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What's really down the hospital plughole?

James Butler[1*] and Mathew Upton[2]

[1]Department of Clinical and Biomedical Sciences, Exeter Medical School, Faculty of Health and Life Sciences, University of Exeter, Exeter, EX1 2LU, UK.
[2]School of Biomedical Sciences, Faculty of Health, University of Plymouth, Plymouth, PL4 8AA, UK.

*Corresponding author: j.m.butler@exeter.ac.uk

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As reported in recent articles in this journal[1] and others, hospital wastewater plumbing systems (WPS) are increasingly being highlighted as an important source of nosocomial infections[2]. Potential interventions to mitigate these risks include changes in patient management through to engineering solutions and modifications to plumbing infrastructure, with recent calls to improve building guidance to mitigate the impact of suboptimal designs on patients and healthcare staff[3]. The WPS is a complex interlinked system of pipework that is intermittently filled with water containing a multitude of solutes and solids. The interface between this system and the environments we occupy is within sink traps, which connect sinks to waste flow in the WPS periphery, preventing the flow of gases from the sewer to the sink and surrounding environment. Sink traps in particular become heavily colonised with (and act as a reservoir for) opportunistically pathogenic bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp. and *Serratia marcescens*[2].

A key aspect of assessing the threats posed by WPS colonised with microbes in clinical areas is accurate identification of the microbes present, because in some cases devastating outbreaks have been caused by persistent, multidrug-resistant strains[2]. The bacteria colonising WPS vary depending on differences in sink use and opportunistic exposure to virulent or persistent species. Understanding the detail of the WPS 'microbiome' will allow interventions to be targeted most effectively at high-risk sites (where virulent, multidrug-resistant or persistent species exist) and accurate characterisation tools could be used to assess the effectiveness of mitigation measures following their introduction.

Culture-based methods have been used to investigate WPS microbes, but it is widely appreciated that these methods do not reflect the true microbial diversity in environmental samples. The use of 16S rDNA amplicon sequencing has led to a better appreciation of biodiversity in microbial ecology, but there are few publications describing its application to hospital sink traps. The approach has been used to

demonstrate that sinks ‘bridge’ clusters of *Enterobacteriaceae*, spreading bacteria within and between hospital wards[4].

In proof-of-concept work, we have used long-read MinION sequencing (Oxford Nanopore Technologies, ONT; https://nanoporetech.com) to characterise the microbial populations in a hospital sink trap by sequencing the entire 16S rRNA gene, which gives higher resolution than conventional 16S rDNA profiling of discrete hypervariable regions. The sink trap was removed from an acute care ward at a UK hospital. Biofilm material was recovered using a sterile swab, and metagenomic DNA extracted using the DNeasy® PowerSoil® kit (Qiagen, Germany) according to the manufacturer’s instructions. DNA yield and purity were quantified using the Qubit™ dsDNA HS Assay Kit (Invitrogen, MA, USA) and a Nanodrop™ spectrophotometer (Thermo Fisher Scientific, UK), respectively. The ONT 16S barcoding kit (SQK-RAB204) and a R9.4.1/FLO-MIN106 flow cell were used according to the manufacturer’s instructions. Basecalling was performed by Guppy version 3.4.5 using the Cloud Infrastructure for Big Data Microbial Bioinformatics (CLIMB) platform. Following demultiplexing and quality filtering, there were 260523 reads, of which 260415 were identified to genus and species level by Kraken2.

The results are shown in Figure 1, indicating a number of detected organisms that have previously been implicated in nosocomial disease and outbreaks linked to WPS. These experimental results indicate that it is feasible to recover biofilm material from a sink trap, isolate DNA and profile the microbes present. It would be feasible to carry this out in near real-time[5]. Full metagenomic DNA sequencing can also be used to identify antimicrobial resistance (and virulence) related genes in samples, bringing an added level of insight into the microbial risks that may be present in sink traps[6].

