

Bath Towel Bacterial contamination and Hygiene practices among Tertiary Students

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Abstract

Bath towels have the ability to support bacterial growth under inappropriate washing and drying conditions and pose health risk to humans. This study investigated bacterial contamination of bath towels used by tertiary students and the washing and drying practices relative to their use. Coliforms (23, 46%) including *Escherichia coli* (18, 36%) were isolated by culture methods using swab samples from standardized area (0.96cm²) of 50 bath towels owned by tertiary students. Male students had a higher rate of coliform contamination on towels (15, 60%) than female students (8, 32%) [$\chi^2(1) = 3.87, p = 0.049$]. *E. coli* contamination was also more common in males (13, 52%) than females (5, 20%) [$\chi^2(1) = 5.44, p = 0.019$]. Mean rank colony counts differed for coliforms in male (29.68) vs. female (21.32) towels (U = 417, p = 0.027) and for *E. coli* in male (30.16) vs. female (20.84) towels (U = 429, p = 0.008). Biochemical tests identified bacteria in seven genera, four coliform species: *Escherichia coli*, *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter* species and three non-coliform species: *Vibrio cholerae*, *Salmonella typhi*, and *Alcaligenes* species. Seven male students (28%) and 16 female students (72%) reported washing their towels within two weeks of use. More males (16, 64%) than females (8, 32%) washed towels one to two months after use. Bacterial contamination of students' towels raises concern about the risk of exposure to potentially harmful bacteria and calls for appropriate towel hygiene practices by students.

Keywords: Bacterial contamination, bath towels, tertiary students

Introduction

The human body serves as habitat for a diverse community of microorganisms, including eukaryotes, archaea, bacteria, and viruses (Kilian et al., 2016; Sender et al., 2016; Shreiner et al., 2015), that influence the human physiology, in the aspect of both health and diseases (Dekaboruah et al., 2020; Wang et al., 2017). Of this complex ecological community, the number of bacteria is estimated to be of the same order as the number of human cells (Sender et al., 2016). These microbiota are derived from variety of sources including contaminated environments, such as hospital settings, soil, as well as fomites, water, food, animals, and humans.

Humans first acquire significant amounts of microbiota from their mothers at the time of birth (Dominguez-Bello, 2010) and through breastfeeding (Lyons et al., 2020; Ruiz et al., 2019). *Staphylococcus*, *Streptococcus*, *Enterococcus*, and enteric Gram-negative bacteria including *Escherichia* and *Enterobacter* dominate the skin microbiota (Younge et al., 2018). Bacterial source tracking indicates that microbiota are commonly exchanged across body sites and the hospital environment (Younge et al., 2018). Microbial contamination of environmental waters (Some et al., 2021; Harwood et al., 2014; Byappanahalli et al., 2012) and drinking water involving coliforms (total coliform, faecal coliform, *Escherichia coli*) [Fida et al., 2023] is well documented.

Microbe-contaminated: fresh fruits and vegetables (Balali et al., 2020), foodstuffs involving pathogenic strains of *Escherichia coli* and *Salmonella* (Machado-Moreira et al., 2019), and animals (Fuhrmeister et al., 2019) are important sources of exposure. *Salmonella* contaminates produce, such as poultry, cattle and their feeds, fruit and vegetable products, and pets, accounting for >50% of the numbers of foodborne illnesses reported in the European Union (Ehuwa et al., 2021). Veterinary faculty restroom surfaces are also reported to have aquatic, human, and animal sources of bacteria (Jabri et al., 2023). Human exposure to these sources contaminates the body and may present health-related issues. However, not all microbial contaminants are harmful or significant to public health. *Staphylococcus epidermidis*, a common skin commensal, contributes to host health by occupying ecological niches on the skin, thus preventing pathogenic colonization and infection, especially on compromised skin and promoting immune function (Grice and Segre, 2011). It rarely causes disease except under specific conditions, such as in immunocompromised individuals or through open wounds. Similarly, gut microbiota like *Bacteroides* and *Lactobacillus* species help with digestion and nutrient synthesis (e.g., vitamin K) while inhibiting pathogen growth (Nicholson et al., 2012).

The body microbiota contaminates materials, such as bath towels and clothes used on daily basis particularly under conditions of poor personal hygiene. Bath towels are able to retain microbes (Hadi et al., 2021), as moist and warm conditions, characteristic of used towels not dried in sunny and airy environment, are favourable conditions for microbial growth. Common bacteria including *Staphylococcus aureus* and *Escherichia coli* are frequently isolated from bath towels, indicating direct transfer from the skin during use (Onemu et al., 2024; Kone and Adegoke, 2022). Microorganisms from environmental sources may also contaminate towels. Microbiota significantly different from that on human skin and clothing have

been reported to contaminate towels (Kato et al., 2023). These materials may not only get contaminated by microbes from external sources, but also endogenous sources (Raineri et al., 2022). The extent of their growth and multiplication in bath towels is influenced by conditions, such as humidity, temperature (Hadi et al., 2021), and hygiene practices relative to bath towel use (Twumwaa et al., 2021).

Temperature and detergents play key roles in fabric-contaminated microbial control (Abney et al., 2021). This study, therefore, assessed bacterial contamination of bath towels used by tertiary students and investigated the washing and drying practices relative to their use.

Materials and methods

Study area, sample and, data collection

The study was undertaken in a tertiary institution in the Ayawaso West Municipal District (N 5° 38' 31.2", W 0° 9' 21.6") of Ghana. With the help of their room numbers, a systematic random sampling, involving selection of sampling rooms at regular intervals, was employed to sample 50 bath towels of male (N=25) and female (N=25) students from which swab samples were taken following their consent to participate in the study.

