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Human Fecal Contamination of Toilet Doors and Food Preparation Surfaces in Households with Poor-Quality Sanitation Facilities in Ibule-Soro, Nigeria

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Abstract

Contaminated surfaces are potential breeding habitats for pathogenic organisms. Toilet doors and food preparation surfaces may serve as fomites in the indirect transmission of pathogens as a result of fecal contamination. This study set out to determine the bacterial communities and levels of enteric bacteria on toilet doors and food preparation surfaces in households with poor-quality toilet facilities in Ibule-Soro, Nigeria. 24 Swab samples from toilet doors and food preparation surfaces were collected from each of the five households over a period of six months within 12 sampling events. The abundance and identity of bacteria in the samples were determined using both culture-based and molecular methods (16S rRNA gene sequencing). Risks factors of fecal contamination were also assessed. Results revealed that the concentration of *Escherichia coli* ranged from 2.15 to 2.64 $\log_{10}$ CFU/ml) while *Salmonella* ranged from 1.07 to 2.15 $\log_{10}$ CFU/ml. The common bacterial phyla detected were Proteobacteria, Actinobacteria and Firmicutes while Proteobacteria was the most abundant (42.45%) phylum. Risk factors such as toilet facilities, most especially 'No toilet' and 'Manageable access to soap' exhibited high significant association with the presence and load of *Enterobacter*, *Klebsiella*, *Proteus.* Toilet doors had higher frequency of occurrence of enteric bacteria than food preparation surfaces but food preparation surfaces had higher load of enteric bacteria than toilet doors. Isolates from toilet doors had higher normal distribution pattern than isolates from food preparation surfaces. In order to protect human health from infections, the quality of the toilet facilities in the households must be improved, adequate hygiene practices must be encouraged and food preparation surfaces must be cleaned and sanitized regularly.

Keywords: Bacteria, Fecal contamination, Fomites, Hygiene, Sanitation

1. Introduction

Managing water, sanitation, and hygiene (WASH) effectively is essential for human health protection. This helps in the prevention and control of waterborne and sanitation-related diseases, which are significant hazards to human health and well-being (Haque & Freeman, 2021; Orimoloye et al., 2015; World Health Organization [WHO], 2019). Poor sanitation remains a major global public health concern and is a significant driver of infectious disease transmission, particularly in low- and middle-income countries. Globally, diarrhoeal diseases are responsible for an estimated 1.6 million deaths annually, with a substantial proportion attributable to unsafe water, inadequate sanitation, and poor hygiene practices (World Health Organization, 2023). The WHO further estimates that over 800,000 deaths each year are directly linked to inadequate water, sanitation, and hygiene (WASH), highlighting fecal–oral transmission as one of the most preventable causes of morbidity and mortality worldwide (World Health Organization, 2023). In Nigeria, the burden is especially severe, as the country consistently ranks among those with the highest prevalence of sanitation-related diseases in sub-Saharan Africa. Diarrhoeal diseases remain a leading cause of illness and mortality, particularly among children under five years of age, with national surveys linking these outcomes to poor sanitation coverage and environmental contamination (World Health Organization and United Nations Children's Fund (UNICEF), 2019). Infectious diseases can be transmitted through several routes, one common way being the fecal-oral route, often arising from poor sanitation and hygiene. Inanimate objects known as fomites can harbour and serve as reservoirs for disease-causing organisms (Tiku et al., 2019). Examples of fomites include doors, food preparation surface, hand lockers, showers, tables, chairs, money, utensils, etc. (Nwankwo & Okochi, 2020). Fecal matter remains a major reservoir of human pathogens which in adverse situations may bring about an outbreak of infections. This may be attributed to poor-quality toilet facilities or the unhygienic use of toilet facilities which results in gross contamination of the door (Andere et al., 2022). Foodborne pathogens, like *Salmonella*, can survive in the moist organic matter that gather on food contact surfaces in kitchens and restaurants, leading to the contamination of other food items (Sibanyoni & Tabit, 2019). Improper sanitation of surfaces and equipment may influence the burden of foodborne microorganisms to a greater extent (Ali et al., 2016). According to research, it can be difficult to completely remove pathogens from the surroundings and tools used in food processing, as many foodborne pathogens are known to be able to attach to food contact surfaces (Lani et al., 2014). Enteric bacteria can serve as an indicator for fecal contamination of surfaces, posing a potential risk to food quality and safety (Gallo et al., 2020). Studies have revealed the presence of bacterial pathogens on hard, non-porous surfaces such as kitchen surfaces, floor surfaces, toilet surfaces, doors etc. (Alonge et al., 2019; Mihucz et al., 2022; Nwankwo et al., 2023; Zhao et al., 2019). Non-porous surfaces of many doors and food preparation counters (particularly under higher relative humidity) have significant higher transfer efficiencies of bacteria (Zhao et al., 2019). Moreover, temperature, pH, and the presence of nutrients influence attachment of microorganisms to the surfaces (Byun et al., 2021). Surfaces with properties that are more conducive to adhesion also promote microbial attachment (Carrascosa et al., 2021). One of the most common sources of infection is poor hand hygiene, particularly after using the restroom. Additionally, contamination can occur when individuals with unclean shoes or clothes come into contact with the doors, and microorganisms can be transmitted from one person or location to another via hands (Bloomfield et al., 2012). Human hands usually harbor microorganisms both as part of the normal microbiota as well as transient microbes contacted from the environment (Oranusi et al., 2013). Fomites may become contaminated with pathogens by direct contact with bodily excretions or secretions (mucus, saliva, blood, etc.). Alternatively, such fluids may be transferred from soiled hands or inadequately washed hands to fomites (Gerba & Pepper, 2015). Despite evidence that Ibule-Soro lacks access to safe water and is characterized by poor hygiene practices and inadequate waste management (Lawal et al., 2024), there is limited empirical data on microbial contamination within household environments in this community. Previous studies have largely focused on sanitation conditions without assessing the presence and distribution of enteric bacteria on frequently contacted household surfaces, such as toilet doors and food preparation areas, which are critical points for fecal–oral transmission. Moreover, few studies have integrated microbiological findings with hygiene practices, sanitation status, and socio-demographic factors to estimate infection risk at the household level (Olalemi et al., 2023). This study addresses this gap by using 16S rRNA gene sequencing to characterize bacterial communities on selected household surfaces and by evaluating associated hygiene and sanitation factors to better understand potential infection risks in rural Ibule-Soro. The aim of the study was to determine the bacterial communities and levels of enteric bacteria on toilet doors and food preparation surfaces in certain households with low quality toilet facilities in rural Ibule-Soro, Nigeria. And it is expected that the metagenomics will reveal a diverse community of fecal-associated bacteria, with contamination levels positively associated with inadequate sanitation infrastructure and poor hygiene behaviors.

