ORIGINAL ARTICLES

Microbial contamination of personal reusable water bottles and associated factors among students

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ABSTRACT

Objectives: To determine the prevalence and level of microbial contamination in personal reusable water bottles among university students and to analyse associations with bottle characteristics, hygiene practices and water source.

Methods: A cross-sectional study was conducted at Van Lang University from April to June 2026. A total of 96 undergraduate students were invited to participate in the study; convenience sampling was used for data collection. Swabs of the internal bottle surfaces were cultured on Blood Agar, MacConkey Agar and Sabouraud Dextrose Agar; organisms were classified presumptively by colony morphology and Gram staining.

Results: The overall microbial contamination rate was 98.0% (95% CI 92.9–99.4); 81.6% of samples had a high microbial density (>300 colonies per swab), with presumptive Gram-positive bacteria, Gram-negative bacteria and fungi detected in 85.7%, 73.5% and 46.9% of samples respectively. None of the eleven factors examined was significantly associated with contamination status; the 95% CI for every risk difference (RD) included zero (−5.5 to +5.9 percentage points). Scratched or worn bottles and non-daily washing showed non-significant trends towards higher microbial density (p = 0.090 and p = 0.063).

Conclusion: Microbial contamination of students' personal water bottles was highly prevalent and of high density, with presumptive fungi in nearly half of the bottles. With only two uncontaminated bottles, the study had little power to detect subgroup differences, and the absence of significant associations should not be read as evidence that these factors are unimportant.

Keywords: *reusable water bottle, microbial contamination, bottle hygiene, university students*

INTRODUCTION

Reusable personal water bottles have become increasingly popular among university students as an alternative to single-use plastic. Cleaning practices, however, have received far less attention than the containers themselves. A water bottle is a continuously moist environment that is re-inoculated with the user's oral and skin flora at every use (1), and because users drink directly from the bottle, contamination represents repeated microbial exposure throughout the day rather than a single event.

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Studies from several countries have reported substantial bacterial contamination. Oliphant et al. found that 64.4% of samples from the bottles of Canadian elementary students exceeded the heterotrophic plate count criterion (2). Among medical students in Malaysia, 46% of samples were coliform-positive and 78% showed high heterotrophic counts, with bottle-mouth design and bottle material identified as associated factors (3). A comparison of stainless steel and plastic bottles in India found significantly higher microbial loads in plastic (4). Together these findings indicate that bottle characteristics and cleaning practices may be modifiable determinants of contamination.

Three gaps limit the applicability of this evidence to Vietnamese university students. First, almost all published work has quantified bacteria alone, using heterotrophic plate counts or coliform indicators in bottle rinse water; fungi have rarely been measured, although they are readily recovered from other drinking-water devices and their counts rise with the interval since cleaning (5). Second, the available studies were conducted in temperate settings, whereas a hot and humid climate year-round may favour microbial regrowth. Third, the comparative evidence on bottle material derives from populations in which plastic bottles predominate, and its relevance to populations where stainless steel is the norm remains untested. In Vietnam, at the time of this study, we were unable to identify any published study of microbial contamination in students' reusable personal water bottles; domestic literature has focused on the microbiological quality of commercially bottled drinking water.

This study was therefore conducted with two objectives: (i) to determine the prevalence and level of microbial contamination in reusable personal water bottles used by students at Van Lang University; and (ii) to analyse the association between microbial contamination and bottle characteristics, cleaning practices and water source. We hypothesised that bottle material, surface condition and cleaning frequency would be associated with the level of contamination.

METHODS

Study Design: Cross-sectional descriptive study.

Study Setting and Period: Van Lang University, Ho Chi Minh City, Viet Nam, April to June 2026.

Study Population: Students who had used a reusable personal water bottle for at least one week and voluntarily agreed to participate. Bottles used for less than one week or too damaged to sample, and incomplete questionnaires, were excluded.

Sample Size and Sampling: The sample size was calculated using the formula for estimating a single proportion ($p = 0.5$, $d = 0.1$, 95% confidence level), giving a minimum of 96 participants; 98 were enrolled by convenience sampling.

Study Variables: Demographic characteristics; bottle characteristics (material, cap type, duration of use, surface condition); cleaning practices (washing frequency, soap use, dedicated brush, disassembly, drying method); primary beverage and, for students drinking filtered

water, its source; and microbiological outcomes. These eleven bottle, cleaning, beverage and water-source variables were examined for association with contamination status. Microbial load was the total colony count per swab across the three media, categorised as 0, 1–100, 101–300 or >300 colonies per swab.

