were loaded onto an agarose gel and, following gel electrophoresis, bands of the correct fragment size (approximately 410 bp) were excised, purified using a QIAquick gel extraction kit (Qiagen, GmbH, Hilden, Germany) and eluted in 30 μl of DNAse free H_2O. The purified PCR products were quantified using a Life Technologies Qubit 2.0 fluorimeter and the fragment size confirmed using an Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA, USA). PCR products were normalised and pooled at equimolar concentrations. The emulsion PCR and the pyrosequencing runs were performed at the University of Manchester DNA Sequencing Facility, using a Roche 454 Life Sciences GS Junior system (454 Life Sciences, Branford, CT, USA).

Taxonomical classification of bacterial and fungal reads

The 454 pyrosequencing reads were analysed using QIIME release 1.8.0 (Caporaso *et al.* 2010). De-noising and chimera

removal was performed in QIIME during operational taxonomic unit (OTU) picking (at 97% sequence similarity) with usearch (Edgar 2010). Taxonomic classification of the 16S rRNA bacterial reads was performed in QIIME using the uclust method (Edgar 2010) against the Greengenes database, release 08/2013 (McDonald *et al.* 2012). Taxonomic classification of the 18S rRNA fungal reads was carried out in QIIME using uclust against the Silva release 111 database (Yilmaz *et al.* 2014). In addition, the closest GenBank match for the OTUs (a representative sequence for each OTU was used) that contained the highest number of reads was identified by Blastn nucleotide search (Altschul *et al.* 1990). OTU clustering was carried out on the log-transformed abundances of the identified OTUs, using the Bray Curtis similarity method. Cluster analysis was carried out using the PRIMER v6 software (PRIMER-E, Plymouth, UK) (Clarke 1993).

All raw sequence data relating to this work have been deposited at the Sequence Read Archive under BioProject number PRJNA267447.

RESULTS

Confirmation of predominant constituents of domestic shower biofilms

Following bacterial 16S rRNA gene amplicon pyrosequencing, more than 20,000 reads were obtained from each sample (Table 1). Analysis of these reads indicated the presence of between 62 and 79 OTUs per sample, at the 97% sequence identity level (Table 1). Taxonomic classification showed that all bacterial communities were dominated by Alphaproteobacteria followed by Actinobacteria. Bacteroidetes related sequences were only present in the NW and Fife samples (Figure 1). All communities were dominated (47 to 80% of the total population) by sequences closely related (99–100% identity) to two Alphaproteobacterial species, the rhizobial, N_2 fixing *Bradyrhizobium japonicum* and the photosynthetic *Blastomonas natatoria* (Table 2). Sequences were also related to aqueous or soil Alphaproteobacteria, including *Novosphingobium* sp., *Sphingopyxis soli*, *Sphingomonas* species and *Porphyrobacter donghaensis* (Table 2).

Samples NW and Fife were characterised by the presence of sequences closely related to two *Mycobacterium gordonae* strains isolated from gastric lavage (25.2% and 16.2% of all reads for each sample, respectively; Table 2). In contrast, 11.6% and 1% of the reads from the SE2 and SE1 samples, respectively, were more closely related to *M. mucogenicum* N248 (Table 2).

Bacteroidetes related sequences were not observed in samples SE1 or SE2. In the NW sample, these were mainly related to *Hydrotalea flava*, recently described in Swedish water samples (Kämpfer *et al.* 2011), and in the Fife sample were affiliated to *Lacibacter cauensis* (Table 2), another recently described aquatic bacterium (Qu *et al.* 2009).

Table 1 | Number of bacterial 16S rRNA and fungal 18S rRNA pyrosequencing reads obtained in this study, reads remaining after denoising and chimera check, and observed OTUs at 97% ID similarity level

Sample	Number of reads	Reads after denoising & chimera check	Observed OTUs	Shannon	Chao1
NW, bacterial	126,502	121,000	76	2.96	81.25
Fife, bacterial	33,977	32,366	62	3.08	62.91
SE1, bacterial	30,496	29,301	64	2.09	73.00
SE2, bacterial	25,865	24,933	79	3.23	84.14
Total bacterial	**216,840**	**207,600**	**129**	NA	NA
NW, fungal	42,381	39,311	27	0.73	27.5
Fife, fungal	31,790	30,182	16	1.36	16
SE1, fungal	33,973	31,366	36	3.50	36
SE2, fungal	21,276	18,362	28	3.12	31
Total fungal	**129,420**	**119,221**	**49**	NA	NA