2. Materials and Methods

2.1 Study setting

The study site was situated in Ibule-Soro, Nigeria. Ibule-Soro is a town located in Ifedore Local Government Area of Ondo State, South West Nigeria and it lies between latitude 7.3173North and longitude 5.1172East. Five households' sites were selected for the study based on the provision of toilet/latrine in the household, presence of children less than five years, type of toilet facilities used in the household, presence of handwashing facilities and the method of water treatment (McGinnis et al., 2019).

2.2 Sample collection

Swab samples were collected biweekly over a period of 6 months (i.e., July 2023 to December 2023) from five different households using a purposive sampling technique. The month of July represents the peak period of heavy rainfall and September is typically the end of wet months, whereas the months of October to December marks the beginning of dry months to the middle of dry period. Dry sterile swab sticks were used to swab the toilet doors and food preparation surfaces, covering about 4cm by 3cm of the surfaces (Dorsa et al., 1996). The food preparation surfaces basically in this study include the surfaces of the table used in preparation of food, and some utensils used in the kitchen like the wooden material (mortar and pestle) and surfaces of pots. Swabbing was carried out for at an average of 30 – 45 seconds during sampling. Selection criteria of samples were in the selection criteria of the households used, households made with mud, with children under the age of 5, and with poor or lack of toilet facility. Swab samples were labeled correctly and transported within 30 minutes to the laboratory at the Department of Microbiology, Federal University of Technology, Akure for analysis.

2.3 Enumeration of enteric bacteria in swab samples

Toilet doors and food preparation surfaces were swabbed, after which the swab sticks were placed into sterile test-tubes filled with 9 ml sterile distilled water and allowed to equilibrate for about 10 minutes. About 1 ml of the serially diluted sample was dispensed into Petri dishes for the enumeration of *Escherichia coli*, *Salmonella*, *Shigella*, *Enterobacter*, *Klebsiella* and *Proteus* on freshly prepared selective media: Eosin Methylene Blue agar (EMB); *Salmonella Shigella* agar (SSA)and MacConkey Agar (MCA)using the pour plate method. The plates were incubated invertedly at 37 for 24 hours. *Escherichia coli* had dark blue colonies on EMB; *Salmonella* had colorless colonies with a black center whereas colonies of *Shigella* were clear, colorless and transparent on SSA. *Enterobacter* had small pink mucoid colonies whereas *Klebsiella* had large pink-red colonies on EMB and MCA. *Proteus* had colorless colonies ON MCA. All colonies were counted, calculated and expressed as the logarithm of colony-forming units per milliliter (log 10 CFU/ml).

2.4 Bacterial genomic DNA extraction from samples

The swab was vortexed in the ZR BashingBead™ Lysis Tube (0.1 and 0.5 mm) with 750 µl of ZymoBIOMICS™ Lysis Solution for 30 seconds to transfer the microbes into solution. A bead beater fitted with a 2 ml tube holder assembly was secured and processed using optimized beat beating conditions. The ZR BashingBead™ Lysis Tubes (0.1 and 0.5 mm) was centrifuged in a microcentrifuge at ≥ 10,000 × g for 1 minute. Approximately 400 µl of supernatant was transferred to the Zymo-Spin™ III-F Filter in a Collection Tube and centrifuged at 8,000 × g for 1 minute. The Zymo-Spin™ III-F Filter was then discarded. 1,200 µl of ZymoBIOMICS™ DNA Binding Buffer was added to the filtrate in the Collection Tube from Step 4 and mixed well. 800 µl of the mixture was transferred to a Zymo-Spin™ IICR Column in a Collection Tube and centrifuged at 10,000 × g for 1 minute. The flow through was discarded from the Collection Tube and the previous step was repeated. 400 µl ZymoBIOMICS™ DNA Wash Buffer 1 was added to the ZymoSpin™ IICR Column in a new Collection Tube and centrifuged at 10,000 × g for 1 minute, then the flow-through was discarded. Afterwards, 700 µl ZymoBIOMICS™ DNA Wash Buffer 2 was added to the ZymoSpin™ IICR Column in a Collection Tube and centrifuged at 10,000 × g for 1 minute and the flow-through was discarded. 200 µl ZymoBIOMICS™ DNA Wash Buffer 2 was added to the ZymoSpin™ IICR Column in a Collection Tube and centrifuged at 10,000 × g for 1 minute. The Zymo-Spin™ IICR Column was transferred to a clean 1.5 ml microcentrifuge tube and 100 µl (50 µl minimum) ZymoBIOMICS™ DNase/RNase Free Water was added directly to the column matrix and incubated for 1 minute and centrifuged at 10,000 × g for 1 minute to elute the DNA. A Zymo-Spin™ III-HRC Filter was placed in a new Collection Tube and 600 µl ZymoBIOMICS™ HRC Prep Solution was added and centrifuged at 8,000 × g for 3 minutes. The eluted DNA was transferred to a prepared Zymo-Spin™ III-HRC Filter in a clean 1.5 ml microcentrifuge tube and centrifuge at exactly 16,000 × g for 3 minutes. The filtered DNA was then suitable for PCR.