Sample Collection and Microbiological Procedures: Data were collected using a structured self-administered questionnaire. Sterile cotton swabs moistened with 0.9% saline were applied to the inner surfaces of the bottle, including the mouth and the cap, one swab per bottle, and transported to the laboratory within two hours or held at 2–8°C if not processed immediately. Samples were cultured on three media: Blood Agar, a non-selective enriched medium for most bacteria including fastidious organisms; MacConkey Agar, selective and differential for Gram-negative bacilli; and Sabouraud Dextrose Agar with chloramphenicol for yeast and filamentous fungi. All media were quality-controlled before use. Colonies were counted on each plate, and plates on which colonies were too numerous to count were recorded as >300 colonies. Organisms were classified presumptively by colony morphology and Gram staining; biochemical identification, coliform confirmation, susceptibility testing and polymerase chain reaction were not performed.

Data Management and Statistical Analysis: Data were entered in Microsoft Excel and analysed in Python. Categorical variables are summarised as frequencies and percentages with 95% confidence intervals (Wilson score method). Associations with contamination status were tested using Fisher's exact test, or the Fisher–Freeman–Halton test with Monte Carlo simulation for variables with more than two categories; microbial load was compared using the Mann–Whitney U test, with Cliff's delta as the effect size. Risk differences are reported with 95% confidence intervals calculated by the Newcombe hybrid score method, and Cliff's delta with 95% confidence intervals derived from the unrestricted (non-null) variance estimator of DeLong. Categories with fewer than ten observations were combined before testing, and participants with missing values on individual items were analysed using available data. Significance was set at $p < 0.05$.

Ethical Considerations: The study was approved by the Ethics Committee of Van Lang University (Decision No. 34/2026/HĐĐĐ-IRB-VN01.078, 16 April 2026). Participation was voluntary, with informed consent obtained after full explanation of the study objectives.

RESULTS

Characteristics of the study sample and personal water bottles

Table 1. Characteristics of the study sample and personal water bottles (n = 98)

Age group	18 - 20 years old	48	49.0
	21 - 23 years old	45	45.9
	> 23 years old	5	5.1
Academic year	1 - 2	53	54.1
	3 - 4	44	44.9
	> 4	1	1.0
Bottle material	Stainless steel	83	84.7
	Plastic	15	15.3
Bottle cap type	Simple	77	78.6
	Sports (valve/straw)	21	21.4
Duration of use	< 3 months	18	18.4
	3 to < 6 months	30	30.6
	6 to < 12 months	14	14.3
	≥ 12 months	36	36.7
Bottle condition	Scratched/worn	64	65.3
	New, no scratches	34	34.7

The study sample predominantly consisted of females (66.3% versus 33.7%). Students in years 1–2 accounted for the highest proportion, followed by years 3–4 and beyond year 4 (54.1%, 44.9% and 1.0%). Most participants used stainless steel bottles (84.7%) rather than plastic bottles (15.3%), and simple caps (78.6%) were more common than sports caps. More than half of the bottles (65.3%) showed signs of surface scratching or wear, and 36.7% had been used for 12 months or longer.

Prevalence and level of microbial contamination

Table 2. Overall microbial contamination rate and distribution of co-occurring microbial groups (n = 98)

Content	Classification	n	%	95% CI / Interpretation
Test result	Positive (≥1 microbial group)	96	98.0	92.9 – 99.4
	Negative	2	2.0	-
Number of co-occurring microbial groups	0 groups	2	2.0	No growth
	1 group	19	19.4	Single-group contamination
	2 groups	48	49.0	Mixed contamination, 2 groups
	3 groups	29	29.6	All 3 groups (Gram-positive, Gram-negative, Fungi)

Note: microbial groups comprise three major groups — presumptive Gram-positive bacteria, presumptive Gram-negative bacteria, and fungi (yeast and filamentous fungi).

The overall microbial contamination rate was very high (98.0%; 95% CI 92.9–99.4), close to saturation within the study sample. Among the 96 positive samples, 77 (80.2%) yielded two or more microbial groups, indicating that multi-group contamination was the rule rather than isolated contamination by a single group.

Table 3. Contamination rate by microbial group (n = 98)

Microbial Group	Microorganism Type	n	%	95% CI
Gram-positive (n=84; 85.7%)	Positive cocci	44	44.9	35.4 – 54.8
	Positive bacilli	61	62.2	52.4 – 71.2
Gram-negative (n=72; 73.5%)	Negative cocci	11	11.2	6.4 – 19.0
	Negative bacilli	64	65.3	55.5 – 74.0
Fungi (n=46; 46.9%)	Yeast	41	41.8	32.6 – 51.7
	Filamentous fungi	6	6.1	2.8 – 12.7

Note: a sample may contain one or several groups and types of microorganisms, so totals may exceed 100%. All classifications are presumptive, based on colony morphology and Gram staining only.