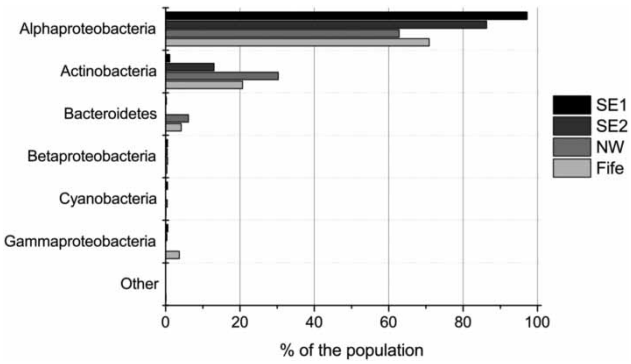

Figure 1 | Bacterial taxonomic classification at the phylum level (class for the Proteobacteria). Only phyla/classes with more than 1% of the total number of reads are shown.

Fungal pathogens are present in samples recovered from domestic shower hoses

Fungal 18S rRNA gene amplicon pyrosequencing also generated more than 20,000 reads per sample (Table 1). The sequence data obtained revealed the presence of between 16 and 36 OTUs per sample, at the 97% sequence identity level (Table 3). The reads obtained were classified predominantly in the Ascomycota and Basidiomycota phyla, and mainly in the Eurotiomycetes, Exobasidiomycetes, Dothideomycetes, Sordariomycetes, Agaricomycetes class of fungi (Table 3; Figure 2). The majority of the fungal pyrosequencing reads from the NW and Fife samples (89% and

Table 2 | The closest phylogenetic relative of the identified bacterial OTUs of this study with the highest number of reads

NW%	Fife %	SE1%	SE2%	Closest relative (accession number; phylum/class)	ID %	Environment/reference
0.1	27.9	58.6	20.0	*Blastomonas natatoria* DSM 3183 NR_040824; *Alphaproteobacteria*	99	Photosynthetic (Hiraishi *et al.* 2000)
25.2	18.9	21.2	28.7	*Bradyrhizobium japonicum* SEMIA 5079 CP007569; *Alphaproteobacteria*	99	Rhizobial, N_2 fixing (Siqueira *et al.* 2014)
32.1	0.2	0.8	4.1	*Bradyrhizobium japonicum* SEMIA 5079 CP007569; *Alphaproteobacteria*	100	Rhizobial, N_2 fixing (Siqueira *et al.* 2014)
2.0	0	8.7	10.1	*Novosphingobium* sp. AKB-2008-TA1 AM989035; *Alphaproteobacteria*	97	Lake water (Berg *et al.* 2009)
0	19.9	0	0	*Sphingopyxis soli* BL03 NR_116739; *Alphaproteobacteria*	99	Landfill soil (Choi *et al.* 2010)
0	0	0.0	7.4	*Sphingomonas soli* T5-04 NR_041018; *Alphaproteobacteria*	99	Soil (Yang *et al.* 2006)
0.4	0	1.0	6.0	*Sphingomonas* sp. AKB-2008-TU3 AM989063; *Alphaproteobacteria*	100	Lake water (Berg *et al.* 2009)
0	0	0	4.3	*Porphyrobacter donghaensis* SW-132 NR_025816; *Alphaproteobacteria*	100	Sea water (Yoon *et al.* 2004)
9.8	12.1	0	0.7	*Mycobacterium gordonae* DSM 44160 NR_114896; *Actinobacteria*	99	Gastric lavage (Bojalil *et al.* 1962; Lefmann *et al.* 2004)
13.5	0.2	0	0	*Mycobacterium gordonae* ATCC 14470 NR_118331; *Actinobacteria*	100	Gastric lavage (Bojalil *et al.* 1962)
1.9	3.9	0	0.1	*Mycobacterium gordonae* ATCC 14470 NR_118331; *Actinobacteria*	99	Gastric lavage (Bojalil *et al.* 1962)
0.1	0.0	1.0	11.6	*Mycobacterium mucogenicum* N248 AY215289; *Actinobacteria*	99	Clinical isolate (Hall *et al.* 2003)
5.4	0	0	0	*Hydrotalea flava* CCUG 51397 NR_117026; *Bacteroidetes*	98	Water (Kämpfer *et al.* 2011)
0.1	4.2	0	0	*Lacibacter cauensis* NBRC 104930 NR_114273; *Bacteroidetes*	93	Lake sediment (Qu *et al.* 2009)
9.4	12.6	8.7	6.9	Other		

