

POEMBAKterie:

A characterisation of the household kitchen sink microbiome

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List of abbreviations

HAIs	Hospital-acquired infections
ICUs	Intensive care units
HCP	Healthcare personnel workstation
ARGs	Antibiotic resistance genes
ONT	Oxford Nanopore Technologies
MALDI-TOF MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
PERMANOVA	Permutational multivariate analysis of variance
NMDS	Non-metric multi-dimensional scaling
LMM	Laboratory of Medical Microbiology
VBNC	viable but non-culturable
AMR	Antimicrobial resistance

1 Abstract

The environmental microbiome plays a well-established role in influencing human health. While bacterial communities associated with hospital sinks have been extensively documented due to their relevance in infection control, the microbiome of sinks in non-clinical, domestic settings remains comparatively underexplored. Therefore, the ongoing POEMBAKterie project in our research group aims to characterise the microbial composition of household kitchen sinks in Antwerp. In this thesis, a citizen science approach was employed to collect household kitchen sink samples and associated household metadata as part of the POEMBAKterie project. Full-length 16S rRNA gene sequencing was performed to characterise bacterial community composition at high taxonomic resolution. In addition, cultivation-based methods using selective media were applied to detect viable opportunistic bacteria, with a focus on the detection of *Escherichia coli*. Sequencing data were analysed to assess bacterial community composition, alpha and beta diversity and differences between sample types and households.

Sequencing revealed diverse microbial communities dominated by the phyla *Pseudomonadota*, *Actinomycetota*, *Bacillota* and *Bacteroidota*, consistent with previously described built-environment microbiomes. At the genus level, taxa such as *Microbacterium*, *Acinetobacter*, *Paracoccus*, *Pseudomonas*, *Moraxella* and *Stenotrophomonas* were most abundant, many of which have previously been associated with water systems, food-related environments, and human microbiota. Significant differences in bacterial community composition were observed between sample types, although substantial overlap and within-group variability were present. In contrast, variation between household locations was limited, likely due to the small sample size. Potential opportunistic bacterial taxa, including *E. coli*, were detected through both sequencing and cultivation-based approaches. However, overlap between the two methods was only partial, highlighting methodological differences related to viability assessment and methodological sensitivity. Importantly, the presence of these taxa alone is insufficient to infer pathogenicity or infection risk.

Overall, these findings indicate that kitchen sinks represent complex microbial ecosystems shaped by multiple environmental and human inputs and demonstrate the value of combining sequencing and cultivation to characterise both microbial diversity and viability.

2 Introduction

2.1 Microbial communities within the urban built environment

As a growing proportion of the global population resides in urban areas, understanding the impact of the built environment on public health has become increasingly important. According to the United Nations, approximately 45% of the population currently lives in cities, with an additional 36% living in smaller urban areas (Figure 1) (1). Within urban environments, humans are exposed to a wide variety of bacteria, referred to as the environmental microbiome. Since the environmental microbiome can influence the human microbiome, understanding its composition and structure is essential.

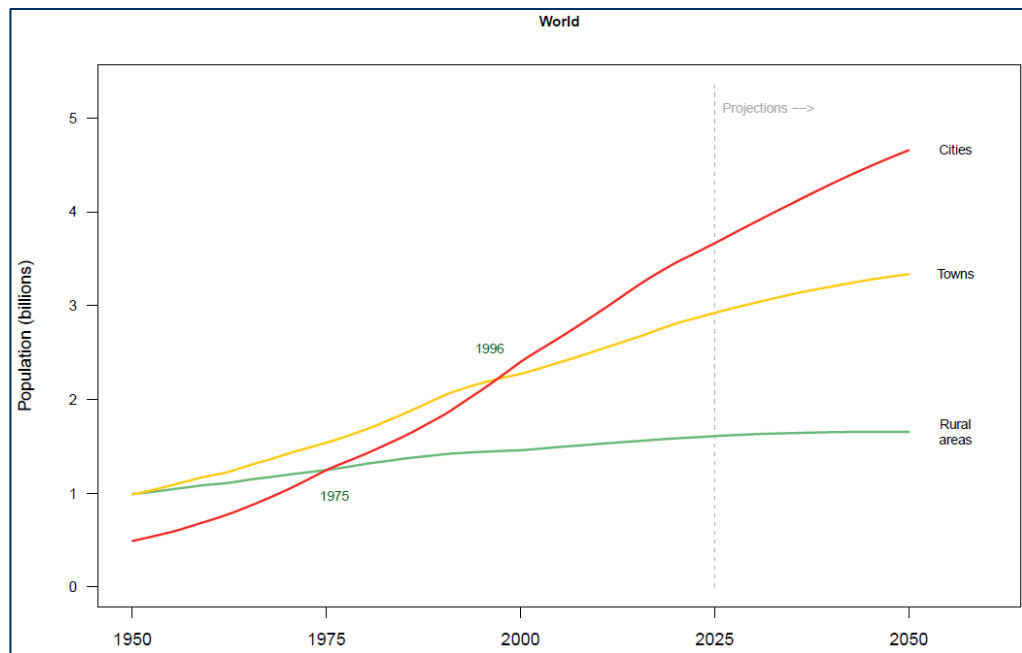


Figure 1. World population living in cities, towns, semi-dense areas and rural areas. Data from 1950-2025 and projections for the next 25 years (1).

Among these urban environments, human-made structures and spaces, such as residential buildings, offices, public institutions, transportation systems, roads and water infrastructures, contain microbial communities that differ substantially from those found in natural environments (2,3). Consequently, research on the urban environment microbiome has been applied in different contexts, such as investigating microbial communities in public transportation systems (4–7) and indoor environments, like households (8–11), university dorms (12) and the workplace (13). These studies have revealed patterns in how microbial communities are structured and have permitted the identification of dominant taxa and comparisons of microbial communities in different spaces (2). Importantly, they show that human activity is a major factor in shaping these communities. A recurring observation is that the majority of bacterial microorganisms found on surfaces originate from human skin and oral sites (2,8). Beyond describing microbial communities, advances in our understanding of the urban microbiome and its relationship with human health have contributed to a shift in how microorganisms are perceived, moving away from a solely negative, pathogenic view towards one where they may also play a beneficial and protective role (2,14).

2.2 Microbiota within indoor environments

Indoor environments support highly diverse and complex bacterial communities, comprising a large number of different taxonomic groups (8,14), and have been shown to contain taxa with pathogenic potential (12). Across surfaces, dominant bacterial phyla are typically *Proteobacteria*, *Firmicutes* and *Actinobacteria* (8,12), with the addition of *Bacteroidetes* in some studies (13,15). At the genus level, *Staphylococcus*, *Corynebacterium* and *Lactococcus* are among the most commonly detected (8,9). Despite these general patterns, bacterial community composition varies significantly between different locations and among different surface types within the same location (8,12).

Indoor microbial community composition is shaped by a combination of different sources, environmental factors, and transmission routes (Figure 2) (10). Sources of indoor bacteria are largely human-derived (8,9,13,15). In public restrooms, skin-associated taxa, as well as bacteria associated with the gut, mouth, and urine, can be found on all surfaces (15). Similarly, in residential settings, a relatively high proportion of bacteria on indoor surfaces is associated with human skin, although bacteria associated with the mouth and stool are also present. In addition to human sources, bacteria from leaves or produce are particularly abundant on door trim surfaces

and, to a lesser extent, on kitchen surfaces, while soil-derived bacteria are widespread throughout the house (8). Overall, the distribution of bacterial sources is often linked to surface use and function, with sampling surface emerging as a major factor structuring indoor microbial communities (8,9). However, the home from which the samples were taken is another factor heavily influencing the microbial communities (8,11). Different households have their own unique bacterial community, and significant differences can be observed between identical sampling locations in different homes, particularly on kitchen surfaces (8). However, studies seem to disagree about whether household or sample surface is the most important influence (8,11,16). Similar patterns are observed in university dormitories, where bacterial community composition differs significantly between functional surfaces (12).

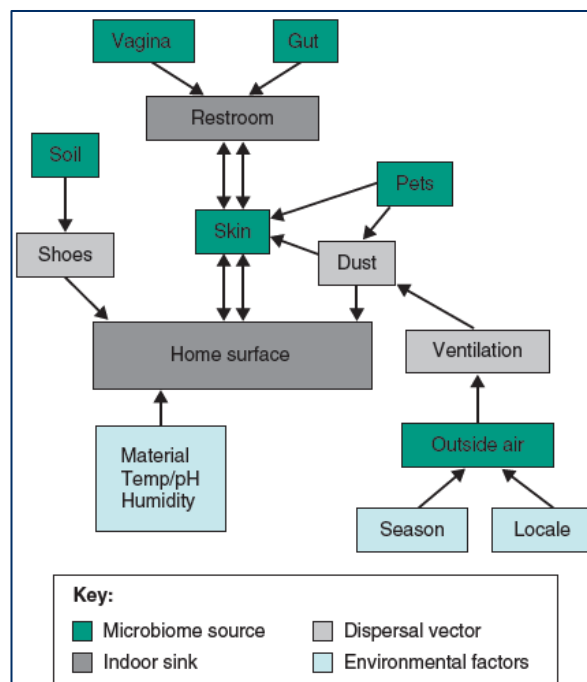


Figure 2. Potential sources and environmental factors influencing indoor microbial communities (10).

Beyond surfaces, the indoor air microbiome is strongly influenced by human occupancy and ventilation strategy. Increased occupancy is associated with a higher relative abundance of human-associated bacterial genera, whereas in well-ventilated spaces, the indoor air microbiome closely resembles that of the outdoor environment regardless of occupancy (17).

Microbial communities on surfaces that are periodically wetted, such as shower walls, showerheads, sinks, and drains, are distinct from those encountered elsewhere in the home (8,18). The presence of moisture, combined with nutrient availability, promotes microbial growth and biofilm formation in water-associated environments (19). These characteristics make water-associated environments potential reservoirs for microbial persistence and dissemination. In tap water and biofilms within water infrastructures, bacteria are the most numerous microorganisms (20).

2.3 The microbiome of sinks in non-domestic settings

Among water-associated environments, sinks represent a frequently used and widely distributed microbial habitat, making them a relevant ecological niche for microbial colonisation. Although research on the microbiome of sinks in a residential setting is limited, sink-associated microbial communities have been studied more frequently in other built environments, including the workplace (16), university campuses (21) and, most extensively, hospitals (22–24).

Sink systems can facilitate microbial persistence and dispersal under favourable conditions. Previous studies have demonstrated that pathogens may spread between interconnected sinks through shared drain pipes when nutrients are available (25,26). Experimental models further indicate that, once established, microorganisms present in colonised sink drains can be transferred to sink surfaces and surrounding areas through both aerosolization and droplet-mediated dispersion (26). During faucet use, water impact on the sink basin and drain can generate shear forces that may facilitate bacterial detachment and dispersal within the sink environment (27), highlighting the potential for splash-back contamination. These populations may originate from biofilm-associated reservoirs within sink drainage systems, from which bacteria can be released during water use and dispersed to more exposed sink surfaces under favourable conditions (26,27).

2.3.1 Hospital sinks as a reservoir for pathogens

Hospitals represent a highly selective environment for the microbiota, shaped by strong selective pressures such as intensive cleaning practices, antimicrobial pressure, and the introduction of new pathogens by patients. As a result, there is a high presence of human-associated bacteria, many of which are opportunistic pathogens, and fewer potentially beneficial microorganisms compared to public and private indoor environments (28). Given the increased risk of infection among patients who may be immunocompromised or have other underlying conditions, extensive research has examined the role of hospital sinks and their microbial composition. Several of these studies demonstrate that hospital sinks serve as reservoirs for opportunistic pathogens (22,29,30) and can act as sources for hospital-acquired infections (HAIs), with genetic relatedness between strains isolated from patients and sink drains suggesting possible bidirectional transfer (22,31,32). Hospital sink drains may contain biofilms that can contaminate sink surfaces (24). While cleaning temporarily reduces bacterial abundance on these surfaces, microbial populations rapidly rebound, and biofilms in drains persist (24,29). Especially, areas that are less frequently cleaned, such as under the drain cover, provide optimal conditions for biofilm formation and colonisation by pathogens and antibiotic-resistant organisms (23). Moreover, because sinks provide a moist environment, they are an ideal reservoir for gram-negative bacteria (22,33).

Correlations have been observed among bacterial loads in the drain, on drain covers, and on the sink basin surface, with the strongest correlation occurring between the drain cover and the sink basin surface, indicating frequent co-contamination. In contrast, bacterial concentrations in tap water are largely independent of those in the drain and on sink surfaces, indicating that bacterial compositions on these surfaces are not primarily driven by incoming water (24). The environment influences sink microbiota, which vary among sink drains within the same hospital, and only a limited number of lineages are more widely distributed. A similar phenomenon is observed for tap water (24). Moreover, distinct microbial communities have been documented between adult and neonatal intensive care units (ICUs) (30). Source tracking indicates that part of the bacteria present on sink surfaces and drain covers originates from the normal human skin microbiome (24). There is a clear transfer pathway from the drain towards the sink surface, with the drain cover being an intermediary (24,34). The drain cover and sink basin host similar communities, indicating microbial exchange and colonisation from shared sources, including tap water (24).

Microbial composition is further influenced by sink usage patterns. Comparisons between patient room and healthcare personnel work station (HCP) sinks revealed that HCP sinks contain higher abundances of microbial organisms, whereas patient sinks harbour a greater percentage of antibiotic-resistant organisms (23). Similarly, hallway sinks, which are more frequently used than sinks in patient rooms, harbour significantly higher bacterial loads in both drains and on sink surfaces, an effect that is not observed in tap water samples (24). Both surfaces around the sink (33) and drain water samples (35) have been shown to have a high presence of antibiotic resistance genes (ARGs). Moreover, ARG profiles in sinks were shown to be similar between different ICUs, despite distinct microbiota and cleaning patterns between these units (30). Furthermore, potential occurrences of horizontal gene transfer of ARGs via plasmids have been observed (32,36), resulting in ARGs persisting in species even in the absence of strain persistence (36).

2.3.2 Sink microbiota in public locations

While hospital sinks can harbour pathogenic bacteria associated with increased health risks, microbiota in other publicly accessible sinks generally pose a lower risk. Bacterial communities in sink drains of communal bathrooms within university buildings are dominated by the phyla *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*, with *Acinetobacter* from the *Moraxellaceae* identified as the dominant genus across all sinks. Despite this apparent consistency, taxonomic profiles at the family level remain highly variable (21). In contrast, sinks in university dormitories portray a slightly different community with *Proteobacteria*, *Actinobacteriota* and *Patescibacteria* identified as the most abundant phyla (12). Overall, bacterial community composition in public restroom sinks varies significantly between buildings, while the primary source of bacterial taxa remains human skin across these environments (21). In university dormitories, environmental factors such as temperature, humidity and sunlight exposure have been shown to correlate significantly with bacterial community composition in sinks. While opportunistic pathogen community composition is correlated with temperature and sunlight exposure, as well as sink height (12). Sinks can also contain a broad range of ARGs, as demonstrated in the workplace environment. Contrary to observations in hospital sinks, workplace sink resistomes have been shown to differ significantly between locations (16).

2.4 The microbiome of sinks and other surfaces in residential kitchens

Although research specifically targeting household kitchen sink microbiomes is very limited, several studies have examined the broader kitchen microbiome, including sink-associated surface sampling (18,37–39). Across these studies, the majority of bacterial genetic sequences detected in residential kitchens belong to four phyla: *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria* (18,37). This is consistent with patterns observed in broader built environment microbiome research, although the order of dominance varies among studies (4,5,8,12,18,37). Flores et al. (2013) sampled over 80 kitchen surfaces across four households in Colorado, USA, using Illumina short-read sequencing. The most abundant bacterial families present were found to be the *Micrococcaceae*, *Flavobacteriaceae*, *Streptococcaceae* and *Moraxellaceae* (18). More recently, Moen et al. (2023) analysed cleaning utensils and kitchen surfaces in 74 residences using Illumina short-read sequencing across five European countries (France, Hungary, Norway, Portugal and Romania) and found eight genera/families to be part of the core microbiota: *Acinetobacter*, *Pseudomonas*, *Enhydrobacter*, *Enterobacteriaceae*, *Psychrobacter*, *Chryseobacterium*, *Bacillus* and *Staphylococcus* (37). These results largely correspond with those previously found in 2013 (18), with some additions.