The portability of the MinION platform is key as deployment on-site in clinical environments is feasible for rapid monitoring in high-risk areas. Although costs are currently prohibitive, multiplexing is possible using barcoded libraries so several sites could be analysed in one run, reducing per-site analysis costs. In addition, newer advances in the ONT technology like the Flongle (https://nanoporetech.com/products/flongle) allow analysis of small samples at a current cost of $90 per run. The technology has been used to analyse blood samples spiked with clinical isolates, giving results for virulence genes and AMR-related targets in 10 minutes to 3 hours[7]. The Flongle may also allow rapid characterisation of environmental microbial populations using 16S rRNA gene sequencing[8].

A full cost-benefit analysis for the use of ONT-based microbial population profiling could provide evidence to justify its introduction in clinical areas. The early and accurate identification of high-risk pathogens in WPS in acute clinical areas could guide rapid deployment of mitigation measures to reduce or control outbreaks. Such interventions are necessary, given the risks posed by WPS microbial populations and

a more detailed investigation of the use of (portable) sequence-based methods in clinical areas is justified.

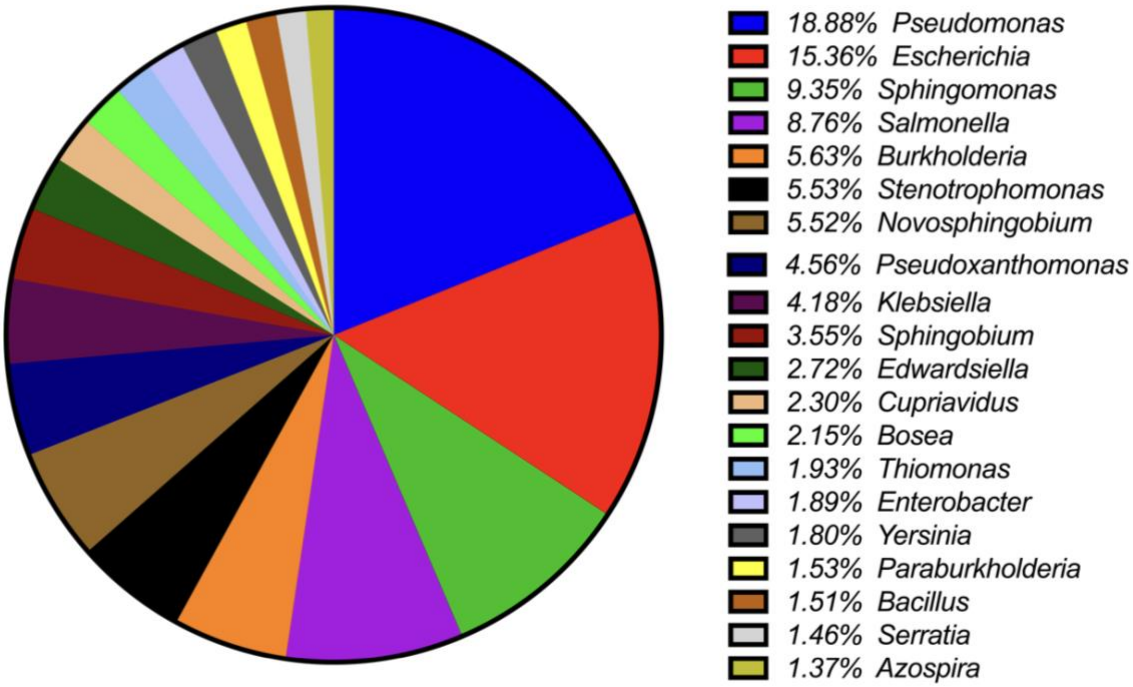

Figure 1: 16S rDNA amplicon survey of a hospital sink trap carried out with long-read MinION sequencing. Results are presented at genus-level as a proportion of total reads and the top 20 genera identified are shown.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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