Table 3 | The closest phylogenetic relative of the identified fungal OTUs of this study with the highest number of reads

NW %	Fife %	SE1%	SE2%	Closest relative (accession number; fungal class)	ID %	Environment/reference
89.3	64.1	19.9	0.4	*Exophiala mesophila* CBS 402.95 JN856016; *Eurotiomycetes*	99	Shower joint, waterborne (de Hoog *et al.* 2011)
0	3.8	20.5	20.9	*Malassezia restricta* CBS 7877 EU192367; *Exobasidiomycetes*	99	Healthy human skin (unpublished)
0	0	9.1	8.9	*Malassezia sympodialis* EU192369; *Exobasidiomycetes*	100	Healthy human skin (unpublished)
5.9	1.4	17.8	16.6	*Leptosphaeria maculans* JN3 NW_003533867; *Dothideomycetes*	99	Plant pathogen (Rouxel *et al.* 2011)
0	0	4.2	28.8	(various *Penicillium* species), e.g. *Penicillium solitum* 20-01 JN642222; *Eurotiomycetes*	100	Soil (Eldarov *et al.* 2012)
1.0	28.7	1.9	0.5	*Fusarium fujikuroi* IMI 58289 HF679024; *Sordariomycetes*	100	Rice pathogen (Wiemann *et al.* 2013)
1.1	0	4.5	1.7	*Cryptococcus vishniacii* AB032657; *Tremellomycetes*	100	Unknown (Takashima & Nakase 1999)
0	0	3.8	1.0	*Aspergillus glaucus* AF548072; *Eurotiomycetes*	100	Airborne (Wu *et al.* 2003)
0.2	0	4.2	0	*Cladosporium uredinicola* CPC 5390 AY251097; *Dothideomycetes*	99	Fungal parasite (Braun *et al.* 2003)
0	0	0	4.1	*Trametes sanguinea* AB084608; *Agaricomycetes*	100	Tree root (Suhara *et al.* 2002)
0	0	0	4.0	*Schizophyllum commune* X54865; *Agaricomycetes*	100	Wood-rotting (Bruns *et al.* 1992)
2.5	1.9	14.1	13.1	Other		

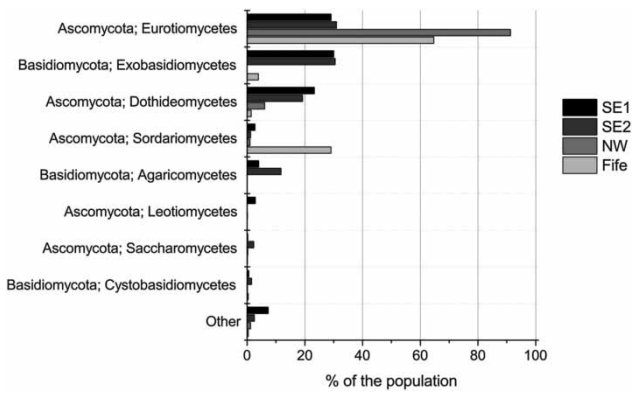

Figure 2 | Fungal taxonomic diversity at the phylum; class level. Only phyla/classes with more than 0.5% of the total number of reads are shown.

64%, respectively; Table 3) had 99% identity to *Exophiala mesophila* CBS 402.95 (JN856016), a waterborne species isolated from a shower joint (de Hoog *et al.* 2011). Only 20% of the reads in sample SE1 were affiliated to this species. In addition, 28.7% of the reads from the Fife sample had 100% identity to the rice pathogen *Fusarium fujikuroi* IMI 58289 (HF679024).

The fungal populations of samples SE1 and SE2 displayed similar compositions (Table 3). Approximately 21% of the reads in these samples were closely related to *Malassezia restricta* CBS 7877 (EU192367). Additional sequences (9%) were related to *Malassezia sympodialis* (EU192369).