Microbial communities differ substantially across various kitchen surfaces, as well as between households (18,38). Kitchen surface communities originating from the same kitchen are more similar to one another than to those from different households, likely reflecting differences in behaviour and bacterial communities of inhabitants, as well as differences in kitchen structure and environment (18). Interestingly, at a larger scale, bacterial communities on kitchen surfaces appear to be more influenced by country of origin than by surface type (37). While sink samples have the highest abundance of bacteria (37), they do not consistently harbour the most diverse bacterial communities. In one study, the kitchen sink was shown to have the least diverse bacterial communities (18), whereas another study identified the most diverse communities around the sink drain (38). In contrast, a third study found that the sink had neither the lowest nor the highest diversity compared to other kitchen surfaces (37).

The general sampling area plays a role in shaping bacterial composition, mainly determined by the two most prevalent phyla: *Proteobacteria* and *Firmicutes*. Moist surfaces, including the sink drain, are more frequently contaminated with gram-negative *Proteobacteria* (18,39), whereas gram-positive *Firmicutes* are more abundant on dry and/or cold surfaces (18). Compared to other kitchen surfaces, the sink drain and kitchen sponge have the highest level of both gram-positive and gram-negative bacterial contamination (39). In accordance with observations in the general indoor environment, the most important source of bacteria in the kitchen is human skin, with a relatively higher contribution on frequently touched surfaces (18). Still, kitchen-associated

communities also had a significant presence of bacteria originating from food items (12). The bacterial sources follow predictable patterns of use, with water contributing more in the sink area and on higher cabinets and food-associated bacteria more prevalent on surfaces regularly touched by food (18).

Genera possibly associated with pathogens have also been identified in the kitchen and are broadly distributed, but their abundance is relatively low (18,37). Furthermore, the majority of bacteria detected in these genera are likely non-pathogenic (18). Among different kitchen surfaces, the sink drain is found to be the most frequently contaminated by foodborne pathogens. Pathogen presence in the kitchen can be linked to inadequate recognition of food contamination as a serious food safety issue (38). Importantly, a link between more frequent disinfection of kitchen surfaces and increased antibiotic resistance could not be established (39).

2.5 *Escherichia coli* as a model organism in sink environments

Within sink-associated microbial communities, *E. coli* is of particular interest due to its dual role as a commensal organism of the intestinal microbiota of many warm-blooded animals and a clinically important opportunistic pathogen (40). *E. coli* is associated with a wide spectrum of infections, including urinary tract infections, gastroenteritis, and invasive bloodstream infections, and represents a major contributor to the global burden of antimicrobial resistance (41–43). In built environments, sink systems have been identified as relevant reservoirs for clinically important gram-negative bacteria, including *E. coli*, particularly in healthcare settings (22,44,45). Model-based studies investigating sink-associated *E. coli* have demonstrated the potential for transmission and dispersion within sink environments, supporting its use as a representative indicator organism in sink microbiology research (25–27). However, its occurrence and population dynamics in non-clinical indoor sink environments remain comparatively poorly characterised compared to hospital sink systems.

Although domestic kitchens and kitchen sink microbiomes have been investigated using sequencing-based approaches, these studies generally focus on overall microbial community structure and provide limited species-level resolution for specific clinically relevant organisms (18,38). Consequently, the presence, persistence, and viability of *E. coli* in such environments remain insufficiently resolved. Moreover, sequencing-based approaches generally do not distinguish between viable and non-viable organisms, limiting the interpretation of detected taxa in relation to potential microbial activity (46).

2.6 Limitations of current research

Despite advances in the understanding of indoor and kitchen-associated microbiomes, several limitations remain. First, many studies of indoor and built-environment microbiota rely on sequencing of short regions of the 16S rRNA gene, which can limit taxonomic resolution and reduce the ability to distinguish closely related bacterial species (47). As a result, species-level composition, including the detection of opportunistic pathogens, often remains unresolved in short-read 16S rRNA microbiome studies. Second, research on sink-associated microbiota has primarily focused on healthcare environments, where sinks have been identified as reservoirs of opportunistic pathogens and, in some cases, antimicrobial resistance determinants (22,23,35). In contrast, residential sink environments remain comparatively understudied. Although several studies have characterised microbial communities in domestic kitchens and household environments (18,37,38), the microbial ecology of household sink systems and the factors shaping these communities remain insufficiently understood. Recent advances in long-read sequencing technologies, including sequencing with Oxford Nanopore Technologies (ONT), have expanded opportunities for species-level microbial characterisation through full-length 16S rRNA gene sequencing (48–50). Nevertheless, methodological limitations related to DNA extraction and sample processing (51), as well as platform-specific variability (49,52) remain important considerations in environmental microbiome research. Finally, sequencing-based approaches generally do not distinguish between viable and non-viable organisms, limiting the interpretation of detected taxa in relation to potential microbial activity (46).

2.7 Study aims and objectives

The overall POEMBAKterie project aims to characterise the microbiome of household kitchen sinks in Antwerp and to gain insight into the factors influencing the composition of the sink microbiome. In this thesis, bacterial community composition within kitchen sinks was characterised and compared across sample types and locations. In addition, the presence of potential opportunistic pathogens was investigated, with a focus on *E. coli*.

We hypothesise that the bacterial composition of kitchen sinks is highly diverse and influenced by factors such as sample type and location. We further expect the most dominant bacterial phyla in kitchen sink samples to include *Actinomycetota*, *Bacteroidota*, *Bacillota* and *Pseudomonadota* (formerly known as *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Proteobacteria*). In the following sections, these updated taxonomic names will be used consistently. In addition, we presume that opportunistic pathogens such as *E. coli* may be present in kitchen sinks.

3 Methodology

3.1 Study design

This study was designed as a cross-sectional observational study using a citizen science approach. Participants collected samples from their own kitchen sinks and completed a questionnaire assessing household characteristics, environmental and behavioural habits. Microbial community composition was analysed using full-length 16S rRNA gene sequencing, allowing taxonomic profiling down to the species level (47). Additionally, opportunistic pathogens such as *E. coli* were isolated by cultivation on selective media and identified using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS).

Survey data were collected from participating households and will be used in the broader POEMBAKterie project to determine environmental and behavioural factors associated with microbial community composition. However, within the context of this thesis, these survey data were not included in inferential statistical analyses due to the limited sample size of the current dataset and were used only for descriptive and exploratory purposes. Statistical analyses in this thesis focused on differences in microbial composition between sample types (household sink, household drain, and office drain) and locations.

3.2 Sample collection

To optimise the methodology for this project, a pilot study was conducted in November 2025, including 12 households. During the pilot study, participants collected two samples from the kitchen sink surface, two from the sink drain and one tap water sample, resulting in five samples per household. Each sample was assigned a unique identification code (PB#####), linked to a household identifier (BE-AN#####).

Before sample collection, participants completed an online survey regarding household composition, dietary preferences, cleaning habits, sink design, general health and socio-demographic factors. These variables will be used in future analyses to identify potential factors influencing the microbial community composition of the kitchen sink. All participants provided informed consent in the survey. Another three samples were taken from the laboratory office kitchen sink drain at varying depths.

Participants who completed the online survey were provided with detailed sampling instructions (Supplementary Figure S1) and a standardised sampling kit to ensure consistency across households. The kit included a sample

registration form where participants could report any inconsistencies or problems that occurred during sampling. Participants were instructed to collect samples before the first use of the kitchen sink and without prior cleaning. Two different sample collection systems with sterile swabs were used: Copan eSwab, containing a transport medium for cultivation-based analyses, and Copan eNAT, containing a nucleic acid preservation medium for sequencing-based analyses. These collection systems have been used and proven effective in other citizen science studies on microbiome profiling, for example, in the Isala project (53).

For the main POEMBAKterie study, sampling was conducted between March and May 2026, in which a selection of members of 862 households located in the city of Antwerp collected their own kitchen sink samples and actively participated in the data collection for this study (54). The upper inner surface of the sink drain was selected as the sampling site for the main study because it represents a frequently wetted and easily reachable area. This area was sampled by rotating the swab along the inner drain surface for approximately 30 seconds while applying gentle pressure. In addition to the drain samples, a tap water sample was collected in a sterile collection tube and used as a negative control for cultivation-based analysis only, resulting in a total of three samples per household.

Following collection, participants transported the samples to designated collection points within 48 hours. From there, samples were collected daily and transported to the laboratory for processing. Upon arrival, samples were stored at 4°C and aliquoted as soon as possible into three aliquots of 300 µL each. eSwab and water samples were transferred into cryotubes containing 250 µL of a 50% glycerol solution, which served as a cryoprotectant to preserve bacterial viability for subsequent cultivation. The eNAT samples were aliquoted into sterile cryotubes without glycerol for long-term storage of nucleic acids and used for DNA-based analyses only. All aliquots were then stored at -80°C until further analysis.

3.3 Full-length 16S sequencing with Oxford Nanopore Technologies

3.3.1 DNA extraction

DNA extraction was performed on the first eNAT aliquots using the DNeasy PowerSoil Pro Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. This kit has previously been shown to provide high DNA yield and purity, along with a relatively long average read length, as well as consistent microbial community representation for full-length 16S rRNA sequencing (51). Samples were processed in batches of 10 to 22 samples per extraction run. Mechanical and chemical lysis were performed using mixed zirconium bead tubes and lysis buffer, followed by horizontal vortexing for ten minutes to facilitate disruption of microbial cell walls. The resulting crude lysate underwent inhibitor removal to eliminate compounds that may interfere with downstream PCR reactions. DNA was purified using a silica membrane spin-column binding in the presence of a high-concentration salt solution, followed by a two-step washing procedure to remove residual contaminants. The purified DNA was eluted using a 10 mM Tris buffer without salts and stored at -20°C until further analysis. The ZymoBIOMICS Microbial Community Standard (D6300), as well as the HM281 mock community, were included as positive extraction controls, while extraction reagents without added sample served as a negative extraction control to monitor extraction efficiency and potential contamination.

3.3.2 Library preparation

Two independent library preparation runs were performed for the pilot samples (Supplementary Table S1). The extracted DNA was thawed and DNA concentrations were quantified using a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, USA). Samples with low DNA concentrations were included in downstream PCR reactions without additional dilution.

The extracted DNA underwent two rounds of PCR amplification before sequencing. The first round of PCR amplified the targeted full-length 16S rRNA gene with incorporation of tailed primers to both ends of the gene (5' *TTTCTGTTGGTGCTGATATTGC-27F* 3' and 5' *ACTTGCCTGTCGCTCTATCTTC-1492R* 3'). The 5' overhangs facilitated the attachment of barcoding primers in the second PCR reaction (48,55). Cycling conditions consisted of an initial denaturation at 95°C for 3 minutes, followed by 23 amplification cycles of denaturation, annealing and extension at 95°C, 57°C and 72°C, respectively. Successful amplification was confirmed by gel electrophoresis, with an expected amplicon size of approximately 1500bp. Samples without visible bands were still retained for downstream analysis.

A second PCR reaction was performed to attach unique barcode sequences to each sample, allowing up to 96 sequencing libraries to be pooled and run together for high-throughput sequencing with ONT MinION. Cycling conditions consisted of an initial denaturation at 94°C for 3 minutes, followed by 18 amplification cycles of denaturation (94°C), annealing (56°C) and extension (65°C), followed by a final extension for 10 minutes at 65°C.

Following barcoding, products underwent end-repair and dA-tailing to prepare the DNA fragments for adapter attachment. After each PCR reaction and the end preparation reaction, the resulting amplicons were purified using magnetic AMPure XP Beads (Beckman Coulter, Brea, USA), and DNA concentrations were measured with a Qubit Fluorometer (Thermo Fisher Scientific, Waltham, USA). Samples were diluted as necessary before proceeding to the next part of the procedure. Finally, the adapter sequences, a leader and hairpin adapter, were attached and the end product was once more purified and quantified prior to sequencing. The ZymoBIOMICS Microbial Community Standard (D6306) was included as a positive control, while reagents without added sample were used as a negative extraction control.

3.3.3 16S rRNA sequencing

Amplicon sequencing of the full-length 16S rRNA gene was performed using the ONT MinION platform to enable species-level taxonomic classification. The 16S rRNA gene contains conserved and hypervariable regions that allow accurate differentiation between bacterial taxa. Sequencing of the full-length gene improves taxonomic resolution compared with short-read approaches and enables classification of more closely related bacterial species (47,48). Sequencing with ONT is based on the measurement of ionic current disruptions generated as DNA strands pass through protein nanopores embedded in a membrane under an applied voltage. These signal changes are subsequently translated into nucleotide sequences through real-time base-calling (52).

3.3.4 Bioinformatics pipeline

Raw signal data were base-called and demultiplexed using the Dorado super-accurate (sup) model. Initial quality control was performed using NanoPlot. Quality trimming of reads was performed using Chopper, applying a minimum quality score threshold of 10. Reads shorter than 1350 bp or longer than 1800 bp were excluded from downstream analyses. Barcodes that were not included in the sequencing run and had very low read counts were removed from downstream analyses. Quality control of the filtered reads was performed using NanoPlot to assess read-length distributions and overall quality metrics.

Filtered reads were subsequently analysed using the epi2me-labs/wf-16s workflow (v1.6.0), executed with Nextflow. Reads were aligned to a reference database using minimap2 (v2.28-r1209), and alignment files were processed using samtools (v1.22.1). Taxonomic classification based on the alignment files was performed using taxonkit (v0.20.0). Where applicable, abundance estimation was refined using Kraken2 (v2.1.3) and Bracken (v3.0.1). Taxonomic assignments were summarised, abundance tables were generated, and a report summarising sequencing and analysis results was provided for each run.

3.4 Identification of *Escherichia coli*

3.4.1 Cultivation on selective media

Selective media were used for the isolation and identification of *E. coli* as a potential opportunistic pathogen. Frozen eSwab and tap water samples obtained during the pilot study were cultured on MacConkey agar, a selective and differential medium that supports the growth of gram-negative enteric bacteria, particularly members of the order *Enterobacterales*, and allows differentiation based on lactose fermentation.

Using a sterile inoculation loop, a small amount of each sample was inoculated on the agar plates and spread using a five-quadrant streaking method, resulting in three plates per household. The plates were then inverted and incubated at 37°C for 18-24 hours under aerobic conditions. Following incubation, colony morphology was evaluated and presumptive lactose-fermenting colonies were selected. A maximum of five pink, well-isolated colonies were selected per plate and subcultured onto fresh agar plates using the same streaking and incubation conditions as before to obtain pure cultures for further identification. These plates were stored at 4°C for later analysis and replated prior to MALDI-TOF MS analysis to ensure fresh bacterial growth.

3.4.2 MALDI TOF-MS identification

Discrete colonies obtained through cultivation on the selective media were picked using sterile toothpicks and applied as thin films onto designated positions on a MALDI target plate in duplicate. Subsequently, 1 µL of α -cyano-4-hydroxycinnamic acid (HCCA) matrix solution was applied to each spot and allowed to air dry. The isolates were analysed using MALDI-TOF MS and a Bruker MALDI Biotyper (Bruker Daltonics).

The resulting mass spectra, representing protein fingerprints of the analysed organisms in the bacterial film, were compared to reference databases using the manufacturer's software, enabling microbial identification based on differences in protein composition among bacterial species. Identification scores were interpreted according to the manufacturer's criteria, where scores between 2.300 and 3.000 were considered highly probable species identification; scores between 2.000 and 2.299 indicated secure genus identification and probable species identification; scores between 1.700 and 1.999 indicated probable genus identification; and scores below 1.700 were considered unreliable. Isolates yielding scores below 2.000 for both duplicate measurements were replated and re-analysed using an additional formic acid application step before matrix application.