2.5 16S rRNA gene amplification and sequencing

The extracted DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Polymerase chain reaction (PCR) amplified 16S gene using 16S Barcoded Forward (341F) and Reverse (805R) primers targeting V3–V4 region to generate the PCR amplicon. The barcoded, 16S gene-specific forward and reverse PCR primers were diluted to 2.5 µM in 10 Tris-HCl pH 8.0-8.5. These were thoroughly mixed using pipetting or vertexing, resulting in a volume of diluted oligo. The diluted primer oligo concentrations were verified by directly measuring the OD260 (with the subscription - UV absorbance at 260 nm for DNA quantification) of each 2.5 μM primer solution using a NanoDrop system (brand-Thermo Fisher Scientific, model-NanoDrop one C). Up to 192 asymmetrically-barcoded 16S metagenomic samples were pooled to generate a single, pooled sample for SMRTbell library construction which occurred for 4 hours. Input mass of pooled sample of ≥500 ng was required for library construction. Samples were sequenced on the Sequel IIe system by Pacific Biosciences (PacBio) and SMRT® (Single Molecule Real-Time) sequencing Technology. Raw sub-reads were processed through the SMRTlink (v11.0) Circular Consensus Sequences (CCS) algorithm to produce highly accurate reads (>QV40). These highly accurate reads were then processed through vsearch (https://github.com/torognes/vsearch) and taxonomic information was determined based on QIIME2 Version 2024.5.0 (Kashchenko et al., 2025).

2.6 Assessment of domestic risk factors of fecal contamination

Data on socio-economic, demographic covariates, sanitation status and hygiene practices were collected through questionnaires given to the members of the selected households. The questionnaire consists of questions based on the type of toilet facility, hygiene practices, access to soap or other cleaning agents, main source of drinking water and availability of water treatment scheme. Questionnaires were interpreted in the local language according to the respondent's preference.

2.7 Statistical analysis

Data obtained were inputted into the Excel workbook using Microsoft Excel 2019 and subjected to descriptive statistics. The socio-economic and sanitary characteristics of residents in selected households were summarized using percentages to reflect the proportion of households meeting specific conditions, while household income was recorded in Nigerian Naira (#). Two-way analysis of variance (ANOVA) was carried out to determine the effects of surface type (toilet doors and food preparation surfaces) and sanitation status of households on the concentration of enteric bacteria, and means were separated by Duncan's new Multiple Range test using SPSS version 27.0. Kolmogorov-Smirnov test was carried to assess the normality of bacterial concentration data to determine the suitability of parametric statistical analysis, and association of risk factors with concentration of enteric bacteria was determined.

3. Results and Discussion

3.1 Detection of enteric bacteria in swab samples

A total of 24 swab samples were collected from each of the five households. 12 from toilet doors and 12 from food processing surfaces. Overall, swab samples from the food processing surfaces had a higher load of enteric bacteria than the toilet doors (Figure 1). In food processing surfaces, the mean load of *E. coli* ranged from 2.15 to 2.64 $\log_{10}$ CFU/ml in 80% of the selected households, *Salmonella* ranged from 1.07 to 2.15 $\log_{10}$ CFU/ml, *Enterobacter* ranged from 1.74 to 2.40 $\log_{10}$ CFU/ml, *Shigella* ranged from 1.14 to 1.4 $\log_{10}$ CFU/ml, *Klebsiella* ranged from 1.2 to 2.2 $\log_{10}$ CFU/ml, and *Proteus* ranged from 2.13 to 2.21 $\log_{10}$ CFU/ml in swab samples, whereas on toilet doors, the mean load of *E. coli* ranged from 1.56 to 2 $\log_{10}$ CFU/ml, *Salmonella* ranged from 1.43 to 1.67 $\log_{10}$ CFU/ml, *Enterobacter* ranged from 1.43 to 1.67 $\log_{10}$ CFU/ml, *Klebsiella* ranged from 1.7to 1.9$\log_{10}$ CFU/ml, and *Proteus* ranged from 1.67 to 1.83 $\log_{10}$ CFU/ml in swab samples. *E. coli* had the highest bacterial count in food processing surfaces, while *Shigella* had the least, whereas, in toilet door samples, *Salmonella* had the least load of bacteria while *E. coli* remained as the highest bacterial count (Figure 1).

