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What's really down the hospital plughole?

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1 What's really down the hospital plughole?

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

28

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

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

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

45 In proof-of-concept work, we have used long-read MinION sequencing (Oxford
46 Nanopore Technologies, ONT; <https://nanoporetech.com>) to characterise the
47 microbial populations in a hospital sink trap by sequencing the entire 16S rRNA gene,
48 which gives higher resolution than conventional 16S rDNA profiling of discrete
49 hypervariable regions. The sink trap was removed from an acute care ward at a UK
50 hospital. Biofilm material was recovered using a sterile swab, and metagenomic DNA
51 extracted using the DNeasy[®] PowerSoil[®] kit (Qiagen, Germany) according to the
52 manufacturer's instructions. DNA yield and purity were quantified using the Qubit[™]
53 dsDNA HS Assay Kit (Invitrogen, MA, USA) and a Nanodrop[™] spectrophotometer
54 (Thermo Fisher Scientific, UK), respectively. The ONT 16S barcoding kit (SQK-
55 RAB204) and a R9.4.1/FLO-MIN106 flow cell were used according to the
56 manufacturer's instructions. Basecalling was performed by Guppy version 3.4.5 using
57 the Cloud Infrastructure for Big Data Microbial Bioinformatics (CLIMB) platform.
58 Following demultiplexing and quality filtering, there were 260523 reads, of which
59 260415 were identified to genus and species level by Kraken2.

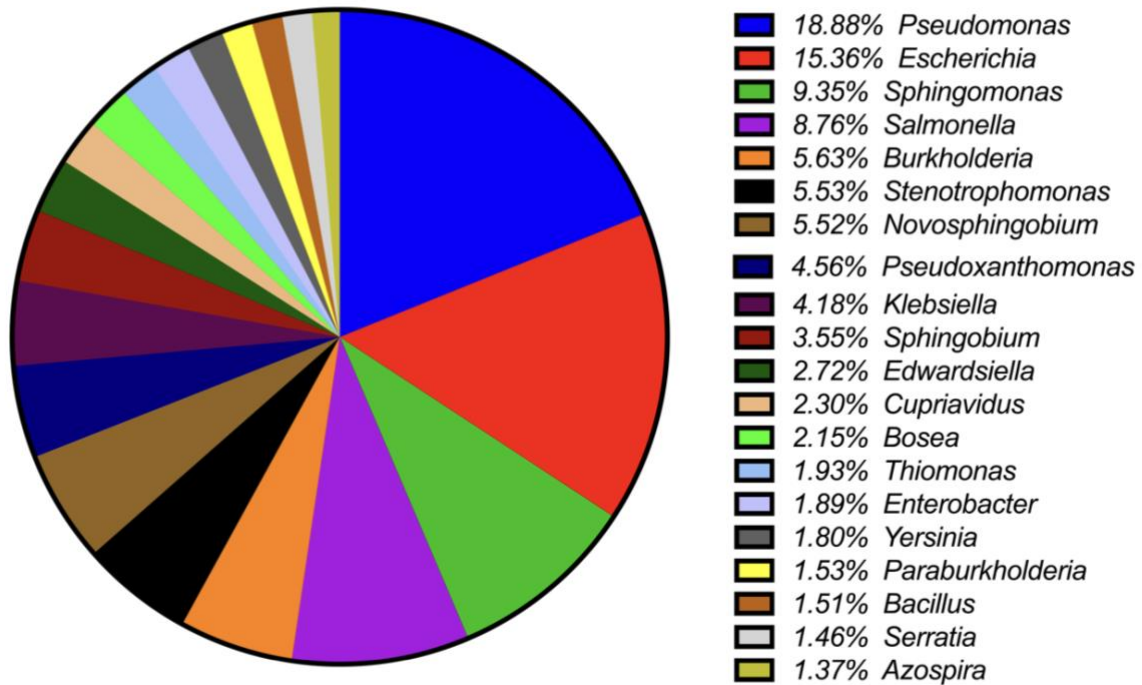
60 The results are shown in Figure 1, indicating a number of detected organisms that
61 have previously been implicated in nosocomial disease and outbreaks linked to WPS.
62 These experimental results indicate that it is feasible to recover biofilm material from
63 a sink trap, isolate DNA and profile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

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

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

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

84



85

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

89 **Conflicts of interest**

90 The authors declare that there are no conflicts of interest.

91

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