For downstream analysis, the highest identification score across technical duplicate measurements for the same isolate was retained, yielding an isolate-level dataset representing the best available identification for each cultured isolate (Supplementary Table S2). To account for multiple isolates originating from the same biological sample, MALDI-TOF MS results were additionally aggregated at the sample level (Supplementary Table S3). At this level, a species was considered present in a sample if it was detected in at least one isolate originating from that sample, regardless of replicate measurements or isolate frequency. This resulted in a biological sample-level dataset based on species occurrence per sample.

3.5 Data analysis

Statistical analysis and graphical visualisations were conducted on data obtained in the pilot study using RStudio in R (v4.4.2). Data processing, statistical analysis, and data visualisation were conducted primarily using packages from the tidyverse, in addition to vegan and rstatix. Sequencing runs were merged prior to downstream analysis, while retaining run-level metadata to assess potential batch effects. Control samples were excluded from downstream analyses and analysed separately to assess potential contamination and sequencing quality.

Taxonomic composition. Phylum- and genus-level abundance tables were converted to relative abundances by dividing read counts by the total number of classified reads per sample. Dominant bacterial taxa were identified based on mean relative abundance across all biological samples. For visualisation purposes, taxa with relative abundances below 1% at the phylum level and 1.5% at the genus level were grouped into an “Other” category.

Controls. Controls were taxonomically analysed at the genus level based on relative abundance tables generated from sequencing read counts. Dominant taxa within each control group (negative controls, ZymoBIOMICS Microbial Community Standards D6300-D6306, and mock community controls HM281A-HM281B) were identified based on the mean relative abundance across both controls per group. Because the HM281 mock community contains a defined set of expected bacterial species without predefined abundance proportions, additional species-level analyses were performed for HM281A and HM281B. Species-level reads were retained only when meeting the same quality filtering criteria applied in the species-level analysis of biological samples, excluding contaminant filtering. Expected species recovery, unexpected taxonomic detections, and reproducibility between replicate controls were assessed using species-level relative abundance profiles.

Differences in relative abundance. Exploratory comparisons of the 15 most abundant genera were conducted between sample types and sequencing runs. Differences between sample types were assessed using the non-parametric Kruskal-Wallis rank sum test, followed by Dunn’s post hoc test with Benjamini-Hochberg correction where appropriate. Differences between sequencing runs were assessed using a Mann-Whitney U test with a Benjamini-Hochberg correction.

Alpha diversity. Alpha diversity (i.e. within-sample diversity) was assessed at the genus level using observed richness, Shannon diversity and Simpson diversity metrics. Diversity metrics were calculated using both relative abundance normalisation and rarefaction to equal sequencing depth. Correlations between both approaches were evaluated using Pearson correlation analysis and showed strong agreement across all diversity metrics (Pearson’s $r = 0.97-1.00$; Supplementary Table S4). Relative abundance-based estimates were therefore retained for downstream analyses to avoid unnecessary data loss associated with rarefaction. Differences in alpha diversity between locations and sample types were evaluated using the Kruskal-Wallis test, followed by Dunn’s post hoc testing with Benjamini-Hochberg correction where applicable. Differences between sequencing runs were assessed using the Mann-Whitney U test with a Benjamini-Hochberg correction.

Beta diversity. Beta diversity (i.e. between-sample diversity) was assessed using Bray-Curtis dissimilarity metrics calculated from genus-level relative abundance data. Differences in bacterial community composition across locations, sample types and sequencing runs were evaluated using permutational multivariate analysis of variance (PERMANOVA) with 999 permutations. Separate models were first applied to each variable, followed by a combined model including all variables to assess their independent contributions while accounting for potential confounding effects. Where significant overall PERMANOVA results were observed, pairwise PERMANOVA comparisons with Benjamini-Hochberg correction were performed to identify differences between individual groups. Homogeneity of multivariate dispersion was assessed using betadisper for variables showing significant PERMANOVA effects.

Species-level analysis. Species-level taxonomic profiles were screened for bacterial species associated with foodborne infections and intoxications according to the Belgian Federal Public Service of Health, Food Chain Safety and Environment (56). Analyses were performed on sample-level taxonomic abundance tables generated by the wf-16S pipeline (v1.6.0). Species-level reads were retained only when they met the quality filtering criteria of $\geq 80\%$ coverage, as reported by the wf-16S workflow, and contained non-zero read counts. Reads were aggregated at the species level per sample, and relative abundances were calculated by normalising read counts to the total number of reads per sample. Putative contaminants were identified by comparing relative abundances of taxa between biological samples and negative controls. Taxa exhibiting higher relative abundance in negative controls than in biological samples were classified as contaminants and excluded from species-level downstream analyses. Positive controls were excluded from all analyses. A species was considered present in a sample when at least one read remained after filtering.

Comparison of cultivation- and sequencing-based detection. Cultivation-based MALDI-TOF MS identifications and sequencing-based taxonomic profiles were compared at the level of household x sample type x species. As both methods were performed on independent biological samples collected from the same household and sampling location, direct sample-to-sample pairing was not performed. Sequencing-based detections were converted to presence/absence following quality filtering and contaminant removal, as described in the species-level analysis section. MALDI-TOF MS identifications were aggregated per household and sample type, with species considered present when detected in at least one isolate. Matches were defined as detection of the same bacterial species by both methodologies within the same household x sample type combination. In addition to the overall comparison of analyses, the occurrence of sequencing-based *E. coli* detections without corresponding cultivation-based identification within the same household x sample type combination was evaluated.

Data validation and statistical assumptions. Normality of datasets was assessed using the Shapiro–Wilk test. As most variables were not normally distributed, non-parametric statistical methods were applied throughout the analyses. Statistical significance was defined as $p < 0.05$.

Survey data analysis. Given the limited sample size, the survey data were analysed descriptively and were not used for statistical analyses. Responses were summarised to characterise the participant population and explore trends in household and demographic variables.

4 Results

4.1 Sample collection and processing

The POEMBAKterie project included 875 participating households. Of these, 862 households were part of the main sampling campaign conducted between March and May 2026, while 12 households participated in an earlier pilot study.

During the main sampling campaign, 862 sampling kits were prepared and distributed to households across the city of Antwerp. Of these, 754 kits were delivered by bicycle, while the remaining kits were sent by post. By the beginning of June 2026, samples had been returned from 87.9% ($n = 758$) of participating households (Figure 3). In the main sampling campaign, each household received a kit containing three sampling tubes. For each returned sample, up to three aliquots were prepared and stored for future analyses. Based on this sampling design, a maximum of 6,822 aliquots could be generated. The actual number of aliquots was slightly lower, as some samples contained insufficient liquid volume to prepare all three aliquots.

The analyses presented in this thesis were performed on a subset of samples collected during the pilot phase of the POEMBAKterie project. A total of 12 households were included, each contributing up to five sample types: sink drain and sink surface samples for both sequencing and cultivation, and a water tube sample for cultivation. One household was missing the sink surface sample for sequencing. For full-length 16S rRNA gene sequencing, 23 household samples were analysed (12 sink drain samples and 11 sink surface samples), along with three additional drain samples collected from the office kitchen at the Laboratory of Medical Microbiology (LMM), resulting in a total of 26 sequencing samples. For cultivation-based analyses, all 12 households contributed sink drain and sink surface samples, as well as water tube samples, resulting in a total of 36 samples.

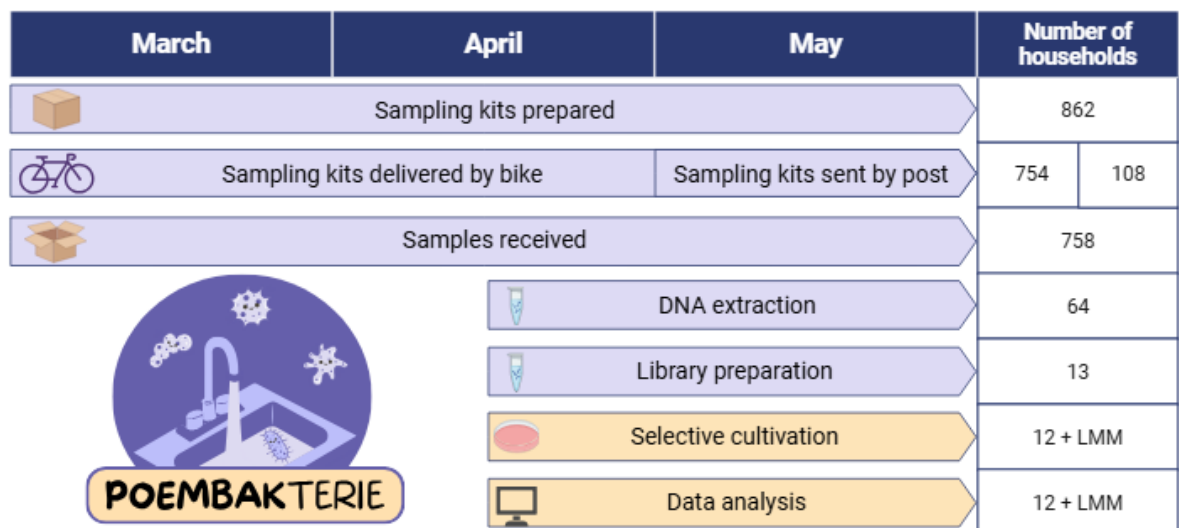


Figure 3. Summary of internship activities conducted between March and May, including the number of households per activity. The first five arrows represent the main study (purple), while the final two arrows (yellow) correspond to the pilot study. LMM refers to the three samples taken from the Laboratory of Medical Microbiology office kitchen sink drain.

4.2 Sequencing output and quality control

Sequencing was performed on the ONT MinION platform across two independent runs, including 26 biological samples, four positive controls, and two negative controls (Supplementary Table S1). Following basecalling and demultiplexing, a total of 5,086,812 reads, corresponding to 7,470,062,042 bases, were obtained across both

runs. Read length distributions were consistent with the expected full-length 16S rRNA gene amplicons (+/- 1500bp), indicating successful amplification and sequencing of target fragments.

Sequencing yield differed slightly between runs, with Run 1 generating 2,623,563 reads and Run 2 producing 2,463,249 reads (Table 1). In Run 1 and Run 2, respectively, 98.6% and 97.1% of reads exceeded a Q10 threshold. Lower-quality reads and sequences outside the expected amplicon length range were excluded from downstream analyses. After quality filtering and trimming, 4,727,963 reads remained for subsequent analysis. Mean read lengths were 1475.6bp for Run 1 and 1479.9bp for Run 2, with corresponding N50 values of 1484bp and 1485bp, respectively. Basecall quality scores were comparable across runs, with mean quality scores of 21.6 (Run 1) and 21.0 (Run 2). Negative controls yielded substantially fewer reads than positive controls and experimental samples (Supplementary Table S1).

Table 1. Summary of sequencing yield and quality metrics for Run 1 and Run 2.

Metric	Run 1	Run 2
Raw reads	2,623,563	2,463,249
Reads after filtering	2,549,526	2,178,437
Reads retained (%)	97.18	88.44
Mean read length (bp)	1475.6	1479.9
Mean quality score	21.6	21.0

4.3 Taxonomic composition

4.3.1 Kitchen sink samples are dominated by *Pseudomonadota*

Taxonomic classification identified members belonging to 28 bacterial phyla and 905 bacterial genera across all kitchen sink samples. At the phylum level, microbial communities were dominated by *Pseudomonadota* (47.85%), followed by unclassified bacteria (21.14%), *Actinomycetota* (17.04%), *Bacillota* (7.91%) and *Bacteroidota* (4.87%) (Figure 4A). All remaining phyla showed a mean relative abundance below 1% and were therefore grouped in the “Other” category for visualisation.

Genus-level analysis revealed the most abundant genera (mean relative abundance >1.5% across all samples) included unclassified bacteria (21.14%), *Microbacterium* (12.74%), *Acinetobacter* (12.03%), *Paracoccus* (11.51%), *Pseudomonas* (3.11%), *Epilithonimonas* (2.56%), *Moraxella* (2.54%), *Pseudoxanthomonas* (1.81%), *Brochothrix* (1.58%) and *Stenotrophomonas* (1.56%) (Figure 4B). Genera with mean relative abundances below 1.5% were grouped into the “Other” category.

When stratified by sample type, visual inspection showed a relatively consistent community composition in office drain samples across different sampling depths. In household samples, differences were observed between drain and surface communities. Drain samples exhibited higher relative abundances of *Microbacterium* and *Paracoccus* compared to the sink surface samples. Household sink surface communities were more diverse in composition and showed a comparatively higher relative abundance of *Acinetobacter* (Figure 4B). In addition, two sink surface samples (PB000021 and PB000026) had a high relative abundance of *Brochothrix* compared to other samples, while sample PB000011 had a relatively high *Pseudomonas* abundance.

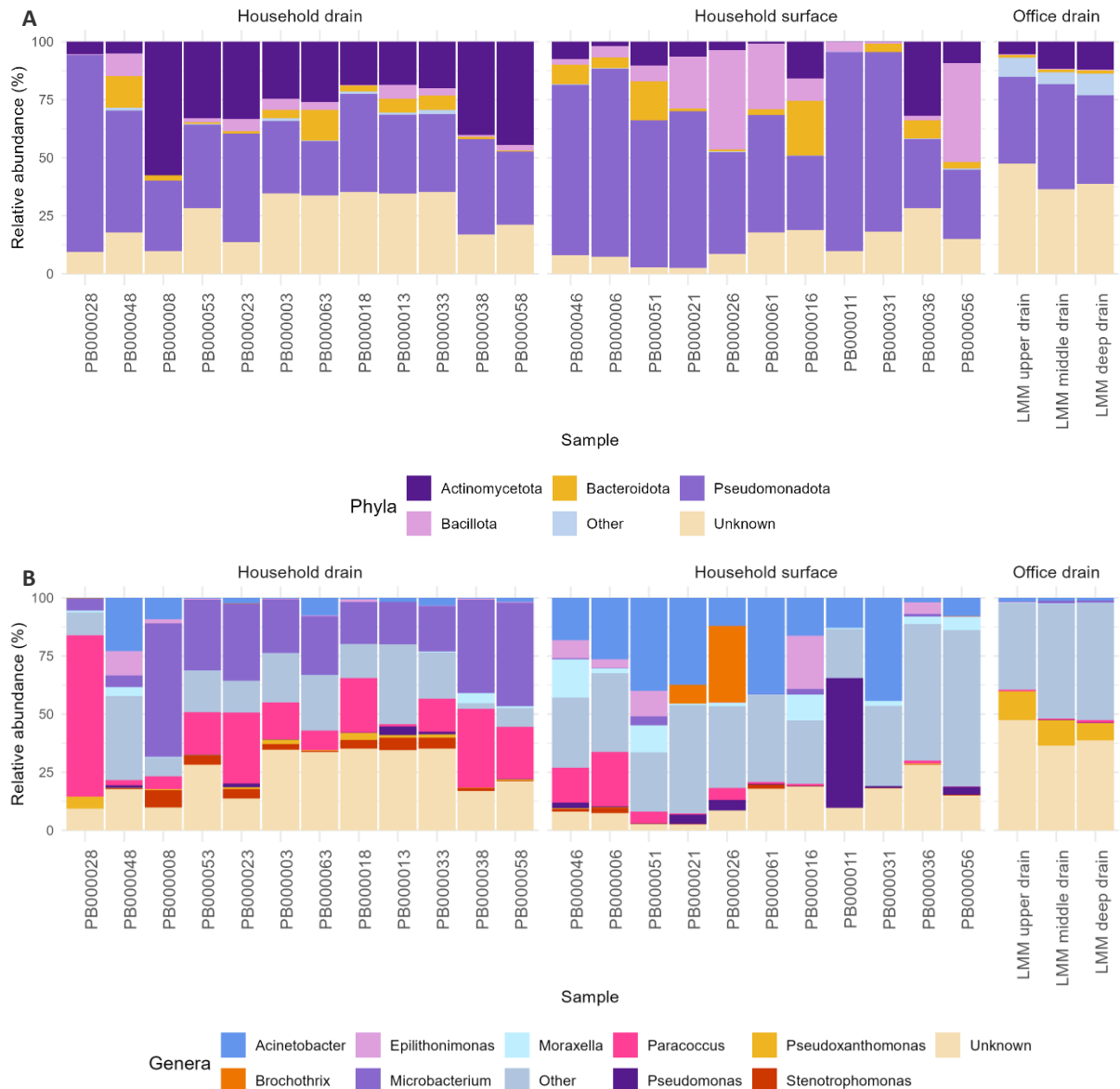


Figure 4. Relative abundance (%) per sample of (A) the five most abundant bacterial phyla and (B) the ten most abundant genera, selected based on their mean relative abundance across all samples. “Unknown” represents the sequences not identified at the genus level, whereas “Other” represents phyla with mean relative abundances below 1% and genera with a mean relative abundance below 1.5%, grouped into a single category.