Contamination rates differed by microbial group, with Gram-positive bacteria highest, followed by Gram-negative bacteria and fungi (85.7%, 73.5% and 46.9% respectively). Gram-negative bacilli were the most frequent organism type (65.3%; 95% CI 55.5–74.0), followed by Gram-positive bacilli (62.2%) and yeasts (41.8%).

Microbial density

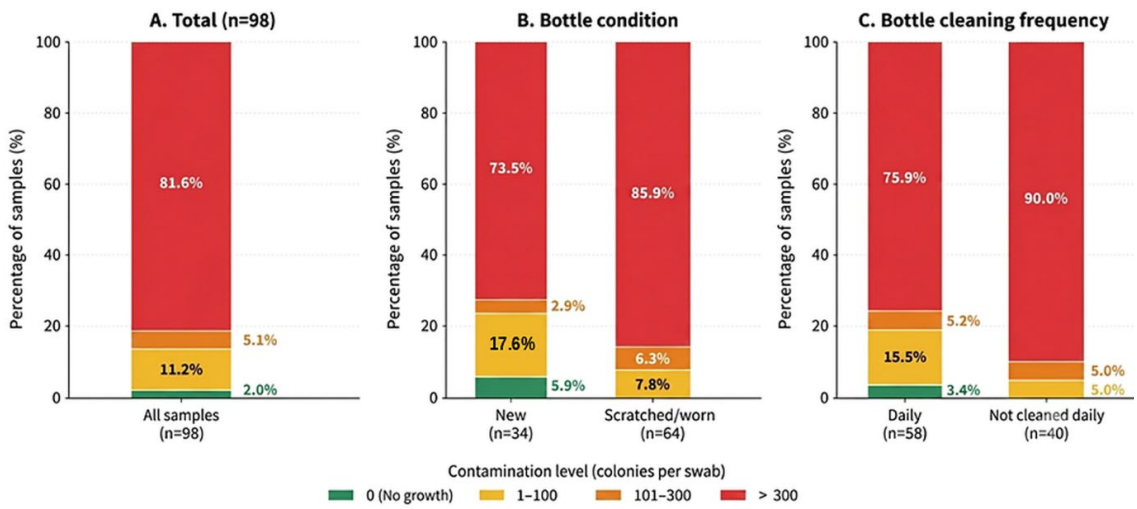


Figure 1. Distribution of microbial density (4 levels) overall (A), by bottle surface condition (B), and by washing frequency (C).

Note: Mann–Whitney U test; Cliff's delta with 95% CI from the unrestricted (non-null) variance estimator. Bottle condition: delta 0.141 (−0.037 to 0.319), p = 0.090. Washing frequency: delta 0.150 (0.006 to 0.294), p = 0.063. For washing frequency the interval marginally excludes zero while the test does not, as the two use different variance estimators (SE 0.074 vs 0.080); both comparisons are interpreted as non-significant trends. Percentages may not total 100% due to rounding.

The total number of colonies across the three-culture media was classified into four density levels. Most samples (81.6%; 95% CI 72.8–88.1) showed a high colony count (> 300 colonies per swab), and only 2.0% had no microbial growth. Scratched or worn bottles and bottles not washed daily both showed higher proportions of samples at the highest density level than their comparison groups, although neither difference reached statistical significance.

Hygiene habits and primary beverages used

Table 4. Hygiene habits and primary beverages (n = 98)

Characteristic	Classification	n	%
Washing frequency	Daily (≥7 times/week)	58	59.2
	3 - 6 times/week	14	14.3
	1 - 2 times/week	23	23.5
	< 1 time/week	3	3.0
Soap/detergent	Yes	76	77.6
	No	22	22.4
Dedicated brush	Yes	50	51.0
	No	48	49.0
Disassembly during washing	Yes	91	92.9
	No	7	7.1
Drying method after washing	Air dry naturally	82	83.7
	Wipe dry with towel	9	9.2
	Cap immediately	6	6.1
	Machine dry (dishwasher)	1	1.0
Primary beverage	Filtered water	74	75.5
	Tea	6	6.1
	Coffee	11	11.2
	Sugary drinks/others	7	7.2

Most students reported relatively good bottle hygiene habits: 59.2% washed their bottles daily, 77.6% used soap or detergent, 51.0% used a dedicated brush, and 92.9% disassembled components during washing. However, the majority (83.7%) allowed bottles to air dry rather than wiping them dry. Filtered water was the primary

beverage for 75.5% of participants.