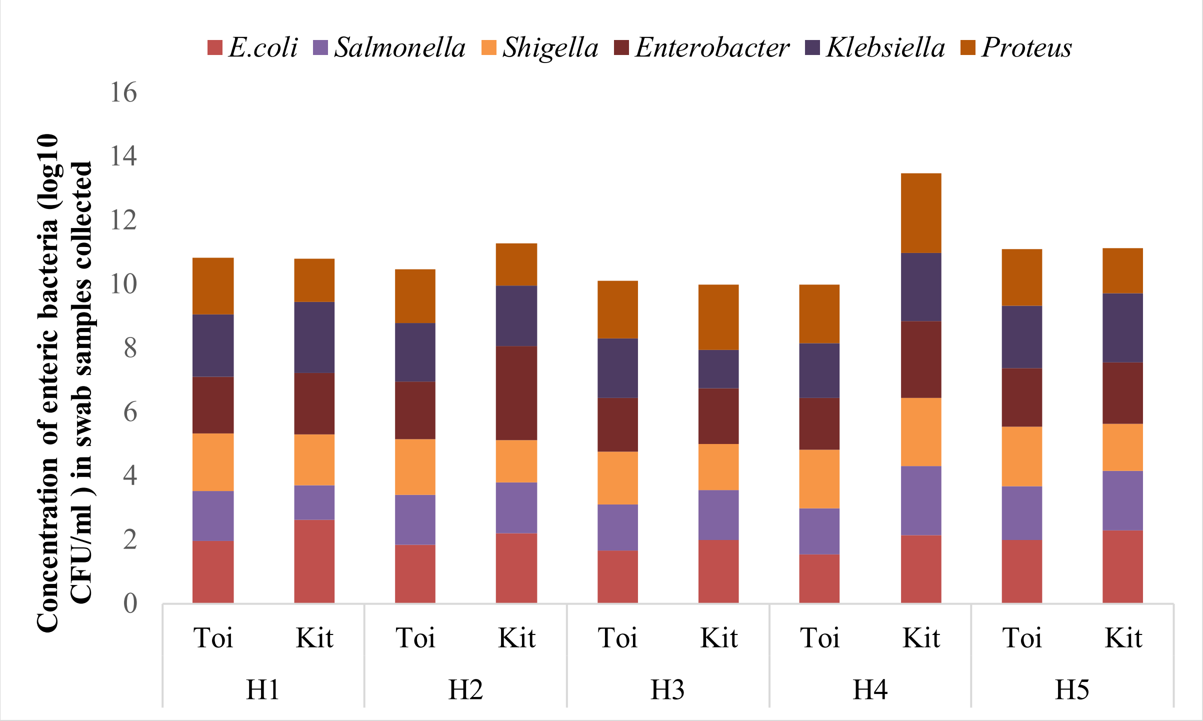

Figure 1. Stacked column showing the mean concentration of enteric bacteria (CFU/ml) in the swab samples (n – 12 samples per source). *Key*: H1 – Household 1; H2 – Household 2; H3 – Household 3; H4 – Household 4; H5 – Household 5; Kit – Kitchen sample; Toi – Toilet door sample

Frequency of occurrence of enteric bacteria in swab samples; PFC $= \frac{No\ of\ specific\ isolate}{Total\ no\ of\ isolates} \times 100\%$

Toilet doors had 100% frequency of occurrence of enteric bacteria, while food preparation surfaces had varying frequencies: 41.6% for *Shigella* and 91.6% for *E. coli*, *Enterobacter*, *Klebsiella* and *Proteus* (Table 1) in the swab samples.

Table 1. Frequency of occurrence of enteric bacteria in swab samples.

Sample source	Isolate	Occurrence	Percentage frequency of occurrence (PFC)%
Toilet doors	*E. coli*	12	100
	Salmonella	12	100
	Shigella	12	100
	Enterobacter	12	100
	Klebsiella	12	100
	Proteus	12	100
Food preparation surfaces	*E. coli*	11	91.6
	Salmonella	7	58.3
	Shigella	5	41.6
	Enterobacter	11	91.6
	Klebsiella	11	91.6
	Proteus	11	91.6

3.2 Distribution pattern of enteric bacteria in samples

The Kolmogorov Smirnov test produced test statistics that were used (along with a degree of freedom parameter) to test for normality for each of the isolated organisms from swabs from toilet doors and food preparation surfaces (Güner et al., 2025). Table 2 shows that all the isolates from toilet doors showed statistical association ($p < 0.001$, 0.041, 0.001 and 0.033) with the load of organisms (*Shigella*, *Enterobacter*, *Klebsiella* and *Proteus*) respectively which gave significant evidence that the distribution pattern of these organisms did not follow a normal distribution, except *E. coli* and *Salmonella* that showed no statistical association with the load of the organisms, and showed a pattern that followed a normal distribution. On the other hand, all isolates from food preparation surfaces showed statistical association with the loads of the organisms except *Enterobacter* ($p = 0.005$), reflecting a pattern for normal distribution. E. coli showed a statistical association ($p = 0.004$), while *Salmonella*, *Shigella*, *Klebsiella* and *Proteus* had a statistical association ($p < 0.001$), all showing no pattern for normal distribution. The Kolmogorov Smirnov statistic value for toilet doors isolates (*E. coli, Salmonella, Shigella, Enterobacter, Klebsiella* and *Proteus*) took 0.110, 0.147, 0.221, 0.163, 0.215 and 0.167 respectively. Statistic value for food preparation surfaces isolates (*E. coli, Salmonella, Shigella, Enterobacter, Klebsiella and Proteus*) also took 0.200, 0.273, 0.341, 0.263, 0.195 and 0.358 respectively. The degrees of freedom for all which equals the number of data points named 30.

Table 2. Kolmogorov-Smirnov test showing the significance in distribution pattern in enteric bacteria microbial count.

Sample source	Isolate	Statistic	df	Sig.
Toilet doors	*E. coli*	0.110	30	0.200*
	Salmonella	0.147	30	0.095
	Shigella	0.221	30	< 0.001
	Enterobacter	0.163	30	0.041
	Klebsiella	0.215	30	0.001
	Proteus	0.167	30	0.033
Food preparation surfaces	*E. coli*	0.200	30	0.004
	Salmonella	0.273	30	< 0.001
	Shigella	0.341	30	< 0.001
	Enterobacter	0.263	30	< 0.001
	Klebsiella	0.195	30	0.005
	Proteus	0.358	30	< 0.001

*. This is a lower bound of the true significance

3.3 Bacterial diversity in samples

Kingdom level classification resulted in 100% of reads in the metagenome datasets being identified as bacteria, which recorded 21694 read count. The order Pseudomonadales had the highest read count of 8608 (39.71%), Micrococcales recorded (19.68%) with 4266 read count. Order Lactobacillales gave a record of (13.43%), Enterobacterales recorded (0.16%) as the 12th in order of dominance, while (16.40%) was unclassified. Enterobacteriaceae recorded 19(1%), meanwhile Moraxellaceae recorded the highest bacterial count 8587, (39.62%). The highest genera recorded include *Acinetobacter* (39.38%), as shown in Table 3, then *Kocuria* (8.91%), *Desemzia* (8.67%), *Staphylococcus* (4.57%) and *Enterococcus* was (1%). At the species level, unknown was recorded to be (84.21%), *Desemzia*_uncultured_*Desemzia* (3.44%), *Kocuria*_uncultured_marine (2.23%), *Kocuria_Kocuria_palustris* (2.05%), *Kocuria_Rothia_koreensis* (1.51%), *Acinetobacter_Acinetobacter_junii* (1.48%), *Desemzia_Mycobacterium_abscessus* (0.62%), while others recorded (4.46%). Table 3 reveals the top ten identification in the taxonomic hierarchy for bacteria in the collected swab samples.