4.3.2 The relative abundance of dominant genera differs between sample types

To assess genus-level differences between sample types, Kruskal-Wallis tests were performed on the 15 most abundant genera. Significant differences between sample types were observed for 11 of the 15 analysed genera, including *Acinetobacter* ($p = 0.0009$), *Comamonas* ($p = 0.037$), *Exiguobacterium* ($p = 0.025$), *Kocuria* ($p = 0.0005$), *Microbacterium* ($p = 0.00007$), *Moraxella* ($p = 0.0098$), *Paracoccus* ($p = 0.0038$), *Pseudoxanthomonas* ($p = 0.0002$), *Stenotrophomonas* ($p = 0.0018$), *Streptococcus* ($p = 0.00022$) and unclassified bacteria ($p = 0.0018$).

Post hoc Dunn tests with Benjamini-Hochberg correction identified several significant pairwise differences between sample types (Table 2). Genera including *Acinetobacter*, *Kocuria*, *Moraxella*, *Pseudoxanthomonas* and unclassified bacteria (“Unknown”) showed significantly different relative abundances between sink surface samples and both household drain and office drain samples. Additionally, the relative abundance of *Microbacterium* and *Streptococcus* differed significantly between household drain samples and sink surface

samples. The relative abundance of the *Exiguobacterium* differed significantly between household sink surface and office drain samples. Moreover, the relative abundance of *Paracoccus* and *Stenotrophomonas* differed significantly between household drains and the two other sample types. Although the Kruskal-Wallis test indicated significant overall differences in the relative abundance of *Comamonas* across sample types, no pairwise comparisons remained significant following Dunn post hoc testing with Benjamini-Hochberg correction.

Table 2. Statistical comparison of the 15 most abundant bacterial genera across samples using Kruskal-Wallis and Dunn post hoc tests. The table includes overall Kruskal-Wallis p-values and Benjamini-Hochberg-adjusted p-values for pairwise Dunn post hoc comparisons between office drain, household drain and household sink surface samples.

Genera	Kruskal-Wallis p-value	Dunn adjusted p-value		
		Household drain vs Household surface	Household drain vs Office drain	Household surface vs Office drain
<i>Acinetobacter</i>	0.000929	0.0021	0.6608	0.0136
<i>Brochothrix</i>	0.128	NA	NA	NA
<i>Comamonas</i>	0.0372	0.0865	0.0776	0.3214
<i>Epilithonimonas</i>	0.373	NA	NA	NA
<i>Exiguobacterium</i>	0.0251	0.154	0.1188	0.0239
<i>Kocuria</i>	0.000505	0.0025	0.273	0.0025
<i>Microbacterium</i>	0.0000721	0.0001	0.0502	0.523
<i>Moraxella</i>	0.00978	0.0238	0.5547	0.0333
<i>Paracoccus</i>	0.00375	0.0109	0.0206	0.5634
<i>Pseudomonas</i>	0.0679	NA	NA	NA
<i>Pseudoxanthomonas</i>	0.000202	0.0034	0.0914	0.0008
<i>Rheinheimera</i>	0.122	NA	NA	NA
<i>Stenotrophomonas</i>	0.00182	0.0305	0.0032	0.0788
<i>Streptococcus</i>	0.000217	0.0002	0.6008	0.0579
Unknown	0.00177	0.0379	0.0522	0.0024

Visual inspection of the genus-level abundance distributions (Figure 5) indicated that several genera were enriched in specific sample types. *Pseudoxanthomonas* was more abundant in office drain samples, whereas *Acinetobacter* exhibited comparatively higher relative abundances in household sink surface samples. *Microbacterium*, *Paracoccus* and *Stenotrophomonas* were more abundant in household drain samples. Unclassified bacteria showed the highest relative abundance in office drain samples, followed by sink drain samples, with the lowest relative abundance in household sink surface samples. Several genera also displayed considerable variability between individual samples within the same sample type. Overall, drain-associated microbiomes were more compositionally similar to each other at the genus level than to sink-surface microbiomes.

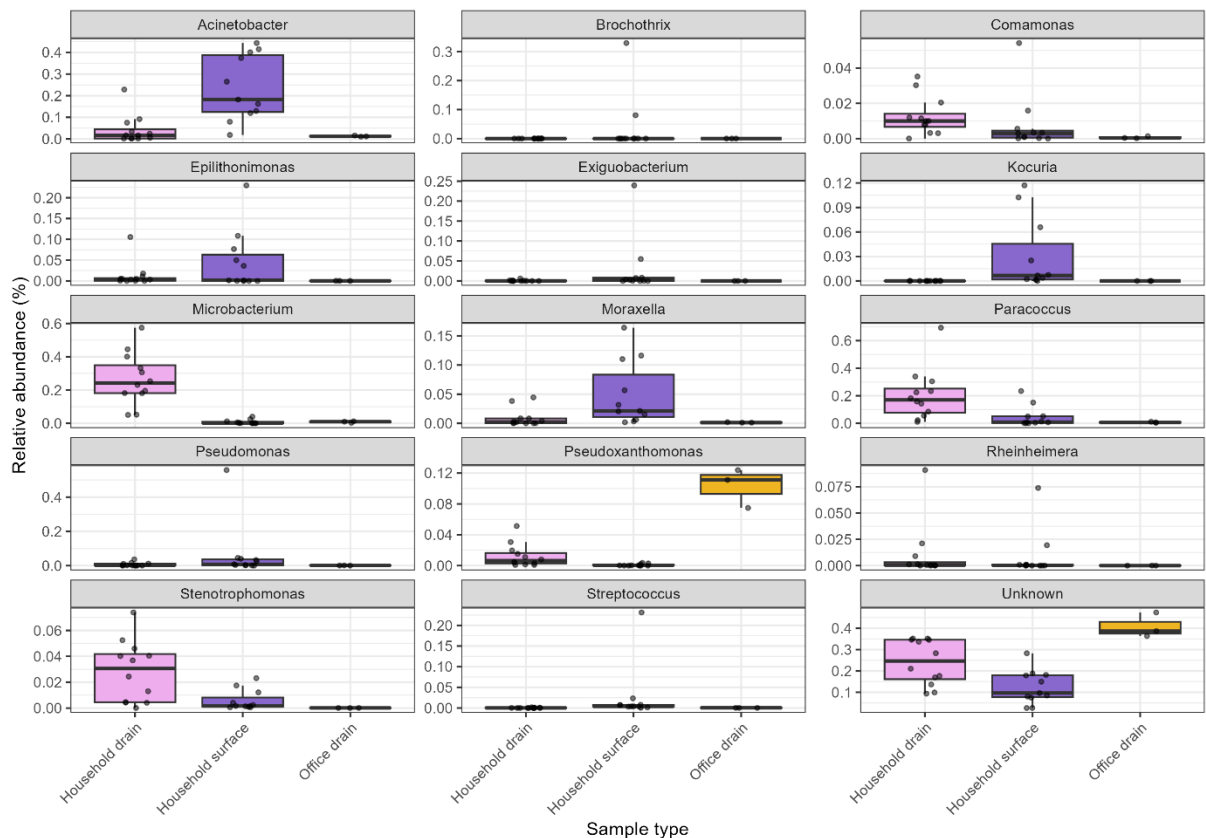


Figure 5. Genus-level abundance distributions of the 15 most abundant bacterial genera across sample types. Boxplots show relative abundance distributions for office drain, household drain and household sink surface samples. Each panel represents a single genus.

An additional Mann-Whitney U test was performed to compare sequencing runs. After Benjamini-Hochberg correction, no significant differences were observed between runs for any of the most abundant genera, indicating limited run-associated variation in the dataset.

4.3.3 Taxonomic composition of positive and negative controls

Negative controls showed low read counts. The negative control of sequencing run 2 was dominated by *Shigella*, while the negative control from run 1 had a more diverse genus composition, although *Shigella* remained the predominant genus. No substantial overlap was observed between dominant taxa in negative controls and the most abundant taxa in the biological samples. Positive control samples from DNA extraction and library preparation, D6300 and D6306, respectively, were dominated by the expected mock community genera *Listeria*, *Pseudomonas*, *Bacillus*, *Escherichia*, *Salmonella*, *Limosilactobacillus*, *Enterococcus* and *Staphylococcus* (Figure 6). However, *Escherichia* and *Pseudomonas* were detected at lower relative abundances than expected based on the theoretical mock community composition.

Positive controls HM281A and HM281B demonstrated consistent genus- and species-level taxonomic profiles across the two sequencing runs. Expected mock community genera, including *Acinetobacter*, *Bacillus*, *Clostridium*, *Enterococcus*, *Lactobacillus*, *Listeria*, *Staphylococcus* and *Streptococcus*, were recovered in both controls. In addition, the unexpected genus *Cutibacterium* was detected among the ten most abundant genera. At the species level, 65% and 75% of the expected species in the mock community were recovered in HM281A and HM281B, respectively. Relative abundances were broadly comparable between sequencing runs, with a mean inter-run abundance difference of 0.14%, indicating good reproducibility of the sequencing and bioinformatics workflow.

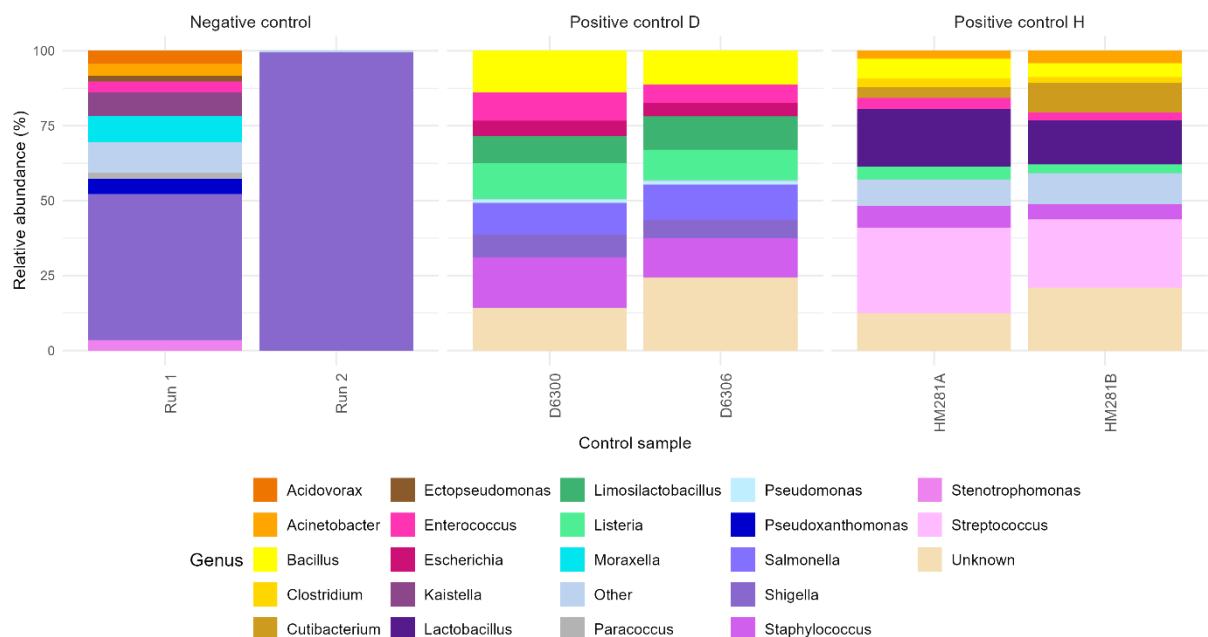


Figure 6. Relative abundance of the 10 most abundant bacterial genera detected in negative and positive control samples across sequencing runs. Genera were selected based on mean relative abundance within each control group.

4.4 Shannon diversity differs between sample types

Alpha diversity was assessed at the genus level based on relative abundance tables and included Shannon diversity, Simpson diversity and richness metrics. Across all samples, Shannon diversity ranged from 1.32 to 2.96, Simpson diversity ranged from 0.50 to 0.90 and richness ranged from 96 to 359 detected genera.

No significant differences in alpha diversity were observed between sequencing runs for any metric, indicating limited batch effects. Similarly, no significant differences were detected between locations for Shannon diversity, Simpson diversity or richness. In contrast, significant differences in Shannon diversity were observed among sample types (Kruskal-Wallis test, $p = 0.033$), whereas Simpson diversity and richness did not differ significantly among groups (Table 3). Dunn post hoc testing identified nominally significant differences in Shannon diversity between household drain samples and both other sample types; these differences did not remain significant after Benjamini-Hochberg correction for multiple testing (Supplementary Table S5).

Table 3. P-values for different alpha diversity metrics (Shannon diversity, Simpson diversity and richness) for sample-type and household (Kruskal-Wallis test) and run (Mann-Whitney U test with Benjamini-Hochberg correction).

P-value	Sample type	Location	Run
Shannon diversity	0.033	0.900	0.668
Simpson diversity	0.137	0.882	0.817
Richness	0.058	0.609	0.959

Visual inspection of alpha diversity distributions revealed variation in median diversity values between sample types. Shannon diversity was highest in office drain samples, followed by household sink surface and household drain samples. A similar pattern was observed for richness. In contrast, Simpson diversity showed the highest median values in household sink surface samples. Overlap between groups was observed across all diversity metrics (Figure 7A).

Across locations, household BE-AN0010 exhibited the highest median Shannon diversity, closely followed by the LMM office drain samples. The remaining households showed lower median diversity values. These two locations also had the highest median Simpson diversity values, but the LMM office drain samples had a median value closer to the other households than to BE-AN0010. Regarding the richness medians, the LMM office had the most genera, although one of the BE-AN0006 samples had the highest overall richness. Substantial within-household variability was observed. One household in particular (BE-AN0006) showed substantial differences between the two samples across all alpha diversity metrics (Figure 7B).