Association between microbial contamination status and factors

Table 5. Association between microbial contamination status and the eleven examined factors (n = 98)

Factor	Classification	Contaminated n (%)	RD % (95% CI)	p-value
Bottle material	Plastic (n = 15)	14 (93.3)	−5.5 (−28.6 to +2.2)	0.284 *
	Stainless steel (n = 83)	82 (98.8)	Ref.	
Bottle condition	Scratched/worn (n = 64)	64 (100.0)	+5.9 (−1.2 to +19.1)	0.118 *
	New (n = 34)	32 (94.1)	Ref.	
Bottle cap type	Sports (n = 21)	21 (100.0)	+2.6 (−13.0 to +9.0)	1.000 *
	Simple (n = 77)	75 (97.4)	Ref.	
Duration of use	< 3 months (n = 18)	17 (94.4)	—	0.634 **
	3 to < 6 months (n = 30)	29 (96.7)		
	6 to < 12 months (n = 14)	14 (100.0)		
	≥ 12 months (n = 36)	36 (100.0)		
Washing frequency	Non-daily (n = 40)	40 (100.0)	+3.4 (−5.7 to +11.7)	0.512 *
	Daily (n = 58)	56 (96.6)	Ref.	
Soap / detergent	No (n = 22)	22 (100.0)	+2.6 (−12.4 to +9.1)	1.000 *
	Yes (n = 76)	74 (97.4)	Ref.	
Dedicated brush	No (n = 48)	47 (97.9)	−0.1 (−9.1 to +8.6)	1.000 *
	Yes (n = 50)	49 (98.0)	Ref.	

Factor	Classification	Contaminated n (%)	RD % (95% CI)	p-value
	Yes (n = 91)	89 (97.8)	Ref.	
Drying method	Other methods (n = 16)	16 (100.0)	+2.4 (−17.0 to +8.5)	1.000 *
	Air dry naturally (n = 82)	80 (97.6)	Ref.	
Primary beverage	Coffee / sugary (n = 18)	18 (100.0)	+2.5 (−15.2 to +8.7)	1.000 *
	Water or tea (n = 80)	78 (97.5)	Ref.	
Water source + **(filtered water)**	Tap water (n = 34)	33 (97.1)	−0.4 (−12.5 to +10.5)	1.000 *

*Note: * Fisher's exact test. ** Fisher–Freeman–Halton test with Monte Carlo simulation. RD = risk difference versus the reference category (Ref.), with 95% CI by the Newcombe hybrid score method. RD is not reported for duration of use because the overall test compares four categories. + Water source was assessed only among students whose primary beverage was filtered water (n = 73; one participant did not report a source).*

None of the eleven factors examined was significantly associated with contamination status (Table 5). Contamination rates were between 93.3% and 100.0% in every subgroup, and risk differences ranged from −5.5 to +5.9 percentage points, with all 95% confidence intervals including zero. These intervals were wide, spanning 17 to 41 percentage points.

DISCUSSION

The contamination rate in our study was 98.0% (96/98; 95% CI 92.9–99.4), with 81.6% of samples at the highest density level. Two features merit emphasis. Contamination was almost universal despite self-reported hygiene that was relatively good — 59.2% washed daily, 77.6% used detergent and 92.9% disassembled components. And presumptive fungi were recovered from 46.9% of bottles, a group rarely quantified in earlier studies, which measured bacterial indicators only.

Our rate exceeds those of Oliphant et al. (64.4% above the heterotrophic plate count criterion) (2) and Wong et al. (78% with high heterotrophic counts, 46% coliform-positive) (3). The difference is partly methodological: those studies analysed rinse water, whereas we swabbed the internal surfaces directly, including the mouth and cap. Colony counts per swab are not numerically comparable with plate counts per millilitre, so our estimate

reflects organisms on the internal surfaces of the bottle, including the areas in most frequent contact with hands and lips, rather than the quality of the water it contains.