Table 3. Top Ten identification in the taxonomic hierarchy for bacteria in swab samples.

	Class	Order	Family	Genus
1	*Gammaproteobacteria* (42.39%)	*Pseudomonadales* (39.71%)	*Moraxellaceae* (39.62%)	Acinetobacter (39.38%)
2	*Bacilli* (28.36%)	*Micrococcales* (19.68%)	*Micrococcaceae* (15.31%)	*Kocuria* (8.91%)
3	*Actinobacteria* (20.80%)	*Lactobacillales* (13.43%)	*Carnobacteriaceae* (10.79%)	*Desemzia* (8.67%)
4	*Alphaproteobacteria* (0.06%)	*Staphylococcales* (6.15%)	*Staphylococcaceae* (6.15%)	*Staphylococcus* (4.57%)
5	SAR324_clade (0.04%)	Bacillales (1.45%)	Dermabacteraceae (1.31%)	Marinilactibacillus (1.26%)
6	Actinomycetes	Oceanospirillales (1.04%)	Enterococcaceae (1.00%)	Brachybacterium (1.22%)
7		Exiguobacterales (0.96%)	Halomonadaceae (0.99%)	Enterococcus (1.00%)
8		Corynebacteriales (0.38%)	Exiguobacteraceae (0.96%)	Exiguobacterium (0.97%)
9		Burkholderiales (0.29%)	Bacillaceae (0.72%)	Salinicoccus (0.85%)
10		Enterobacterales (0.16%)	Enterobacteriaceae (0.09%)	Bacillus (0.67%)

3.4 Characteristics of selected households and risk factors

More than 60% of the households had more than 10 inhabitants, with an overall average of 8 persons. There was no statistical significance ($p = 0.053$) in the number of households when more than 10, between 5 and 10, and less than 5 members of each household when compared. Similarly, there was no statistical significance ($p = 0.302$) in the number of children in each household (Table 2). About 20% of the residents earned an average income more than ₦200,000 every month, 40% earned between ₦100,000 and ₦200,000 per month, 20% earned between ₦50,000 to ₦100,000. The residents were majorly farmers, traders, civil servants, and operators (Figure 2). About 60% had access to good toilet facilities, 80% had access to good hygiene practice and 80% had access to cleaning agents. All had access to clean and safe drinking water. About 40% used treated water, 40% used treated water once in a year and 20% lacked access to treated water (Table 4).

Table 4. Socio- economic and sanitary characteristics of residents in selected households.

Risk factors	**Swab sources**						
	H1	H2	H3	H4	H5	Total	Percentage (%)
Type of toilet facility ($p = 0.302$)							
Water closet	0	0	1	1	1	3	60
Squat toilet	0	0	0	0	0	0	0
Pit latrine	1	0	0	0	0	1	20
No toilet	0	1	0	0	0	1	20

Hygiene practice ensured (*p* = 0.052)							
Hand washing	1	1	1	1	0	4	80
Daily bathing	1	1	1	1	1	5	100
Daily washing of dishes	1	1	1	1	1	5	100
Hand washing after handling pets	0	0	1	0	1	2	40
Mouth covering when sneezing	1	1	1	1	1	5	100
Access to soap or other cleaning agents (*p* = 0.053)							
Sufficient	0	1	1	1	1	4	80
Manageable	1	0	0	0	0	1	20
Not enough	0	0	0	0	0	0	0
Main source of drinking water (*p* = 0.002)							
Boreholes	0	0	0	0	0	0	0
Wells	1	1	0	1	1	4	80
Rainfall	0	1	0	0	0	1	20
Spring and River	0	0	0	0	0	0	0
Bottled water	0	0	1	0	0	1	20
Availability of water treatment scheme (*p* = 0.576)							
Frequently	0	0	0	0	0	0	0
Occasionally	1	1	0	0	0	2	40
Once in a year	0	0	1	0	0	1	20
None	0	0	0	1	1	2	40

Key: H1 – Household 1, H2 – Household 2, H3 – Household 3, H4 – Household 4, H5 – Household 5