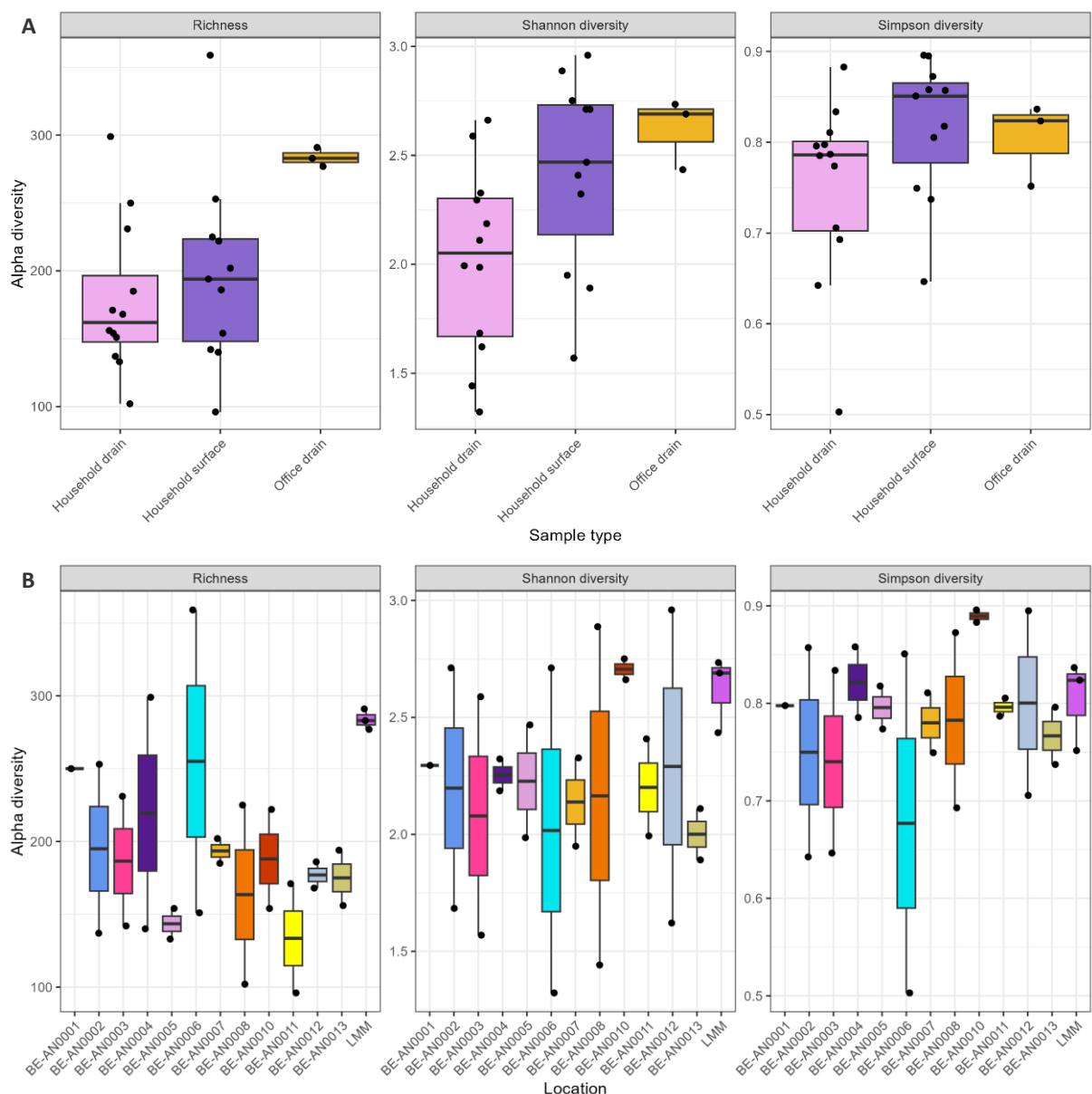


Figure 7. Alpha diversity of the genus-level microbial communities detected in Antwerp households (n = 12) and one laboratory office (LMM) by (A) sample type and (B) participant or sampling location as represented by Shannon's diversity, Simpson's diversity and richness index parameters.

4.5 Microbial community composition differs between sample types

Non-metric multi-dimensional scaling (NMDS) ordination resulted in a stress value of 0.13, indicating an acceptable two-dimensional representation of community dissimilarities. Visual inspection of the NMDS ordination revealed partial clustering according to sample type (Figure 8A). In particular, office drain samples formed a relatively distinct cluster, whereas household drain and sink surface samples showed a slight overlap.

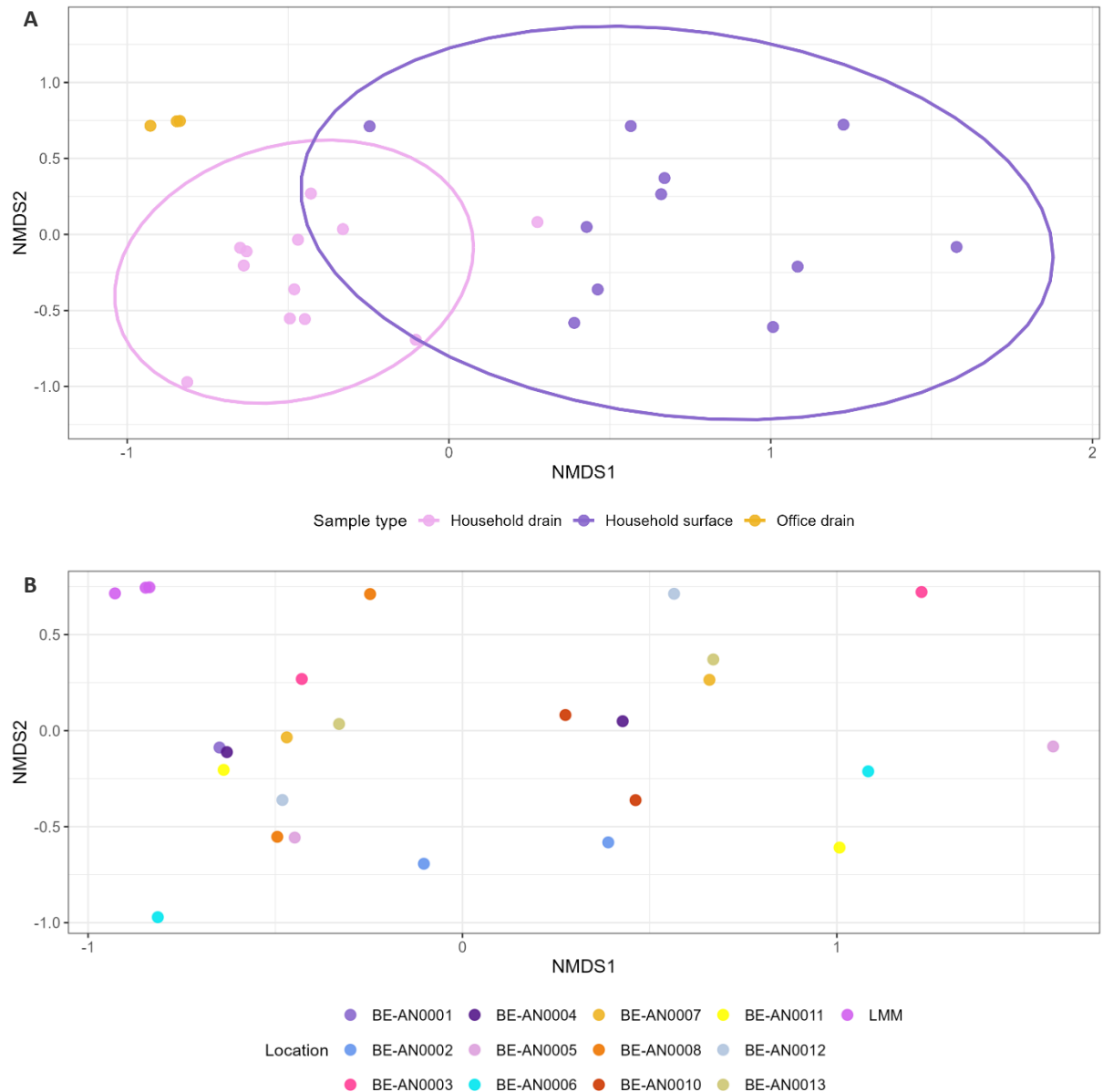


Figure 8. Non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarity of the genus-level microbial communities detected in Antwerp households (n = 12) and one laboratory office (LMM) by sample type (A) and sampling location (B).

PERMANOVA analysis indicated that sample type explained a significant proportion of variation in microbial community composition ($R^2 = 0.395$, $p = 0.001$). In contrast, no significant differences in community composition were observed between locations or runs (Table 4). Similar patterns were observed in the NMDS ordination, where no clear clustering according to these variables was detected. A combined PERMANOVA model including household, sample type and sequencing run confirmed that sample type remains significantly associated with microbial community composition after accounting for potential confounding effects ($R^2 = 0.231$, $p = 0.001$). Pairwise PERMANOVA comparisons identified significant differences in community composition between all

combinations of sample types. The largest pairwise effect size was observed between office drain and household drain samples ($R^2 = 0.373$), whereas the remaining pairwise comparisons showed similar effect sizes (Table 5).

Test results for homogeneity of multivariate dispersion indicated significant differences in within-group variability across sample types ($p = 0.002$). This suggests that microbial composition differed not only between sample types, but also in the degree of variation observed within each sample type.

Table 4. Results of PERMANOVA testing the effects of sample type, household and sequencing run on microbial community composition based on Bray-Curtis dissimilarities. Reported values include R^2 values and associated p-values.

Variable	R^2	P-value
Type only	0.395	0.001
Run only	0.040	0.379
Household only	0.478	0.520
Type combined model	0.231	0.001
Run combined model	0	NA
Household combined model	0.250	0.447

Table 5. Pairwise PERMANOVA results comparing microbial community composition between sample type groups based on Bray-Curtis dissimilarities. Reported values include R^2 and p-values, as well as Benjamini-Hochberg adjusted p-values.

Group1	Group2	R^2	P-value	Adjusted p-value
Office drain	Household drain	0.373	0.002	0.003
Office drain	Household surface	0.318	0.003	0.003
Household drain	Household surface	0.295	0.001	0.003

4.6 Potential opportunistic foodborne bacterial species are detected at low relative abundance across kitchen sink samples

Samples were screened for 17 bacterial species associated with foodborne infections and intoxications, of which ten target species were detected across the dataset ($n = 26$) (Figure 9). Target species were identified in all but two samples, one office drain sample and one household drain sample.

The most frequently detected species was *Bacillus cereus*, which was present in 84.6% of samples ($n = 22$), including 10 household drain samples, 10 household surface samples and 2 office drain samples. Other frequently detected species included *Salmonella enterica* (61.5%), *Shigella boydii* (50.0%), *Escherichia coli* (42.3%) and *Shigella dysenteriae* (34.6%). In general, these species were more frequently detected in drain samples than in surface samples. *Staphylococcus aureus* was detected exclusively in sink surface samples.

Household drain samples showed the highest mean number of detected target species per sample (4.17 ± 1.59), followed by household surface samples (3.00 ± 1.41), whereas office drain samples showed lower numbers of detected target species (1.00 ± 1.00). Several species were detected only sporadically, including *Campylobacter jejuni* and *Vibrio parahaemolyticus*, which were each detected in a single sample. Overall, detections were heterogeneous across samples, with no single species detected in all samples. Detected targets were generally present at low relative abundances, with 98.8% of detections occurring at <1% relative abundance in the sample in which they were detected. Only one detection exceeded this threshold (2.86%), indicating that potential opportunistic pathogen occurrences were predominantly low-level across samples.

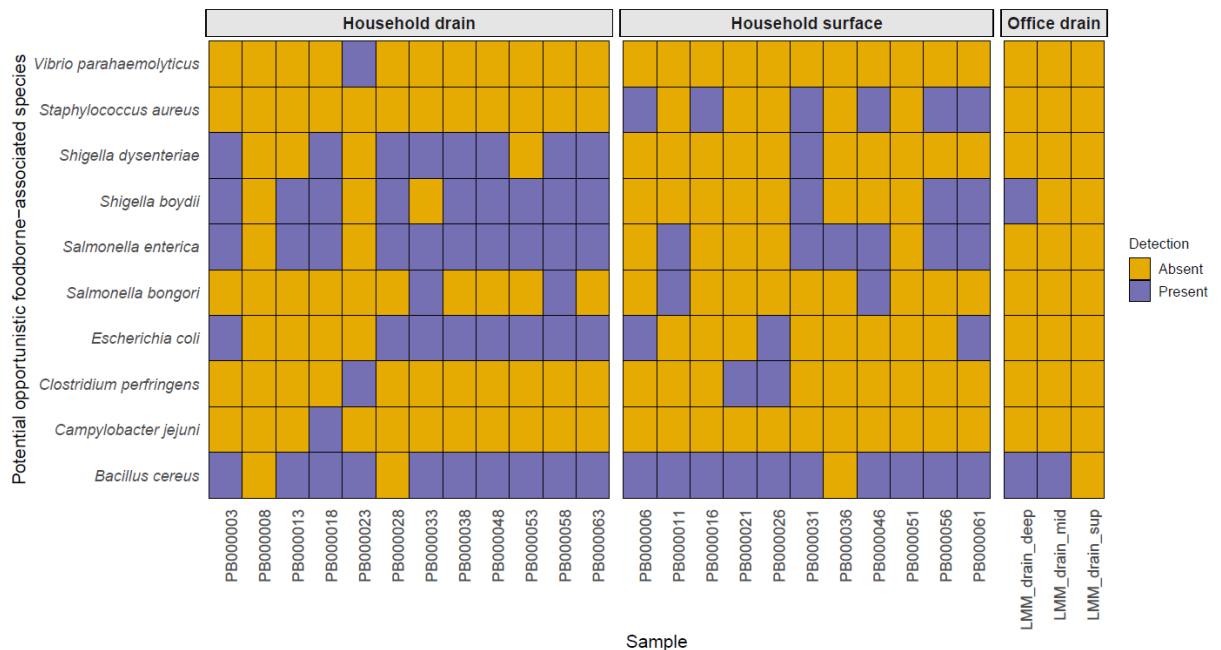


Figure 9. Presence of potential opportunistic foodborne species. Heatmap showing presence (purple) or absence (yellow) of selected bacterial species associated with foodborne infections and intoxications across samples based on species-level 16S rRNA gene sequencing data. Samples are grouped by sample type (sink surface, sink drain, and office drain).

4.7 Comparison of culture-based and sequencing-based detection

4.7.1 Viable *E. coli* is detected in household kitchen sinks using cultivation-based methods

Growth on MacConkey agar was observed in 18 out of 36 inoculated samples, none of which originated from water tube samples. All households exhibited growth on MacConkey agar in at least one sample. In six households, both sink surface and sink drain samples showed growth, whereas in five households, growth was detected only in drain samples and in one household, only in the sink surface sample.

A total of 48 bacterial isolates obtained from selective cultures were analysed using MALDI-TOF MS over two technical runs. Over these two runs, all isolates were measured in duplicate. During the initial MALDI-TOF MS analysis, 20.8% ($n = 10$) of isolates achieved genus-level identification (score 1.700–1.999), while another 20.8% ($n = 10$) yielded unreliable identification (score <1.700). These 20 isolates were thus replated to ensure fresh cultures and re-analysed in a second MALDI-TOF MS experiment.

Following quality control of the second MALDI-TOF experiment, technical duplicate measurements were consolidated by retaining the highest identification score for each isolate (Supplementary Table S2). Based on the obtained identification scores after consolidation of both analyses, 72.9% ($n = 35$) of isolates were identified at the species level (score ≥ 2.000), of which 10.4% ($n = 5$) achieved highly probable species identification (score ≥ 2.300). Additionally, 12.5% ($n = 6$) of isolates were identified at the genus level (score 1.700–1.999), while 14.6%

(n = 7) yielded unreliable identification (score <1.700) (Table 6). Species-level frequency analysis was restricted to isolates with identification scores ≥ 2.000 . At the isolate level, the most frequently identified species were *Escherichia coli* (n = 16), *Klebsiella oxytoca* (n = 6) and *Pantoea agglomerans* (n = 2).

Table 6. Percentages of isolates within the MALDI-TOF MS identification score ranges at the isolate level for the two separate analytical runs and the combined best-score dataset.

Score	Run 1	Run 2	Consolidated
≥ 2.300	10.4%	0%	10.4%
≥ 2.000	58.3%	35%	72.9%
1.700–1.999	20.8%	10%	12.5%
<1.700	20.8%	55%	14.6%

For biological sample-level analysis, only MALDI-TOF MS identifications with species-level identification scores (≥ 2.000) were retained (n = 25). Among these retained detections, 20% (n = 5) achieved highly probable species-level identification scores (≥ 2.300). Across the 17 biological samples, MALDI-TOF MS identified 14 distinct species, corresponding to 25 species detections across samples. In sink surface samples, 10 distinct species, accounting for 11 of the 14 present species detections, were observed. In contrast, in sink drain samples, 5 distinct species, corresponding to 14 of the 14 present species detections, were identified. Across all biological samples, the most frequently detected species were *Escherichia coli*, detected solely in sink drain samples (n = 8), and *Klebsiella oxytoca*, detected in both sink drain (n = 3) and sink surface samples (n = 2). All remaining species were detected in only one sample.