Plant pathogen *Leptosphaeria maculans* JN3 (NW_003533867) was represented by 17% of sequences. In sample SE2, 29% of the reads were affiliated to various *Penicillium* species (Table 3). Other fungal species detected in one or more of the samples of this study included airborne *Cryptococcus vishniacii*, *Aspergillus glaucus*, *Cladosporium uredinicola* CPC 5390, *Trametes sanguinea* and wood-rotting *Schizophyllum commune*, an emerging cause of sinusitis (Lorentz *et al.* 2012) (Table 3).

OTU clustering analysis (Figure 3) and taxonomic classification (Tables 2 and 3) revealed similarities in the bacterial and fungal communities in samples SE1 and SE2, whereas samples NW and Fife were more similar to each other than the two samples from the Southeast.

DISCUSSION

The environment around us hosts a significant number of microbes and, in domestic settings, it is suggested that the

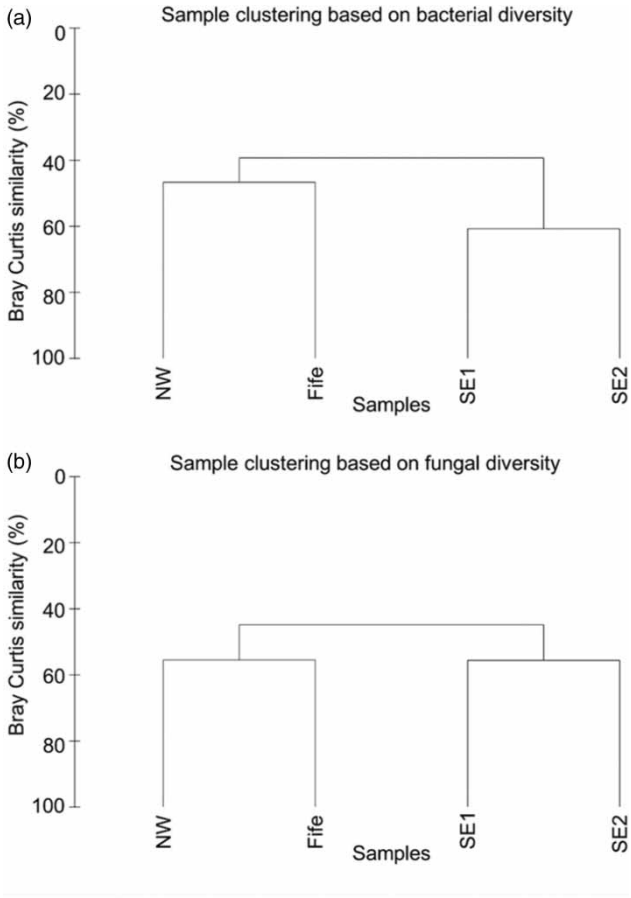

Figure 3 | Sample clustering based on (a) bacterial or (b) fungal diversity. Clustering was carried out on the log transformed abundances of the identified OTUs, using the Bray Curtis similarity method.

majority are of human origin (Hospodsky *et al.* 2012). Previous studies have highlighted the importance of waterborne *Legionella* species in domestic and healthcare settings (Fields *et al.* 2002; Leoni *et al.* 2005; Decker & Palmore 2013), but there is now growing interest in other bacteria that may infect immuno-compromised hosts. In the current study, we have used pyrosequencing approaches to characterise the established bacterial and fungal inhabitants of domestic shower hoses from four sites in England and Scotland, demonstrating the presence of bacteria and fungi that may represent a threat to immuno-compromised hosts, and certain fungi that can be more overtly pathogenic. Although limited in sample numbers, this work was carried out to give an indication of diversity over a geographically dispersed area, supplied by different water sources, rather than to generate a comprehensive catalogue of microbial diversity in this environment.

Amplicon pyrosequencing revealed a maximum of 79 bacterial and 36 fungal OTUs per sample, with most of the sequences clustered within a small number of OTUs. These relatively low levels of diversity in the samples examined is similar to that reported for showerhead communities in the USA and probably results from the low nutrient environment that is present in the water supplying the hoses (Feazel *et al.* 2009).