Several mechanisms may explain the uniformly high rate. The mouthpiece and cap are re-inoculated with oral and skin flora at every use, so cleaning removes organisms only until the bottle is next opened (6), and heterotrophic counts in reusable bottles can reach one to two million per millilitre within a day (7); consistent with this, contamination did not differ by water source. Beverage type may add to it: Sun et al. found significantly higher heterotrophic and coliform counts in bottles used for drinks other than water (1), and all 18 bottles containing coffee or sweetened drinks in our sample were contaminated, with presumptive yeasts the commonest fungal type (41.8%). Scratched surfaces and silicone components allow biofilm to persist through washing; in recovered plastic bottles reused for food packaging, Mouafo et al. found biofilm in every bottle, dominated by Pseudomonas but also containing Candida, which routine cleaning failed to remove (8). Finally, Ho Chi Minh City is hot and humid year-round, favouring regrowth, and 83.7% of students air-dried their bottles, so residual moisture may matter more than washing frequency.

None of the eleven factors was significantly associated with contamination status, for three reasons. First, the outcome had almost no variability: with two negative bottles no comparison has meaningful power, and our risk differences carry confidence intervals spanning 17 to 41 percentage points, still compatible with substantial associations. Second, self-reported hygiene is subject to misclassification that biases estimates towards the null. Third, and most consequentially, we recorded how often bottles were washed but not how long had elapsed since the last wash; Tischner et al. found fungal counts in water dispensers correlated with the interval since the last cleaning (5), and two students both reporting daily washing may have been sampled one hour and twelve hours after cleaning. Other unmeasured exposures — drying conditions, access to hot water, storage in closed bags, hand hygiene, and socioeconomic constraints on replacing a worn bottle — may act in the same way.

Two factors nonetheless showed a consistent gradient: scratched or worn bottles and bottles not washed daily had more samples at the highest density level (85.9% versus 73.5%, Cliff's delta 0.141, 95% CI −0.037 to 0.319, $p = 0.090$; 90.0% versus 75.9%, delta 0.150, 95% CI 0.006 to 0.294, $p = 0.063$). Both effect sizes are small and their intervals include or closely approach zero. Both directions fit the mechanisms above and merit study using an outcome more variable than binary contamination status.

Interpretation of the health significance requires care. Without species identification, many organisms are probably the user's own commensal flora and carry low risk. Even so, Hubbard et al. isolated a multidrug-resistant, biofilm-forming Klebsiella grimontii from a reusable bottle (9), and Candida from drinking groundwater can form biofilms with reduced azole susceptibility, mainly threatening immunocompromised people (10). The concern therefore centres on

immunocompromised users, shared bottles and reservoirs of antimicrobial resistance rather than acute illness in healthy students. Practically, the message should move from washing more often to washing more effectively: Wilson et al. reported lower contamination with soap and hot water (11) and Hariharan and Sankar found a structured wash reduced load in both plastic and stainless-steel bottles (4), whereas routine procedures often leave biofilm intact (8). Guidance should emphasise hot water and detergent, cleaning of removable parts, complete drying with the cap left open, and replacement of worn bottles. Campaigns promoting reusable bottles in Vietnamese universities should carry hygiene instructions alongside their environmental message.

This study has several limitations. First, 98 bottles were sampled at a single university by convenience sampling; the sample was adequate for estimating prevalence but left small subgroups, and because stainless steel bottles predominated (84.7%), the findings may not transfer to settings where plastic bottles are the norm. Second, and more limiting than sample size, only two bottles were uncontaminated; with the outcome this close to saturation, a larger study using the same binary measure would face the same constraint. Third, organisms were classified presumptively from colony morphology and Gram staining alone, so species-level misclassification cannot be excluded and neither pathogenicity nor antimicrobial resistance could be assessed, leaving the clinical significance of these counts undetermined. Finally, colony counts from swabs are semi-quantitative, and hygiene practices were self-reported.

CONCLUSION

Microbial contamination was near universal among the reusable personal water bottles of students at Van Lang University: 98.0% of bottles were contaminated (95% CI 92.9–99.4), 81.6% showed high colony counts, and presumptive fungi were recovered from 46.9%.

Because only two bottles were uncontaminated, the outcome had almost no variability and the study had little power to distinguish between subgroups. None of the eleven factors examined was significantly associated with contamination status, and all risk differences had wide confidence intervals including zero; this indicates an absence of detectable association rather than evidence that these factors are unimportant. Bottle surface condition and washing frequency showed non-significant trends towards higher microbial density.

As a cross-sectional study without species-level identification or measured health outcomes, this work cannot establish causality or clinical significance. It nevertheless provides baseline Vietnamese data on a topic not previously reported, and indicates that future studies should record the interval since last cleaning and adopt outcomes with greater variability than binary contamination status.

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Conflict of Interest

The authors declare that they have no conflicts of interest with any organization, institution, or entity related to this research.

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