4.7.2 Cultivation and sequencing show partial agreement in detecting sink-associated bacteria

Comparison of cultivation-based MALDI-TOF MS identifications with sequencing-based taxonomic profiles demonstrated 19 matching species-level detections out of the 25 retained MALDI-TOF MS identifications at the biological sample level (Table 7). Matches were defined as detection of the same species by both methods within the same household and sample type combination. Overall, six MALDI-TOF MS identifications were not detected in sequencing data (Table 8). Of these, one identification could not be matched due to the absence of a corresponding sequencing sample (sink surface, household BE-AN0001). Another unmatched identification was attributed to a genus-level MALDI-TOF MS result, which could therefore not be matched at the species level (PB000005 A, Supplementary Table S3). At the household level, one household (BE-AN0002) showed no exact matches between MALDI-TOF MS and sequencing-based species identifications, including the genus-level MALDI-TOF MS result that could not be matched to sequencing data.

Among the unmatched MALDI-TOF MS identifications, two corresponded to *E. coli* identifications, both originating from drain samples. Sequencing data additionally identified five *E. coli* occurrences that were not detected by MALDI-TOF MS (Table 8). Of these, two originated from household drain samples in households where no corresponding MALDI-TOF MS identification of *E. coli* was observed. In contrast, the three remaining sequencing-only *E. coli* detections were obtained from household sink surfaces and belonged to households where *E. coli* was detected by MALDI-TOF MS in the sink drain samples. However, no direct match was observed at the same household x sample type combination. One of these indirect matches was from household BE-AN0002, which had no exact matches.

Table 7. Exact species-level matches between sequencing-based (full-length 16S rRNA gene) and cultivation-based (MALDI-TOF MS) identifications, aggregated by household, sample type and species.

Household	MALDI sample	Sequencing sample	Species	Type
BE-AN0001	PB000002	PB000003	<i>Escherichia coli</i>	Household drain
BE-AN0003	PB000010	PB000011	<i>Citrobacter freundii</i>	Household surface
BE-AN0003	PB000010	PB000011	<i>Klebsiella oxytoca</i>	Household surface
BE-AN0004	PB000015	PB000016	<i>Raoultella ornithinolytica</i>	Household surface
BE-AN0005	PB000022	PB000023	<i>Lelliottia amnigena</i>	Household drain
BE-AN0006	PB000027	PB000028	<i>Escherichia coli</i>	Household drain
BE-AN0007	PB000032	PB000033	<i>Klebsiella oxytoca</i>	Household drain
BE-AN0007	PB000030	PB000031	<i>Aeromonas media</i>	Household surface
BE-AN0007	PB000030	PB000031	<i>Klebsiella oxytoca</i>	Household surface
BE-AN0007	PB000030	PB000031	<i>Leclercia adecarboxylata</i>	Household surface
BE-AN0008	PB000037	PB000038	<i>Escherichia coli</i>	Household drain
BE-AN0010	PB000047	PB000048	<i>Acinetobacter pittii</i>	Household drain
BE-AN0011	PB000052	PB000053	<i>Escherichia coli</i>	Household drain
BE-AN0012	PB000057	PB000058	<i>Escherichia coli</i>	Household drain
BE-AN0012	PB000057	PB000058	<i>Klebsiella oxytoca</i>	Household drain
BE-AN0013	PB000062	PB000063	<i>Escherichia coli</i>	Household drain
BE-AN0013	PB000062	PB000063	<i>Klebsiella oxytoca</i>	Household drain
BE-AN0013	PB000060	PB000061	<i>Enterobacter hormaechei</i>	Household surface
BE-AN0013	PB000060	PB000061	<i>Serratia nematodiphila</i>	Household surface

Table 8. Unmatched species-level identifications from MALDI-TOF MS identifications and full-length 16S rRNA sequencing (*Escherichia coli*). Including household, sample ID, species identification and sample type.

Unmatched MALDI-TOF MS identifications			
Household	Sample	Species	Type
BE-AN0001	PB000000	<i>Pantoea agglomerans</i>	Household surface
BE-AN0002	PB000005	<i>Pantoea sp</i> (genus)	Household surface
BE-AN0002	PB000007	<i>Escherichia coli</i>	Household drain
BE-AN0002	PB000007	<i>Klebsiella pneumoniae</i>	Household drain
BE-AN0003	PB000010	<i>Escherichia hermannii</i>	Household surface
BE-AN0004	PB000017	<i>Escherichia coli</i>	Household drain
Unmatched full-length 16S sequencing <i>E. coli</i> detections			
Household	Sample	Species	Type
BE-AN0002	PB000006	<i>Escherichia coli</i>	Household surface
BE-AN0006	PB000026	<i>Escherichia coli</i>	Household surface
BE-AN0007	PB000033	<i>Escherichia coli</i>	Household drain
BE-AN0010	PB000048	<i>Escherichia coli</i>	Household drain
BE-AN0013	PB000061	<i>Escherichia coli</i>	Household surface

4.8 Household characteristics and kitchen sink usage

One household (BE-AN0003) did not complete the full survey and was therefore excluded from the survey analysis. In addition, one household submitted the questionnaire twice; only the most recent submission was retained. All participants of the pilot study were Belgian and had obtained a higher education degree. Most participants lived with a partner, while one participant was a single parent. Ten households had children and seven had animals. Most households reported no recent acute infections ($n = 8$), allergies ($n = 7$) or chronic illnesses ($n = 6$) among household members, although gastrointestinal problems were reported in six households. Most participants worked full-time ($n = 7$) and some part-time ($n = 2$), with employment distributed across diverse professional sectors.

Among the eleven households included in the survey analysis, sink configurations vary between single-basin sinks ($n = 3$) and sinks with one large and one small basin ($n = 8$). All kitchens were equipped with a cooking hood for ventilation, while eight households also reported frequently opening windows and two households had additional mechanical ventilation. Sink age varied across households, with some sinks being newer than five years and others older than 15 years. Most sinks were stainless steel ($n = 9$), while a minority were plastic ($n = 2$). Drain filters were present in nearly all households ($n = 10$). Most sinks drained efficiently, although some households occasionally experienced slower drainage. Kitchen temperatures were generally reported between 18–22°C.

Sink use frequency ranged from 3-5 times per day (n = 3) to more than 5 times per day (n = 8). All households used the sink to wash dirty dishes, food products, and hands. Additional uses included caring for flowers and plants (n = 9) and refreshing pet water bowls (n = 5). Dishwashing practices varied between households, with most relying primarily on dishwashers (n = 8) and others combining dishwasher use with manual washing (n = 3). Air-drying of sinks after use was commonly reported. Sink cleaning frequency varied from multiple times a day to monthly. All households reported using dishcloths during sink cleaning activities. Some households additionally used sponges (n = 7) and dishwashing brushes (n = 5). Food preparation practices also varied. Households prepared fruits and vegetables daily or multiple times a day and reported washing fruits and vegetables before use. Meat was prepared multiple times a week, except for one household that prepared it seldom. Most households did not wash raw meat (n = 10) or its packaging (n = 8), although cleaning practices for utensils and preparation materials ranged from always (n = 6) to sometimes (n = 4) to never (n = 1). Fish preparation and associated cleaning practices showed greater variability across participants. All households prepared milk products or eggs at least once a week.

5 Discussion

This study provides a characterisation of the bacterial microbiome in household kitchen sinks in Antwerp using full-length 16S rRNA gene sequencing combined with cultivation-based detection of opportunistic bacteria, with a specific focus on *E. coli*. This approach enabled the simultaneous assessment of overall microbial community structure and the detection of viable opportunistic bacteria, offering a more complete perspective on sink-associated microbiota than either method alone.

The findings suggest that household kitchen sinks harbour diverse microbial communities, dominated by bacterial phyla commonly reported in built-environment microbiomes, specifically *Pseudomonadota*, *Actinomycetota*, *Bacillota* and *Bacteroidota*. At the genus level, many of the detected taxa have previously been associated in the literature with water systems, food-associated environments, and human-associated microbiota, highlighting the multisource nature of sink microbial communities. A key finding is the significant variation between sample types, suggesting that microhabitat plays a role in shaping microbial communities. Although some variation between households was observed, the limited number of samples per household restricts the ability to draw definitive conclusions about household-specific effects.

Both sequencing and cultivation approaches detected taxa containing opportunistic pathogens, including viable *E. coli*. However, the study design did not allow assessment of infection potential, virulence, or antimicrobial resistance. The partial overlap between sequencing and cultivation results underscores the importance of combining complementary methods: sequencing captures broad community-level patterns but cannot determine viability, whereas cultivation confirms the presence of living bacteria but detects only a subset of the community.

5.1 Sink microbiomes are dominated by *Pseudomonadota* and include water-, food- and human-associated bacterial genera

The bacterial community composition observed in household kitchen sinks in this study was largely consistent with patterns previously identified in built environment microbiome studies. At the phylum level, the community was dominated by *Pseudomonadota*, *Actinomycetota*, *Bacillota* and *Bacteroidota*, which are commonly reported as dominant bacterial phyla in indoor environments and on household surfaces (8,12,13,15). Similar phylum-level compositions have been described in residential kitchen microbiome studies, where these four phyla represent the dominant bacterial groups across different kitchen surfaces, including kitchen sinks (18,37). Interestingly, *Pseudomonadota*, the most abundant phylum observed in this study, has previously been reported

as dominant on moist kitchen surfaces (18,57). These findings suggest that the household kitchen sink microbiome largely reflects broader microbial patterns characteristic of indoor built environments.

At the genus level, the sink microbiome reflects a convergence of multiple environmental reservoirs, consistent with the mixed-source nature of indoor microbiomes (8,9). The most abundant classified genera included *Microbacterium*, *Acinetobacter*, *Paracoccus*, *Pseudomonas*, *Epilithonimonas*, *Moraxella*, *Pseudoxanthomonas*, *Brochothrix*, *Stenotrophomonas*, *Comamonas*, *Exiguobacterium*, *Kocuria*, *Rheinheimera* and *Streptococcus*. Several of these genera have been previously associated with environments relevant to the household kitchen sink ecosystem, including moist built environments, water systems, food-associated environments, soils and human-associated microbiota.

Multiple of these genera have previously been reported as abundant taxa in household kitchen-associated microbiomes. *Acinetobacter* and *Pseudomonas* were identified as dominant genera in household kitchen microbiomes, while *Kocuria* and *Streptococcus* were detected at lower relative abundances (37). In kitchen sponge microbiomes, *Acinetobacter* and *Moraxella* have similarly been reported as dominant taxa (57). In addition, several dominant genera identified in the present study, particularly *Acinetobacter*, *Pseudomonas* and *Stenotrophomonas*, have previously been reported as abundant in other moist, water-associated microbiomes, including hospital sink drains, plumbing systems and wastewater (24,35,58,59). Collectively, the predominance of these genera in the present study may reflect ecological characteristics of the kitchen sink environment itself, including persistent moisture, intermittent nutrient availability and regular water flow. A number of the dominant water-associated genera identified in this study, including *Pseudomonas*, *Acinetobacter* and *Stenotrophomonas*, are also known to form biofilm in moist environments, which is consistent with the ecological characteristics of sink drains (19,24,58).

The dominant genera observed in this study have also been associated with food-processing environments. Genera including *Brochothrix*, *Pseudomonas*, *Stenotrophomonas*, *Microbacterium*, *Kocuria*, *Streptococcus* and *Acinetobacter* have previously been associated with food products and food-processing microbiomes (60–62). Their presence may partially reflect repeated exposure of the sink environment to microorganisms originating from raw food products, food residues and food-handling activities.

In addition to water- and food-associated taxa, genera linked to human-associated microbiota were also identified as dominant. *Moraxella* and *Streptococcus* are commonly associated with the human upper respiratory tract microbiota (63–65), while *Kocuria* is frequently associated with the human skin and mucosal microbiota (66). Their detection may reflect the microbial input through direct human contact during daily sink use. Finally, several dominant genera, including *Pseudomonas*, *Exiguobacterium* and *Comamonas*, have been reported to be prevalent in soils (67,68), further illustrating the ecological diversity of the taxa identified in this study. Their presence in kitchen sinks indicates outdoor microbial inputs, potentially introduced via produce, shoes, pets, or airborne particles. However, many of the dominant genera identified are not exclusive to a single ecological source and may originate from multiple environmental reservoirs.

Previous studies have demonstrated that indoor microbial communities, including those in kitchens, are shaped by multiple interacting sources, including human occupants, outdoor air, water systems, ventilation and food-associated microorganisms (8,9,17,18). The findings of the present study are consistent with these observations and support the view that household kitchen sinks represent dynamic microbial ecosystems shaped by multiple interacting environmental sources. Overall, household kitchen sinks appear to represent complex microbial ecosystems, which may be particularly influenced by water- and food-associated microorganisms, as well as human-associated taxa and surrounding environmental sources.

5.2 Microbial diversity and community composition vary across sample types

Understanding the diversity and community structure of microbial communities in household sinks is essential for interpreting the ecological processes shaping these environments. In this study, differences in bacterial diversity and community composition were observed between household sink surfaces, household drains and office drains, indicating that microhabitat-specific ecological conditions may contribute to the selection of distinct microbial communities. Kitchen environments contain multiple microhabitats across different surfaces that vary in nutrient availability, exposure to human contact, and moisture retention, all of which can influence microbial community composition (8,9,18).

Alpha diversity analyses revealed significant differences in Shannon diversity between sample types, whereas Simpson diversity and richness did not differ significantly. This suggests that community evenness, rather than the presence or absence of taxa, varies across microhabitats. Shannon diversity is sensitive to both richness and evenness, while Simpson diversity is more strongly influenced by dominant taxa. The observation that Shannon diversity differed, whereas Simpson diversity did not, implies that the relative distribution of taxa is more variable than the identity of dominant taxa. Household drain samples generally exhibited lower Shannon diversity compared to sink surfaces and office drains, indicating reduced evenness.

This pattern is consistent with the ecological characteristics of drain environments, which commonly support biofilm-associated microbial communities adapted to persistent moisture and fluctuating nutrient availability (19,21,24). Biofilms within plumbing and drainage systems are influenced by conditions associated with wastewater exposure, including intermittent nutrient inputs and localised oxygen gradients, which can favour water-associated genera such as *Pseudomonas*, *Acinetobacter* and *Stenotrophomonas* (19,24,58). In contrast, sink surfaces are characterised by more intermittent water exposure, variable organic inputs, and regular direct human contact, which may contribute to the higher microbial heterogeneity observed at the genus level relative to drain-associated samples. The observed diversity patterns are consistent with previous studies showing that microbial communities in built environments vary across sample types and surface categories, likely reflecting differences in environmental conditions, disturbances, and microbial source inputs across ecological niches (8,18,37).

Beta diversity analyses further supported the presence of sample-type-associated differences in microbial community composition. NMDS ordination revealed partial clustering according to sample type, with office drain samples forming a distinct cluster, while household drains and sink surfaces showed more overlap. The distinct clustering of the office drain samples may be explained by the fact that these samples originated from the same drain, with samples collected at three different depths. PERMANOVA analyses similarly indicated that sample type explained a significant proportion of variation in microbial community composition, whereas household location and sequencing runs were not significantly associated with community variation. Together, these findings suggest that local sink microenvironmental characteristics may influence microbial community composition. Although the possible contribution of the degree of variation observed within each sample type should also be taken into account. Furthermore, it is not possible to safely conclude from this study that household location has no significant influence on microbial community composition or that sample type exerts a stronger influence than household location. The limited number of samples collected per household likely prevented reliable estimation of within-household variability. These findings are broadly consistent with previous studies reporting that microbial community composition in indoor environments is shaped by multiple interacting factors, including surface type and household identity, although their relative contributions vary across systems and spatial scales (8,9,11,16,18,37,38).

Genus-level relative abundance comparisons revealed significant differences between sample types for 11 of the 15 most abundant genera. These findings provide further evidence that microhabitat characteristics influence microbial community composition at a finer taxonomic scale. Several clear patterns emerged. *Acinetobacter*, *Kocuria*, *Moraxella* and *Pseudoxanthomonas* differed significantly between sink surfaces and both drain types

(household and office), while *Microbacterium* and *Streptococcus* showed significant differences between household drains and sink surfaces. *Exiguobacterium* differed significantly between household sink surfaces and office drains, and *Paracoccus* and *Stenotrophomonas* differed significantly between household drains and the other sample types. These patterns likely reflect differences in environmental conditions across sample types, including differences in moisture, nutrient availability, and exposure to human-associated inputs. Sink surfaces, which are more frequently exposed to human contact and food residues, showed higher relative abundances of *Kocuria*, *Moraxella*, and *Streptococcus*. However, *Streptococcus* signals were largely driven by a single outlier sample (63–66). In contrast, household drains provide conditions more favourable to biofilm-associated taxa such as *Stenotrophomonas*, which are adapted to persistently moist environments with intermittent nutrient supply (58). Office drains showed a more restricted pattern of differences, including a higher relative abundance of *Pseudoxanthomonas*, which may reflect differences in water flow or cleaning frequency.