Cluster analysis and taxonomic classification of all the microbial communities revealed that two geographically distant samples (NW and Fife) shared some similarities and a number of bacterial and fungal species were present across all samples (in some cases in abundance). Environmental Alphaproteobacteria, Actinobacteria and Bacteroidetes dominated the bacterial communities. Several examples of the *Sphingomonadaceae* were observed (e.g. *Blastomonas*, *Novosphingobium*, *Sphingopyxis*, *Sphingomonas* and *Porphyrobacter*). Fungal sequence reads were mainly related to the Eurotiomycetes, Exobasidiomycetes, Dothideomycetes, Sordariomycetes and Agaricomycetes. This may indicate that specific organisms preferentially colonise and establish within the biofilm communities that develop over time within domestic shower hoses.

The differences in the bacterial and fungal communities observed in the samples could be attributed to a range of factors, such as water composition in the different regions; water in Northwest England and Fife is soft, whereas it is generally medium-hard to hard in Southeast England (waterwise.org.uk). A larger and more detailed analysis of the impact of different physicochemical parameters of water on shower biofilm populations is warranted, though the similarities seen between the samples examined here suggest that some genera are widely dispersed, irrespective of water characteristics.

Members of the above-mentioned bacterial phyla have been described in shower associated biofilms (Kelley *et al.* 2004; Feazel *et al.* 2009) and are only very rarely associated with infections. However, some form extensive biofilms and it is suggested that waterborne members of the *Sphingomonadaceae* from hospital and domestic environments may be a reservoir for antibiotic resistance genes (Narciso-da-Rocha *et al.* 2014). *Porphyrobacter donghaensis* has been associated

with environmental and hospital water in previous studies (Yoon *et al.* 2004; Furuhata *et al.* 2007), but not with infection.

Samples contained sequences related to *Mycobacterium gordonae* and *M. mucogenicum*. *M. gordonae* has been reported in showerhead populations (Feazel *et al.* 2009). Members of the NTM are ubiquitous in the environment and have recently been associated with shower aerosol related infection in Australia where strains of NTM from patients were linked to their domestic environments (Thomson *et al.* 2013a, 2013b). In a different study of showerheads and hose samples, Rhodes *et al.* (2014) demonstrated the widespread occurrence of *Mycobacterium avium* subspecies *paratuberculosis* (Map), which is significantly associated with development of Crohn's disease. Their findings supported the suggestion that shower aerosols may have an under-recognized role in the acquisition of Crohn's disease.

Although other molecular- or culture-based studies have identified *Legionella* species in domestic water systems (Leoni *et al.* 2005; Bauer *et al.* 2008; Whiley *et al.* 2015), in the current study, no such sequences were detected. Interestingly, a similar study reported only low-level occurrence of *Legionella* species 16S rRNA sequences (0.1% of total sequences), while qPCR was unable to detect *L. pneumophila* specific sequences in any samples (16 from water and 20 swabs) recovered from domestic showerheads (Feazel *et al.* 2009). Taken together, this may indicate that (pathogenic or non-pathogenic) *Legionella* species are not expected to be universally present in all domestic water systems.

This study reports, for the first time, the fungal flora of shower associated biofilms. In some cases, there was a predominance of *Exophiala mesophila*, an organism associated with cutaneous and subcutaneous infections (Zeng *et al.* 2007), was first isolated from silicone seals in a hospital shower room (Listemann & Freiesleben 1996) and has been reported in dental unit water lines (Porteous *et al.* 2003). *Exophiala* species have been recovered from drinking water in Germany (Göttlich *et al.* 2002) and Slovenia, where the occurrence of these organisms has been studied in detail (Novak Babič *et al.* 2016). *Exophiala* species are known for biofilm formation (Heinrichs *et al.* 2013) and they have been observed in high numbers in sauna facilities (Matos *et al.* 2002), domestic dishwashers (Gümral *et al.* 2015) and washing machines (Novak Babič *et al.* 2015). In the latter environments, conditions like temperature and pH will be different to those found in showers and there will be a plentiful supply of nutrients in the form of detergents, which may support dense biofilm development. Shower hoses are low nutrient environments, so *Exophiala* phenotypes from these sources would be expected to differ and the role of other flora in succession and biofilm development may be key in shower hoses. Although *Exophiala* species rarely cause infections, these can be difficult to treat (Rimawi *et al.* 2013) and their true prevalence may be underestimated given their slow growth and difficulties in identification (Porteous *et al.* 2003). In studies of domestic washing machines, which can be heavily colonised with these fungi, it has been suggested that detergent drawers should be regularly cleaned with bleach, in line with manufacturers instructions to reduce the possibility of opportunistic infection (Novak Babič *et al.* 2015).