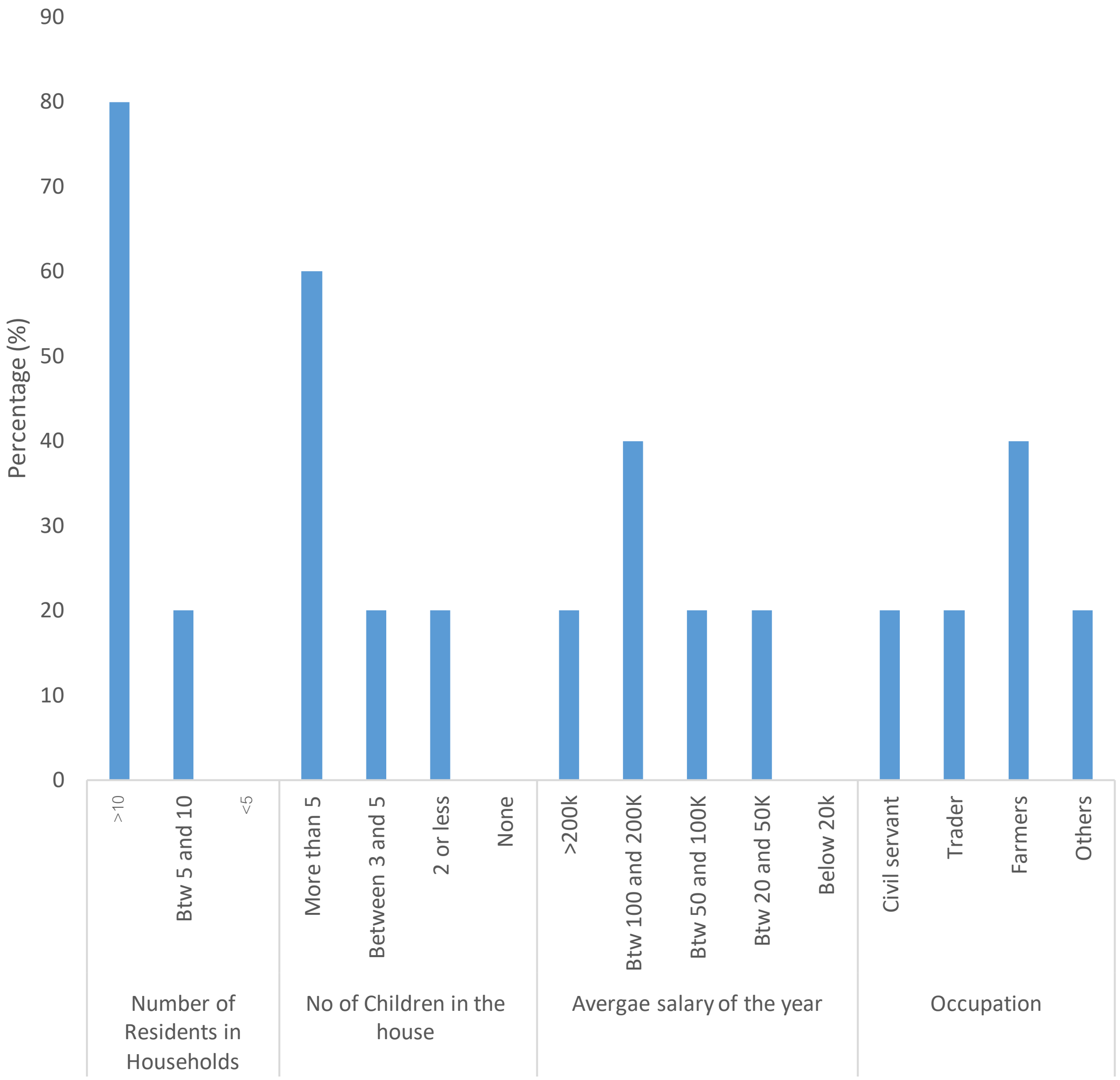

Figure 2. Socio- economic characteristics of residents in selected households.

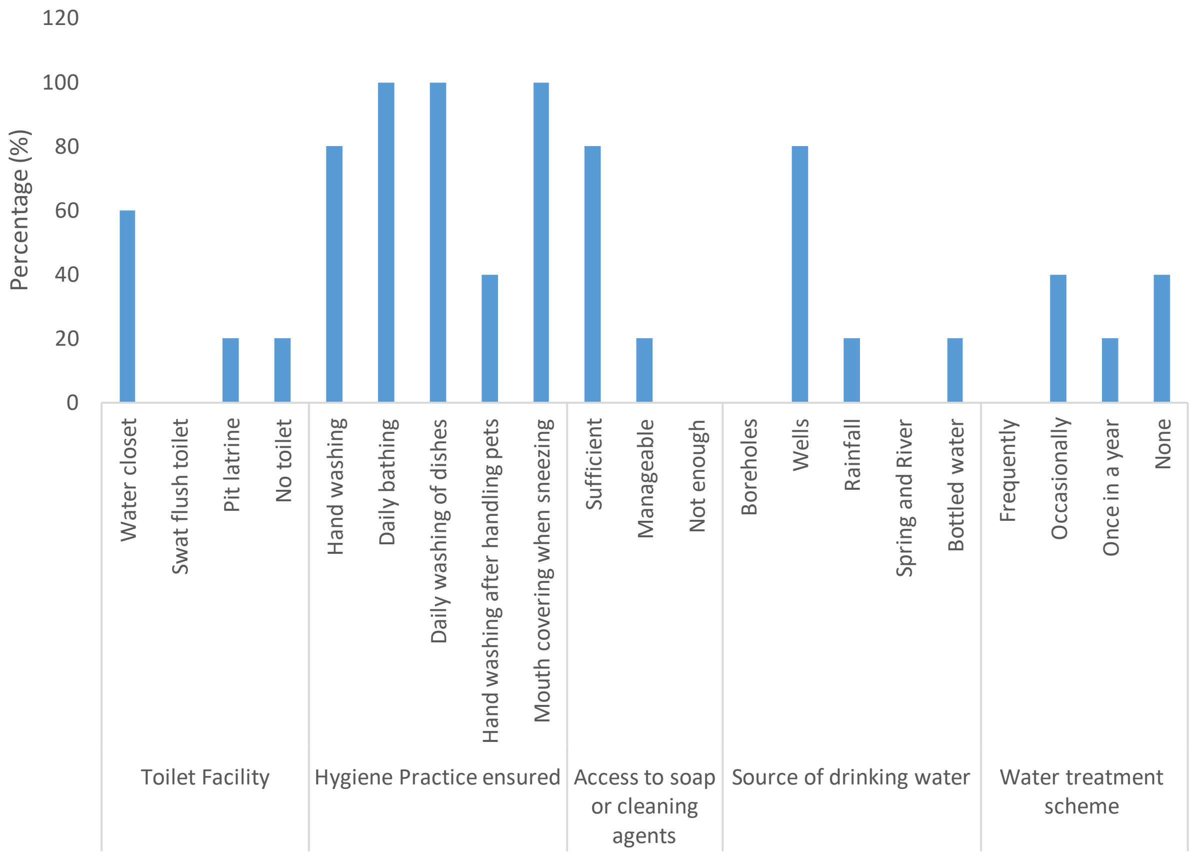

Figure 3. Hygiene practice characteristics of residents in selected households.

3.5 Association of risk factors with concentration of enteric bacteria in samples

‘No toilet’ and 'Manageable access to soap’ showed significant ($p = 0.715, 0.715, 0.834$) and ($p = 0.834, 0.565, 0.565$) association with the presence of *Enterobacter*, *Klebsiella* and *Proteus* respectively. ‘Rainfall’ as drinking water showed significant ($p = 0.693, 0.693$) association with the presence of *Salmonella* and *Shigella* respectively. ‘Pit latrine’, and 'Once in a year water treatment scheme' showed no statistical significance ($p < 0.05$) with all the isolated organisms (Table 5).