Altogether, these results suggest that sample type plays an important role in shaping bacterial community composition at the genus level, in line with patterns reported in built environment microbiome studies (8,9,11,18). The observed differences between sample types likely reflect a combination of ecological factors, including variation in moisture, nutrient availability, and human contact, which influence microbial community assembly in sink-associated environments. In addition, despite efforts to standardise sampling procedures, citizen science-based sampling may introduce minor procedural variability inherent to distributed sample collection. However, the overall patterns observed in this study remain consistent with ecological expectations for sink-associated microbiomes.

5.3 Potential opportunistic pathogenic taxa, including viable *E. coli*, are present at low-abundance

The detection of taxa containing potential opportunistic pathogenic species is an important aspect of this study, given the potential public-health implications of microbial contamination in household environments. Both sequencing- and cultivation-based approaches identified genera that include opportunistic pathogens, including viable *E. coli*. In addition, species-level screening of taxonomic profiles identified taxa containing species associated with foodborne infections and intoxications, according to the Belgian Federal Public Service of Health, Food Chain Safety and Environment (56).

These organisms were generally detected at low relative abundances, consistent with observations from kitchen microbiome studies showing that potential opportunistic and food-associated taxa are present but typically form a minor component of residential microbial communities (18,37,38). Their presence may reflect contamination originating from raw food products, produce, or direct human contact during routine kitchen activities. Although the targeted species were detected in most samples, their consistently low relative abundance suggests that they are not dominant members of the kitchen sink microbiome. However, because samples were collected at a single time point, it is not possible to determine whether kitchen sinks serve as persistent reservoirs of these organisms or whether their presence reflects repeated, transient introductions from external sources.

The cultivation-based detection of viable *E. coli* indicates that sink-associated environments can support short-term survival of enteric bacteria under certain conditions. This finding aligns with previous studies in hospital environments identifying sink systems as reservoirs for multidrug-resistant *Enterobacteriaceae*, with evidence of persistence and recurrent detection in sink-associated biofilms (22,24,36). However, household environments differ substantially from hospital environments in microbial load, antimicrobial selection pressures, and cleaning practices. As such, the presence of viable *E. coli* in household sinks does not necessarily indicate long-term persistence or increased clinical risk.

Importantly, the detection of bacterial DNA or viable isolates does not directly imply an increased infection risk or pathogenic potential. Neither amplicon sequencing nor cultivation-based methods used in this study provide information on strain-level identity, virulence determinants, antimicrobial resistance genes or transmission

dynamics. Higher-resolution approaches, such as whole-genome sequencing or metagenomic sequencing, could be performed in future analyses to assess these features. Consequently, detection of potential opportunistic pathogens in this context reflects microbial presence and dispersal within sink ecosystems, rather than an assessment of infection risk.

From an ecological perspective, the detection of opportunistic pathogens reflects the open and dynamic nature of sink microbiomes, which receive microbial inputs from multiple sources. The presence of these taxa, including potential opportunistic pathogens, does not necessarily indicate ecological dominance or direct health risk, but instead reflects their capacity to persist or be introduced into built environments. This underscores the importance of understanding colonisation and persistence in sink-associated microbial communities.

5.4 Sequencing and cultivation provide complementary views of sink microbiomes

A central methodological feature of this study was the combined use of full-length 16S rRNA gene sequencing (ONT MinION) and cultivation-based detection of viable *E. coli*. This dual-approach design provided complementary perspectives on the microbial communities inhabiting household kitchen sinks. Sequencing offered a culture-independent overview of bacterial diversity, whereas cultivation enabled targeted detection of viable organisms. The integration of these methods is particularly valuable for distinguishing between viable and non-viable microorganisms, thereby improving the interpretation of ecological relevance (46).

5.4.1 Full-length 16S sequencing captures broad diversity but cannot resolve viability

Full-length 16S rRNA gene sequencing offers several advantages over short-read amplicon approaches, including improved taxonomic resolution, the ability to span multiple hypervariable regions, and enhanced species-level classification potential (47,50). Nanopore sequencing additionally provides real-time data generation, long read lengths, and device portability (50,52).

However, several limitations should be considered when interpreting sequencing-based results. First, DNA-based methods cannot distinguish between viable and non-viable cells, meaning that taxa detected through sequencing may not represent active members of the community. This is particularly relevant in built environments where cleaning, disinfection, and water flow can leave behind DNA from dead cells (46). Second, PCR amplification bias may influence observed community profiles, as primer specificity, GC content, and differential amplification efficiency can affect the detection and relative abundance of taxa (47). Third, while Nanopore sequencing offers advantages such as long reads and portability, it has historically been associated with higher raw error rates compared to short-read platforms, particularly in homopolymer regions. However, advances in base-calling algorithms have substantially improved its accuracy over time (52,69), although misclassification remains possible, particularly at the species level.

A further limitation relates to reference database completeness. Environmental microbiomes often contain taxa that are underrepresented or absent in existing databases, contributing to the proportion of unclassified reads observed in this study (47). Finally, sequencing-based approaches generate read counts that are typically normalised to relative abundance values rather than providing direct measures of absolute cell abundance, meaning that changes in one taxon may appear to influence others due to the compositional nature of the data (47).

Despite these limitations, full-length 16S sequencing remains a powerful tool for characterising microbial communities in built environments, offering broad taxonomic coverage and high sensitivity for detecting diverse bacterial lineages.

5.4.2 Cultivation detects viable bacteria but captures only a fraction of microbial diversity

Cultivation-based methods complement sequencing by providing direct evidence of microbial viability. In this study, selective cultivation targeted viable *E. coli*, a clinically relevant enteric bacterium and a common indicator of faecal contamination. One major strength of cultivation is its ability to detect only organisms capable of growth, which is critical for assessing potential health relevance (70,71). Cultivation also enables downstream analyses such as antimicrobial susceptibility testing or whole-genome sequencing, although these were beyond the scope of this study.

Selective media further enhance specificity, allowing targeted detection of specific organisms even within complex microbial communities. However, cultivation has well-established limitations. It detects only organisms capable of growing under the specific laboratory conditions provided, and a substantial proportion of environmental bacteria are difficult or impossible to culture (70–72). Because cultivation in this study focused solely on *E. coli*, it provides no information about the viability of other taxa detected through sequencing.

Cultivation outcomes can also be sensitive to sampling variation, including small differences in swabbing location or pressure, which may contribute to discrepancies between sequencing and cultivation results. Additionally, environmental bacteria exposed to detergents, temperature fluctuations, or nutrient deprivation may enter a viable but non-culturable (VBNC) state, remaining metabolically active but undetectable by standard cultivation methods (73).

5.4.3 Integrating sequencing and cultivation improves ecological interpretation

Despite their respective limitations, sequencing and cultivation provide complementary insights into sink-associated microbial communities. Sequencing enables broad characterisation of microbial diversity and community structure, while cultivation confirms the presence of viable organisms and allows targeted detection of specific taxa. The combined use of these approaches therefore provides a more comprehensive understanding of microbial communities in built environments, particularly in contexts where DNA-based sequencing alone cannot distinguish between microbial presence and viability.

5.5 Methodological considerations and limitations

Beyond the method-specific limitations of full-length 16S rRNA gene sequencing and cultivation discussed above, several additional methodological considerations should be taken into account when interpreting the results of this study.

A key limitation of this study is the relatively small sample size and the limited number of samples collected per household. With only two samples per household, the study cannot fully capture within-household variability or reliably assess household-specific effects. This is particularly relevant given that previous studies have shown that indoor microbiomes can vary substantially within individual households depending on factors such as surface type, human activity, ventilation, and cleaning practices (8,11). A larger sample size would enable more robust comparisons between households and allow the integration of metadata such as cleaning frequency, household demographics and behaviours and sink usage patterns, which are known to influence indoor microbial communities (9). However, within a citizen science framework, it is important that the sampling procedure remains clear and easy to follow in order to maximise participant engagement and compliance. Limiting the number of samples per household therefore reduces participant burden and allows the available resources to be allocated toward sampling a larger number of households rather than increasing sampling depth within households.

Secondly, the citizen-science sampling design used in this study offers clear advantages in terms of scalability, accessibility, and community engagement. However, it may also have introduced additional variability in sample collection despite the use of standardised sampling instructions and collection materials. Differences in sampling duration, applied pressure, or adherence to sampling protocols may have contributed to variation between samples.

Quality control considerations also influenced data interpretation. Low-level contamination was detected in the negative controls, which contained *Shigella* and a small number of other taxa. Such signals may arise from trace laboratory contamination, barcode cross-talk, or index misassignment during sequencing. In addition, the mock community showed minor deviations from its expected composition, likely reflecting amplification bias, sequencing error, or limitations in reference database coverage. These issues are important considerations in microbiome studies and highlight the importance of rigorous quality control procedures, contamination-aware analytical pipelines, and cautious interpretation of low-abundance taxa (74).

Finally, amplicon-based DNA sequencing approaches provide limited direct information regarding microbial viability or metabolic activity and do not directly resolve functional traits or virulence potential. Without complementary approaches such as cultivation, metagenomics, or metatranscriptomics, it is difficult to determine whether detected taxa are viable, metabolically active, or possess pathogenic potential. This limitation is particularly relevant in built environment microbiomes, where DNA originating from dead cells may persist on surfaces following cleaning or disinfection (46).

5.6 Future research directions

The findings of this study provide a strong foundation for future research into the microbial ecology of household kitchen sinks. However, they also highlight several important avenues for further investigation. This potential further research would be able to address the limitations identified in this study and deepen our understanding of the ecological, behavioural, and infrastructural factors that shape sink-associated microbiomes.

Future research within the POEMBAKterie project will build on the findings of this study by increasing the number of sampled households and broadening geographic coverage across Antwerp. This will improve statistical power and enable integration of survey-derived metadata to investigate environmental and behavioural factors associated with sink microbiome composition. Previous studies have shown that indoor microbiomes are shaped by a combination of environmental and behavioural factors (8,9,17) and a larger dataset would allow these relationships to be explored in greater detail.

A recently launched sister study of POEMBAKterie, named “Leven in Leidingen”, will investigate microbial communities in public sinks located in cultural buildings, sports facilities, and public events, extending this research beyond the household environment. This provides a unique opportunity to compare residential and public sink microbiomes using the same sequencing methodology. Public sinks differ from household sinks in multiple ways, including terms of frequency and type of use, cleaning and disinfection practices and exposure to diverse human populations. These differences may contribute to the development of distinct microbial communities, as suggested by studies of hospital, industrial, and communal sink systems (16,21,24). Direct comparison of residential and public sinks will help identify which microbial patterns are universal and which are context-dependent.

Beyond broader cross-sectional sampling, a possible future direction is the implementation of longitudinal sampling, which would allow the examination of temporal dynamics in sink microbiomes. Temporal studies would help assess whether sink microbiomes are stable over time, how behavioural factors such as cleaning, food preparation, and sink usage influence microbial communities and whether opportunistic pathogens persist or appear only transiently.

Beyond taxonomic profiling, future work should also place greater emphasis on antimicrobial resistance (AMR) within sink-associated microbial communities. Given the growing global burden of antimicrobial resistance and its substantial impact on public health (43), a better understanding of environmental reservoirs of resistance determinants is increasingly important. ARGs are widespread in environmental bacteria, including non-pathogenic taxa, and constitute part of the broader environmental resistome. (75). These genes can be associated with mobile genetic elements and may be transferred between bacteria via horizontal gene transfer, facilitating their dissemination within microbial communities (76). Further sequencing efforts could therefore target known ARGs to assess the AMR potential of the kitchen sink microbiome. This would provide a more detailed understanding of the presence and distribution of resistance determinants and improve assessment of the clinical relevance of this environment.

While this study focused on the cultivation of *E. coli*, future work could expand cultivation efforts to include additional opportunistic pathogens and environmentally relevant taxa to improve understanding of viable bacteria present in sink environments. Combining selective cultivation with assays for antimicrobial resistance and virulence traits would further enhance assessment of the potential clinical relevance of sink-associated taxa. Moreover, biofilm formation assays could be incorporated to evaluate the ability of isolates to form and persist within biofilm structures, providing further insight into their persistence potential. In addition, whole-genome sequencing of potential opportunistic pathogens isolated from the samples obtained within the POEMBAKterie project would enable strain-level resolution and allow assessment of antimicrobial resistance genes and virulence-associated determinants, as well as the spatial distribution and potential recurrence of specific strains across different locations, particularly for organisms such as *E. coli*.

Finally, sink microbiomes may also be relevant in the broader context of microbial transmission and infection dynamics in built environments. Sinks represent frequent points of contact between humans, water, and food-related materials and may therefore play a role in the dissemination or exchange of microorganisms within households and public settings. From this perspective, sink microbiomes could be explored not only as ecological systems but also as potential indicators for surveillance of opportunistic pathogens and antimicrobial resistance in the built environment. Such an approach could inform targeted hygiene strategies and intervention measures aimed at reducing the spread of clinically relevant microorganisms.

6 Conclusion

This study characterised the bacterial microbiome of household kitchen sink environments in Antwerp using full-length 16S rRNA gene sequencing combined with cultivation-based detection of viable *E. coli*. In addition, this thesis contributed to the collection and storage of samples from 862 households for subsequent analysis within the main POEMBAKterie study.

The results show that kitchen sinks harbour diverse and heterogeneous microbial communities dominated by taxa commonly associated with built environments, water systems, food-related environments, and human-associated microbiota. This reflects the combined influence of multiple microbial input sources within domestic sink ecosystems.

Clear differences in microbial diversity and community composition were observed between household sink surfaces, household drains and an office drain, suggesting that local microenvironmental conditions contribute to shaping community structure within kitchen sinks. These differences likely reflect variation in moisture exposure, nutrient availability, and microbial input pathways within sink microhabitats. In addition, both sequencing- and cultivation-based approaches identified bacterial taxa containing potential opportunistic pathogens, including viable *E. coli*. However, their clinical relevance could not be determined within the scope of this study. The combined use of sequencing and cultivation provided complementary insights into both overall

community composition and the presence of viable bacteria, highlighting the value of integrating culture-dependent and culture-independent approaches in built-environment microbiology.

A key limitation of this thesis is the limited sample size, which restricted the integration of household metadata and thereby limited the ability to evaluate environmental and behavioural factors influencing sink microbiome composition. Future analyses within the POEMBAKterie project will address this limitation by analysing samples from over 850 households. As such, this thesis, based on the analysis of the pilot study samples, provides a foundational dataset for the broader POEMBAKterie study and contributes to the development of a larger-scale investigation of sink microbiomes in Antwerp.

Overall, the results of this study contribute to a clearer understanding of sink-associated microbial ecology in domestic environments and provide a baseline for future studies investigating microbial persistence, transmission pathways and functional potential. Importantly, the presence of opportunistic and potentially pathogenic bacteria in sink environments highlights their potential relevance as reservoirs in built environments, where microbial exchange between humans, surfaces, and water systems may occur. This underscores the importance of further investigating the dissemination dynamics of clinically relevant microorganisms, including potential antimicrobial-resistant strains, in order to better understand their role in household and public health contexts.

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8 Supplementary documents

HOE GA IK TE WERK?

Vorbereidende stappen en info

Dankjewel voor jouw deelname aan POEMBAKteriel

Op de **voor- en achterkant van deze flyer** vind je alle instructies om stalen te nemen.

Lees alles zorgvuldig door **voordat je begint**. Heb je liever een visuele uitleg? Bekijk dan het **instructiefilmpje**!