Also observed were sequences related to *Fusarium fujikuroi*. Members of the *Fusarium fujikuroi* species complex are predominantly plant pathogens, but they are being increasingly recognized as aetiological agents of infection in immunocompromised and immunocompetent hosts (Kebabci *et al.* 2014; Tortorano *et al.* 2014) and infection with some *Fusarium* species has been linked to colonization of hospital water systems (Anaissie *et al.* 2001). It has been demonstrated that the concentration of airborne of filamentous fungi, including *Fusarium* and *Aspergillus* species, was significantly reduced by washing water-related surfaces in hospital bathrooms immediately prior to showering (Anaissie *et al.* 2002). These authors also suggested that the findings reported would be applicable to domestic shower environments, where discharged immunocompromised patients may be at risk of fungal infections.

An additional potential risk with *Fusarium* species is the generation of trichothecenes, protein synthesis inhibitors that are documented to cause intoxications in humans following consumption of food products colonised with *Fusarium* species (Murphy *et al.* 2006) and may lead to an increase in respiratory tract infections (Bhat *et al.* 1989). Given the toxicity of trichothecenes, concentrations in water emerging from showers should be investigated.

In samples from the Southeast of England, over 20% of the reads were related to *Malassezia restricta*, an organism

associated with severe opportunistic infections (Arendrup *et al.* 2014), although they are also members of skin flora. However, given recent evidence for an association between *Malassezia restricta* and dandruff and seborrheic dermatitis (Gemmer *et al.* 2002; De Angelis *et al.* 2005; Clavaud *et al.* 2013), the implications of significant numbers of these organisms in the shower hose biofilms is clear. Overall, members of the *Malassezia* genus are the predominant flora of many parts of the human skin, where they have a complex interaction with other microorganisms and the host (Gaitanis *et al.* 2012). When these interactions are disturbed, *Malassezia* species can cause a wide range of conditions; they are among the most common causes of superficial mycoses (Crespo-Erchiga & Florencio 2006) and dermatoses (Jagielski *et al.* 2014) and are also associated with atopic eczema and allergic reactions (Gaitanis *et al.* 2012).

Although sample numbers are small, there are suggestions of geographical and temporal associations for some flora. *Agaricomycetes* are only seen in the older southern sample, SE2, and the *Sordariomycetes* are only seen in high numbers in Fife, while the *Dothideomycetes* and *Exobasidiomycetes* were predominant in the South. Similarly, *Actinobacteria* are only present in small numbers in sample SE1 and *Bacteroidetes* are not seen in the southern samples at all. The findings warrant more detailed and extensive studies into these associations, particularly with a focus on fungal flora and exploration of the dynamics of community succession in the biofilm.

The UK Department of Health suggests that flexible hoses should not be used in high-risk situations (Best Practice Guidance; HTM 04-01–Addendum: *Pseudomonas aeruginosa*–Advice for Augmented Care Units, March 2013). Such guidelines are implemented to reduce infection with *Pseudomonas aeruginosa*, but the data presented here suggests that effective management of biofilms in flexible hoses may control several additional opportunistic bacterial and fungal pathogens.

CONCLUSIONS

Previous studies have extensively catalogued the bacterial populations associated with showerheads and shower curtains, and our data corroborate these findings. Ours is the first study to describe in detail the fungal community members, some of which give cause for concern. Although our findings from a small collection of samples are preliminary, more detailed investigations are justified. The role of different shower water supplies (e.g. tank vs mains feed) and shower hose materials should be established. The impact of frequency of shower use on biofilm populations and the rate of biofilm accumulation should be investigated. We support the suggestion in previous studies that potential pathogens residing in shower hose biofilms will periodically slough off the inner surface, being released and distributed in shower aerosols (Falkinham *et al.* 2008; Rhodes *et al.* 2014). A recent study with a model shower system demonstrated that drying the hose between uses did not reduce the load of bacterial pathogens including *Legionella* species and members of NTM (Whiley *et al.* 2015). It may be prudent to routinely decolonise or regularly replace shower hoses in domestic and health-care settings, where immuno-compromised individuals are present.

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