Table 5. Association of risk factors with enteric bacteria in swab samples.

Risk factors	***E. coli*** ***p* value**	***Salmonella*** ***p* value**	***Shigella*** ***p* value**	***Enterobacter*** ***p* value**	***Klebsiella*** ***p* value**	***Proteus*** ***p* value**
Pit latrine (TL)	0.007	0.001	0.001	0.007	0.019	0.003
No toilet (TL)	0.019	0.076	0.167	**0.715**	**0.715**	**0.834**
Manageable access to soap	0.003	0.033	0.081	**0.834**	**0.565**	**0.565**
Well (DW)	0.001	0.117	0.240	0.076	0.033	0.117
Rainfall (DW)	0.001	**0.693**	**0.693**	0.167	0.081	0.240
Once in a year water treatment scheme	0.001	0.001	0.001	0.001	0.001	0.001

Key: TL – Type of toilet facility, DW – Main source of drinking water. Values in bold indicate correlation.

Pathogenic microorganisms are continually being introduced into surfaces via people, food, pets, insects, contaminated water supplies and the air, food processing surfaces and toilet doors inclusive. Previous works and studies have shown that toilet doors and walls are prone to contamination by bacteria, fungi and viruses which in turn serve as reservoirs for cross infection and recontamination of washed hands (Olaitan, 2020). In this study, food processing surfaces and toilet doors of some selected households in rural neighborhoods of Ibule-Soro in Akure, Ondo State, Nigeria were evaluated for bacterial communities and level of enteric bacteria. Bacterial loads were expressed as $\log_{10}$ CFU/ml and interpreted using international hygiene benchmarks, which consider counts above 4.0 log 10 CFU/ml indicative of poor sanitary conditions and increased risk of infection (Gume et al., 2023). The presence of bacteria such as, fecal coliforms, *Salmonella*, *Shigella*, *Klebsiella*, and *Proteus*, among others from these sources in the selected households raises significant concerns about the water quality and hygiene practices in the examined households. These bacteria are commonly associated with fecal contamination and can pose serious health risks if ingested. Some of these bacteria are potential pathogens and can be transferred from person to person or may result in autoinoculation (Olaitan, 2020). The findings of this study are in agreement with Borrusso and Quinlan (2017) where the authors isolated *Salmonella*, *Escherichia coli*, *Campylobacter jejuni*, and *Listeria monocytogenes* from food processing surfaces. The presence of Escherichia coli, Proteus mirabilis, Salmonella species and Klebsiella pneumoniae infer contamination by fecal matter. This might be possible since most people go to toilet and end up contaminating their hands, food processing environments and cooking utensils with fecal and urinary material. A study recorded that most people lack the concept of hand washing as a simple means of stopping spread of infectious agents (Olaitan, 2020). Infection by *Escherichia coli* may cause diseases like urinary tract infection (UTI) and gastrointestinal disease,zz pneumonia etc. *E. coli* emerged as the most prevalent bacterium in swab samples from food processing samples with dominance in Households 1 and 5 while *Enterobacter* in toilet doors samples with dominance in same households among others, indicating potential fecal contamination. The consistent presence of *E. coli* in these households being an indicator of fecal pollution, emphasizes the urgent need for improved hygiene practices and water quality management to mitigate health risks associated with waterborne diseases. *E. coli*, and *Proteus* spp. were isolated frequently both in dry and wet seasons from the samples' sources during this study. About 100% of the households were positive for *E. coli*, *Salmonella*, and *Proteus*, with variation in their total counts. This observation is in agreement with past studies which detected *Salmonella* and *Proteus* in dishcloths, sponges and kitchen sinks (Borrusso & Quinlan, 2017). While *Shigella* was the least isolated bacterium in food processing surfaces, it's noteworthy that household 4 still showed to be most contaminated because it exhibited the highest average microbial count, however, *Salmonella* appeared to be the least isolated bacterium in toilet door samples across the households. A study conducted in European countries revealed the prevalence of *Campylobacter* and *Salmonella* in kitchens which was attributed to causing cross-contamination events that disseminate infectious doses of pathogen (Møretrø et al., 2021). However, the presence of *Shigella* and *Salmonella* in these households raised concerns about water safety and the potential for waterborne diseases associated with these bacteria (Kumar et al., 2021).

The varied prevalence of *Klebsiella* and *Proteus* in different households suggests environmental heterogeneity. *Klebsiella* had the highest average bacterial count in household 1 in both food processing and toilet doors swab samples, in contrast *Proteus* was least isolated in household 2 for both swab samples. These variations may be attributed to specific environmental conditions, water sources, or individual hygiene practices in each household (Nguyen et al., 2021). Understanding these differences is crucial for tailoring interventions to address specific bacterial threats in different settings.

Metagenomics of the households' swab samples revealed insights into the bacterial genomes present. All reads were classified at the kingdom level as bacteria, totalling 21,694 reads. The order Pseudomonadales had the highest read count (39.71%), followed by Micrococcales (19.68%), and Lactobacillales (13.43%). Enterobacterales ranked 12th in dominance with 0.16% reads. Enterobacteriaceae accounted for 1% of reads, while Moraxellaceae had the highest bacterial count (39.62%). The most abundant genera were *Acinetobacter* (39.38%), *Kocuria* (8.91%), *Desemzia* (8.67%), *Staphylococcus* (4.57%), and *Enterococcus* (1%). At the species level, unknown species dominated (84.21%), followed by *Desemzia*_uncultured_*Desemzia* (3.44%), *Kocuria*_uncultured_marine (2.23%), and others.