Bekijk het **instructiefilmpje** op www.poembakterie.be/instructies of scan de QR-code:



Breng jouw stalen zelf naar het adres op de achterzijde op werkdagen tussen 8-18u. Lukt dit jou echt niet? Stuur ons een mail **vóór** je jouw stalen neemt, zodat we een oplossing kunnen zoeken.

Ga je keukenspoelbak of "poembak" **niet extra poetsen** voor het nemen van de stalen, het is belangrijk om de poembak te analyseren zoals hij is!



Neem je stalen **kort nadat je wakker wordt**, voordat je de poembak gaat gebruiken.



Bestaat jouw poembak uit meerdere delen? Neem dan alle stalen uit het **meest gebruikte deel**.



Inhoud van de envelop

- 1. Formulier geïnformeerde toestemming
- 2. Instructies staalname
- 3. Registratieformulier staalname

Neem de papieren zakjes en lege tube uit het ziplock-zakje, maar houd ze nog gesloten.

1 =  +  Roze swab (of "eSwab") en etiket voor het oppervlak van de **spoelbak**

2 =  +  Blauwe swab* (of "eNat") en etiket voor het oppervlak van de **spoelbak**

3 =  +  Roze swab (of "eSwab") en etiket voor het bovenste gedeelte van de **afvoer**

4 =  +  Blauwe swab* (of "eNat") en etiket voor het bovenste gedeelte van de **afvoer**

 Lege tube voor **kraanwater**

*Wees voorzichtig: de vloeistof in het buisje mag niet in contact komen met de huid of ogen. Is er toch contact, raadpleeg dan een arts. Houd het buisje buiten bereik van kinderen.

1

STAALNAME

Hulp nodig? Heb je een vraag?

Contacteer ons:
poembakterie@uantwerpen.be

Was je handen vóór je begint. Let op, gebruik hiervoor niet de spoelbak waarvan je een staal neemt!
Neem achtereenvolgens staal 1, staal 2 tot en met staal 5.



SPOELBAK OPPERVAK



30s



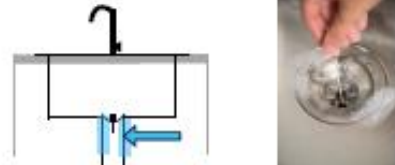
Staal 1

- 1 Zet een timer van 30 seconden klaar.
- 2 Neem de plastic verpakking van **staal 1** (roze dop) en het etiket uit het papieren zakje. Open de verpakking en neem het buisje eruit, laat het wattenstaafje nog even zitten. **Plak het etiket** op het buisje.
- 3 Haal het wattenstaafje uit de verpakking zonder het gedeelte onder het rode lijntje aan te raken.
- 4 Start de timer en wrijf met het wattenstaafje over het volledige oppervlak van de spoelbak gedurende 30 seconden, zonder hard te duwen. Draai de swab ondertussen rond zijn as, zodat alle kanten het oppervlak raken.
- 5 Plaats het wattenstaafje in de tube en breek het stokje af bij de rode lijn door het voorzichtig te buigen. Gooi de rest van het stokje in de vuilbak.



Staal 2

- 6 Sluit de tube goed af met de dop. Leg de tube in het ziplock-zakje met absorberend papier.
- 7 Herhaal deze stappen voor **staal 2** (blauwe dop).



AFVOER



30s



Staal 3

- 1 Zet een timer van 30 seconden klaar.
- 2 Neem de plastic verpakking van **staal 3** (roze dop) en het etiket uit het papieren zakje. Open de verpakking en neem het buisje eruit, laat het wattenstaafje nog even zitten. **Plak het etiket** op het buisje.
- 3 Haal het wattenstaafje uit de verpakking zonder het gedeelte onder het rode lijntje aan te raken.
- 4 Haal het zeefje uit de spoelbak. Start de timer en breng het wattenstaafje via het putje in de afvoer. Wrijf de swab gedurende 30 seconden langs de binnenkant van de afvoer terwijl je hem ronddraait. Duw niet te hard en hou het wattenstaafje goed vast zodat het niet in de afvoer valt.
- 5 Plaats het wattenstaafje in de tube en breek het stokje af bij de rode lijn door het voorzichtig te buigen.



Staal 4

- 6 Sluit de tube goed af met de dop. Leg de tube in het ziplock-zakje met absorberend papier.
- 7 Herhaal deze stappen voor **staal 4** (blauwe dop).



KRAANWATER

Open de kleurloze lege tube en vul deze ongeveer voor de helft met kraanwater. Sluit de tube goed af en leg deze in het zakje.

Staal 5

We verwachten geen bacteriën in kraanwater te vinden, daarom is het een goede extra controle!

STALEN BEWAREN EN INLEVEREN

Lees en handteken het formulier **geïnformeerde toestemming**.

Vul het formulier **registratie stalen in**.

Bewaar jouw stalen in de koelkast.

Breng ze binnen 2 dagen naar het labo, op een werkdag tussen 8-18u:



Labo Medische Microbiologie
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— Gebouw S verdieping 6

Supplementary Figure S1. Sampling instructions provided to participants during the pilot study.

Supplementary Table S1. Overview of samples included in each run, linked to barcode, sample type, household and number of reads.

Run 1				
Barcode	Sample ID	Type	Location	Reads
barcode01	LMM_drain_sup	Office drain	LMM	195442
barcode02	PB000028	Household drain	BE-AN0006	207589
barcode13	LMM_drain_mid	Office drain	LMM	145683
barcode14	PB000046	Household surface	BE-AN0010	175627
barcode25	LMM_drain_deep	Office drain	LMM	142465
barcode26	PB000048	Household drain	BE-AN0010	158061
barcode37	PB000006	Household surface	BE-AN0002	193898
barcode38	PB000051	Household surface	BE-AN0011	187953
barcode49	PB000008	Household drain	BE-AN0002	170580
barcode50	PB000053	Household drain	BE-AN0011	190192
barcode61	PB000021	Household surface	BE-AN0005	193391
barcode62	pos_con_HM281A	Control	NA	249534
barcode73	PB000023	Household drain	BE-AN0005	157548
barcode74	neg_control2	Control	NA	253
barcode85	PB000026	Household surface	BE-AN0006	181310
Run 2				
Barcode	Sample ID	Type	Household	Reads
barcode01	neg_control3	Control	NA	383
barcode04	PB000061	Household surface	BE-AN0013	149364
barcode13	pos_con_D6306	Control	NA	152379
barcode15	PB000003	Household drain	BE-AN0001	91237
barcode16	PB000063	Household drain	BE-AN0013	82614
barcode27	PB000016	Household surface	BE-AN0004	66161
barcode28	PB000011	Household surface	BE-AN0003	110957
barcode39	PB000018	Household drain	BE-AN0004	133983

barcode40	PB000013	Household drain	BE-AN0003	123234
barcode51	PB000031	Household surface	BE-AN0007	222968
barcode52	PB000036	Household surface	BE-AN0008	100412
barcode63	PB000033	Household drain	BE-AN0007	150862
barcode64	PB000038	Household drain	BE-AN0008	147313
barcode75	PB000056	Household surface	BE-AN0012	104459
barcode76	pos_con_HM281B	Control	NA	182771
barcode87	PB000058	Household drain	BE-AN0012	310242
barcode88	pos_con_D6300	Control	NA	49098

Supplementary Table S2. Isolate-level consolidated MALDI-TOF MS identification results for all analysed isolates, including sample type classification (sink surface or sink drain), best and second-best organism matches, and retained highest identification scores.

Isolate	Sample type	Organism best match	Score value	Organism second best match	Score value
PB000000 A	Surface	<i>Pantoea agglomerans</i>	2.17	<i>Pantoea agglomerans</i>	2.14
PB000000 B	Surface	<i>Pantoea agglomerans</i>	2.08	<i>Pantoea agglomerans</i>	2.03
PB000002 A	Drain	<i>Escherichia coli</i>	2.2	<i>Escherichia coli</i>	2.2
PB000002 B	Drain	<i>Escherichia coli</i>	2.11	<i>Escherichia coli</i>	2.06
PB000002 C	Drain	<i>Escherichia coli</i>	2.26	<i>Escherichia coli</i>	2.22
PB000005 A	Surface	<i>Pantoea sp</i>	2.02	<i>Pantoea agglomerans</i>	1.8
PB000005 B	Surface	not reliable identification	1.62	not reliable identification	1.62
PB000007 A	Drain	<i>Escherichia coli</i>	2.09	<i>Escherichia coli</i>	2.08
PB000007 B	Drain	no peaks found	0	not (yet) present	0
PB000007 C	Drain	<i>Escherichia coli</i>	2.18	<i>Escherichia coli</i>	2.17
PB000007 D	Drain	<i>Klebsiella pneumoniae</i>	2.3	<i>Klebsiella pneumoniae</i>	2.21
PB000007 E	Drain	<i>Escherichia coli</i>	2.17	<i>Escherichia coli</i>	2.14
PB000010 A	Surface	<i>Escherichia hermannii</i>	2.05	<i>Escherichia hermannii</i>	1.83

PB000010 B	Surface	<i>Klebsiella oxytoca</i>	2.25	<i>Klebsiella oxytoca</i>	2.18
PB000010 C	Surface	<i>Citrobacter freundii</i>	2.07	<i>Citrobacter freundii</i>	2.05
PB000015 A	Surface	<i>Raoultella ornithinolytica</i>	2.24	<i>Raoultella planticola</i>	2.19
PB000015 B	Surface	not reliable identification	1.61	not reliable identification	1.56
PB000015 C	Surface	<i>Aeromonas caviae</i>	1.78	not reliable identification	1.56
PB000015 D	Surface	<i>Enterobacter roggenkampii</i>	1.98	<i>Enterobacter roggenkampii</i>	1.97
PB000017 A	Drain	<i>Escherichia coli</i>	2.08	<i>Escherichia coli</i>	2.05
PB000017 B	Drain	<i>Escherichia coli</i>	2.17	<i>Escherichia coli</i>	2.15
PB000022 A	Drain	no peaks found	0	not (yet) present	0
PB000022 B	Drain	<i>Pseudescherichia vulneris</i>	1.93	<i>Pseudescherichia vulneris</i>	1.92
PB000022 C	Drain	<i>Lelliottia amnigena</i>	2.27	<i>Lelliottia amnigena</i>	2.23
PB000027 A	Drain	<i>Escherichia coli</i>	2.12	<i>Escherichia coli</i>	2.11
PB000030 A	Surface	<i>Klebsiella oxytoca</i>	2.35	<i>Klebsiella oxytoca</i>	2.18
PB000030 B	Surface	<i>Aeromonas media</i>	2.01	<i>Aeromonas media</i>	1.97
PB000030 C	Surface	<i>Leclercia adecarboxylata</i>	2.02	<i>Leclercia adecarboxylata</i>	1.99
PB000030 D	Surface	no peaks found	0	not (yet) present	0
PB000032 A	Drain	<i>Klebsiella oxytoca</i>	2.27	<i>Klebsiella oxytoca</i>	2.2
PB000032 B	Drain	<i>Leclercia adecarboxylata</i>	1.94	<i>Leclercia adecarboxylata</i>	1.92
PB000032 C	drain	no peaks found	0	not (yet) present	0
PB000035 A	surface	no peaks found	0	not (yet) present	0
PB000037 A	drain	<i>Escherichia coli</i>	2.14	<i>Escherichia coli</i>	2.1
PB000047 A	drain	<i>Klebsiella variicola</i>	1.9	<i>Klebsiella variicola</i>	1.88
PB000047 B	drain	<i>Acinetobacter pittii</i>	2.38	<i>Acinetobacter pittii</i>	2.33
PB000052 A	drain	<i>Escherichia coli</i>	2.23	<i>Escherichia coli</i>	2.2
PB000052 B	drain	<i>Escherichia coli</i>	2.14	<i>Escherichia coli</i>	2.03

PB000052 C	drain	<i>Escherichia coli</i>	2.15	<i>Escherichia coli</i>	2.09
PB000052 D	drain	<i>Escherichia coli</i>	2.29	<i>Escherichia coli</i>	2.17
PB000057 A	drain	<i>Escherichia coli</i>	2.34	<i>Escherichia coli</i>	2.21
PB000057 B	drain	<i>Klebsiella oxytoca</i>	2.31	<i>Klebsiella oxytoca</i>	2.31
PB000060 A	surface	<i>Enterobacter hormaechei</i>	2.1	<i>Enterobacter hormaechei</i>	2.08
PB000060 B	surface	<i>Serratia nematodiphila</i>	2.12	<i>Serratia marcescens</i>	2.01
PB000062 A	drain	<i>Escherichia coli</i>	2.16	<i>Escherichia coli</i>	2.09
PB000062 B	drain	<i>Klebsiella oxytoca</i>	2.09	<i>Klebsiella oxytoca</i>	2.08
PB000062 C	drain	<i>Enterobacter asburiae</i>	1.99	<i>Enterobacter kobei</i>	1.95
PB000062 D	drain	<i>Klebsiella oxytoca</i>	2.26	<i>Klebsiella oxytoca</i>	2.25

Supplementary Table S3. MALDI-TOF MS identification results consolidated at biological sample-level, including sample type (sink surface or sink drain), best organism matches with a score ≥ 2.000 , retained highest identification scores and the number of isolates per sample assigned to each identified species.

Sample	Sample type	Organism best match	Score value	Number of isolates
PB000000	surface	<i>Pantoea agglomerans</i>	2.17	2
PB000002	drain	<i>Escherichia coli</i>	2.26	3
PB000005	surface	<i>Pantoea sp</i>	2.02	1
PB000007	drain	<i>Escherichia coli</i>	2.18	3
PB000007	drain	<i>Klebsiella pneumoniae</i>	2.3	1
PB000010	surface	<i>Citrobacter freundii</i>	2.07	1
PB000010	surface	<i>Escherichia hermannii</i>	2.05	1
PB000010	surface	<i>Klebsiella oxytoca</i>	2.25	1
PB000015	surface	<i>Raoultella ornithinolytica</i>	2.24	1
PB000017	drain	<i>Escherichia coli</i>	2.17	2
PB000022	drain	<i>Lelliottia amnigena</i>	2.27	1

PB000027	drain	<i>Escherichia coli</i>	2.12	1
PB000030	surface	<i>Aeromonas media</i>	2.01	1
PB000030	surface	<i>Klebsiella oxytoca</i>	2.35	1
PB000030	surface	<i>Leclercia adecarboxylata</i>	2.02	1
PB000032	drain	<i>Klebsiella oxytoca</i>	2.27	1
PB000037	drain	<i>Escherichia coli</i>	2.14	1
PB000047	drain	<i>Acinetobacter pittii</i>	2.38	1
PB000052	drain	<i>Escherichia coli</i>	2.29	4
PB000057	drain	<i>Escherichia coli</i>	2.34	1
PB000057	drain	<i>Klebsiella oxytoca</i>	2.31	1
PB000060	surface	<i>Enterobacter hormaechei</i>	2.1	1
PB000060	surface	<i>Serratia nematodiphila</i>	2.12	1
PB000062	drain	<i>Escherichia coli</i>	2.16	1
PB000062	drain	<i>Klebsiella oxytoca</i>	2.26	2

Supplementary Table S4. Pearson correlation coefficients and associated p-values between alpha diversity metrics calculated from relative abundance and rarefied genus-level abundance tables.

Index	Pearson r	p-value
Shannon	0.999962	<0.001
Simpson	0.9999614	<0.001
Richness	0.9792481	<0.001

Supplementary table S5. Pairwise Dunn's post hoc test p-values and Benjamini-Hochberg adjusted p-values for alpha-diversity metrics across sample types. Results are presented for all pairwise sample type comparisons and all evaluated diversity metrics.

Groups	Dunn p-value	Adjusted p-value	Index
Household drain vs Household surface	0.032	0.057	Shannon
	0.048	0.145	Simpson
	0.491	0.491	Richness
Household drain vs Office drain	0.038	0.057	Shannon
	0.380	0.570	Simpson
	0.017	0.051	Richness
Household surface vs Office drain	0.496	0.496	Shannon
	0.693	0.693	Simpson
	0.054	0.081	Richness