Differences observed between culture-based bacterial counts and 16S rRNA gene sequencing results are expected and reflect the inherent methodological distinctions between these approaches. Culture-based methods quantify only viable and culturable bacteria capable of growing under specific laboratory conditions, and therefore may underestimate the total bacterial diversity present on environmental surfaces. In contrast, 16S rRNA gene sequencing detects both culturable and non-culturable bacteria, including viable-but-non-culturable (VBNC) organisms and bacterial DNA from recently inactivated cells, providing a broader representation of microbial community composition. Environmental stresses such as desiccation, ultraviolet exposure, and temperature fluctuations—particularly across wet and dry seasons—can reduce bacterial culturability without eliminating bacterial DNA, thereby contributing to discrepancies between CFU counts and sequencing-based detection (Rossi et al., 2025). As a result, surfaces may show lower culture-based bacterial counts while still exhibiting substantial bacterial diversity in molecular analyses. Similar discrepancies have been widely reported in environmental and sanitation studies, where molecular methods consistently as reveal higher microbial diversity than culture-based techniques (Delbès et al., 2007).

Olalemi et al. (2023) had earlier reported that water quality was generally poor for some water supplies in Ibule-Soro. In this study, many of the selected households lacked access to good water systems and sound sanitary facilities which definitely have contributed to the high amount of bacterial count in all the households, and household 4 being the most dangerous zone to inhabit, having the highest average microbial load. A lack of cleaning products in the home, including paper and cloth towels, was strongly associated with several types of contamination, including coliforms, fecal coliforms, *E. coli*, and *S. aureus* (Carstens et al., 2022). These findings suggest that a significant transfer of these organisms occurs in domestic kitchens, partly due to failure of the households' occupants to wash their hands correctly. In other cases, this transfer may occur via the cleaning utensils, such as sponges and dishcloths, because a past study found a significant association between contamination on kitchen sponges and dishcloths and contamination in other parts of the kitchen of the same home (Borrusso & Quinlan, 2017). High-touch surfaces such as toilet doors, latrine handles, and food preparation areas have been shown to harbor enteric bacteria capable of facilitating fecal–oral transmission within households (Abney, 2022; Abney et al., 2021; Appiah et al., 2025). In sub-Saharan Africa, inadequate access to improved sanitation facilities, poor waste management, and limited hand hygiene practices have been strongly associated with elevated microbial contamination of domestic environments (Bishoge, 2021). In Nigeria specifically, several studies have reported widespread fecal contamination of household environments, water sources, and food contact surfaces in rural and peri-urban communities, linking these conditions to increased risks of diarrhoeal diseases and other enteric infections (Aydamo et al., 2024; Kelly et al., 2023; Potgieter et al., 2020). Environmental microbiological studies have further shown that contamination levels are often higher on sanitation-associated surfaces than on general household surfaces, underscoring the role of toilets and surrounding fomites as critical points of pathogen transmission (Chard et al., 2020).

Since the study covered both dry and wet sessions, variation in results showed that there was a massive increase in the average bacteria count from the first month to the second month. And this was similar in all the households. Then, a gradual and consistent decrease was recorded from the third month henceforth and this may be as a result of changes in environmental conditions (Nguyen et al., 2021). The observed reduction in bacterial counts during the dry season compared to the wet season highlights the influence of seasonal variation on environmental microbial contamination. The higher bacterial loads recorded during the rainy months (July–September) can be attributed to increased surface moisture, runoff, and flooding, which facilitate the spread of fecal matter from latrines, refuse dumps, and contaminated soils onto household surfaces. During the wet season, poor drainage and water splashing around toilet areas further enhance the survival and dissemination of enteric bacteria, increasing the likelihood of surface contamination. In contrast, the dry season (October–December) is characterized by reduced moisture and higher ambient temperatures, conditions that are less favorable for bacterial persistence and survival on exposed surfaces, thereby explaining the lower bacterial counts observed during this period.

Similar seasonal trends have been reported in other studies from low-income settings, where microbial contamination of household environments and water sources was significantly higher during the rainy season due to increased fecal runoff and environmental exposure (Bain et al., 2014). Seasonal variation in enteric pathogen prevalence has also been linked to increased diarrhoeal disease incidence during rainy periods, particularly in communities with inadequate sanitation and waste management systems (Troeger et al., 2017).

The microbial assessment via combined use of culture-based enumeration and 16S rRNA gene sequencing in this study provides complementary insights, allowing both quantitative assessment of viable enteric bacteria and qualitative characterization of overall bacterial communities and underscores the need for comprehensive water and sanitation management strategies at the household level. The prevalence patterns observed highlight specific areas for improvement in hygiene practices, water sources, and sanitation infrastructure. Tailored interventions and educational programs aimed at addressing the specific challenges identified in each household can contribute to a significant reduction in microbial contamination, ultimately enhancing the overall health and well-being of the communities involved.

4. Conclusions

This study showed that proper hygiene practice and sanitation was generally poor in Ibule-Soro, but that food processing surfaces were worse than toilet doors, that is, higher load but not occurrence, and are therefore, a higher priority for action. *E. coli* and *Enterobacter* were detected more in all swab samples, irrespective of the source, but *Shigella* and *Salmonella* were the least detected in swab samples collected from the food preparation surfaces and toilet door respectively. The load of enteric bacteria in swab samples was higher in food preparation than in toilet doors. The molecular analysis revealed the presence of Enterobacteriaceae family which all the organisms of interest belong to. The presence of *E. coli* showed more association with risk factors than *Enterobacter* in both food preparation and toilet doors swab samples, while *Salmonella* showed less than *Shigella* in food preparation compared to toilet doors swab samples. *E. coli* and *Salmonella* appeared to be normally distributed in toilet doors, while other organisms did not. Similarly, only *Enterobacter* showed a normal distribution pattern in food preparation surfaces while others showed no normal pattern of distribution.

Conflict of Interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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Ethical Approval

Ethical approval was obtained from Karis Life Hospital and Diagnostic Centre, Akure, Nigeria. Letter of permission was written to the regent of Ibule-Soro through the Head of Department of Microbiology, Federal University of Technology, Akure and was submitted to the palace of the regent. Prior to sample collection, verbal consent was obtained from the regent and heads of each of the selected households. (Date: July 7, 2023)

Publication Ethic

The manuscript has not been previously published by or under review by another print or online journal or source.

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