Using sterile cotton swab moistened with sterile physiological saline (0.85%), a standardized area of 0.96cm² of each towel was swabbed, placed in its tube, labelled, and transported on ice to the laboratory for refrigeration at 4°C and for further microbial analysis. Questionnaires were also given to gather data on bath towel washing and drying practices among the students. The questionnaire for each student had identification corresponding to the label of the student's towel swab sample to match the responses to the results of the microbial analysis of that swab sample.

Bacteria culture and isolation

Sevenfold serial dilutions were prepared

twice at eight levels for each processed sample to decrease the bacterial concentration to a required concentration for a specific test method (Ben-David and Davidson, 2014). Each swab sample was washed in Eppendorf tube with 1000 μ l of sterile saline solution (stock solution). The stock solutions were serially diluted and plated for total aerobic count using the Plate Count, Desoxycholate Lactose, Brilliant-green Phenol-red Lactose, and Eosin Methylene Blue Media for the general bacteria, coliform, *Salmonella* spp., and *E. coli* counts respectively following the pour plate method and bacterial growth (Vial and Geoffray, 1981) in colony forming units. Each sterile Petri dish with medium was swirled gently, allowed to set for about 10mins while covered, and inverted. The media were incubated for 24 hours at 37°C except for the Eosin Methylene Blue Media, which was incubated at 44°C. The colonies were counted using a colony counter for the first set of four dilutions which had growth. The same method was repeated for the second set and an average of the two was taken and recorded.

Nutrient agar was prepared and put into the various sterile Petri dishes of colony forming units using the pour plate method and purified, employing the streak plate technique (Zhou et al., 2019). Colonies on the coliforms agar plates were picked aseptically, using sterilized metal loop, streaked on nutrient agar plate, and incubated overnight at 37°C for discrete colonies (purified cells).

Biochemical tests and identification of enterobacteria species

The identification of the pure bacteria isolates were done by motility test, and biochemical tests (indole, urease, and Triple Sugar Iron (TSI). The results obtained from the biochemical tests were matched on a chart in Bergy's manual for Enterobacteria species (Brenner et al., 2005).

Data analysis

Descriptive statistics in Statistical Package for Social Sciences (SPSS v20) were used

to establish frequencies of responses of participants on bath towel washing and drying practices. Proportions of bacteria isolated from bath towels of male and female participants were compared using N-1 Chi-squared test, in MedCalc Statistical Software (Version 22.009), as recommended by Campbell (2007). The colony counts for the type of bacteria were compared in both male and female towel samples by first checking for the skewness and kurtosis as well as normality using Shapiro-Wilk Test in SPSS (version 20). The data were positively skewed and, therefore, not normally distributed. Therefore, Mann-Whitney U test was performed to compare the mean ranks of bacterial colony counts between the male and female towel samples.

Results

Table 1 presents bath towel washing and drying practices among 50 tertiary students 18 years and above. Seven male students (28%) and 16 female students (72%) reported washing their towels within two weeks of use. More males (16, 64%) than females (8, 32%) washed towels one to two months after use. Both groups had comparable modes of towel washing and drying place. Figure 1 presents the proportion of male and female student towels contaminated with bacteria (CFU/100 μ l per 0.96cm² of towel). Male students had a higher rate of coliform contamination on towels (15, 60%) than female students (8, 32%) [$\chi^2(1) = 3.87$, $p = 0.049$]. Similarly, *Escherichia coli* contamination was also more common in males (13, 52%) than females (5, 20%) [$\chi^2(1) = 5.44$, $p = 0.019$].

A Mann-Whitney U test showed that the mean ranks for total aerobic bacterial colony counts in male and female towel samples were not significantly different. However, coliform and *E. coli* 98colony counts in both groups were significantly different (Table 2). *E. coli*, *Serratia marcescens*, *Citrobacter freundii*, and *Enterobacter* species are coliform species that were identified by biochemical tests (Table 3). Non-coliform species, *Vibrio*

TABLE 1
Bath towel washing and drying practices among 50 tertiary students

Variable	Number of male (N=25) responses (%)	Number of female (N=25) responses (%)	Total (N=50) responses (%)
Frequency of washing towel			
Once a week	1 (4)	11 (44)	12 (24)
Twice a week	0 (0)	0 (0)	0 (0)
Once 2 weeks	6 (24)	6 (24)	12 (24)
Once a month	13 (52)	8 (32)	21 (42)
once 2 months	3 (12)	0 (0)	3 (6)
Other (state)	2 (8)	0 (0)	2 (4)
Mode of towel washing			
Cold water and soap/detergent	20 (80)	21 (84)	41 (82)
Hot water and soap	0 (0)	3 (12)	3 (6)
Washing machine with dryer	5 (20)	1 (4)	5 (12)
Towel drying place			
Bathroom	0 (0)	3 (12)	3 (6)
Room	2 (8)	1 (4)	3 (6)
Balcony	15 (60)	13 (52)	28 (56)
Dry lines in the sun	8 (32)	7 (28)	15 (30)
Others	0 (0)	1 (4)	1 (2)

N: sample size; %: percentage

cholerae, *Salmonella typhi*, and *Alcaligenes* species, were also identified.

Discussion

Bath towels are essential for personal hygiene, but under poor hygiene conditions, they can harbor microbes, providing ideal

conditions for growth and posing a risk of exposure and infection (Bloomfield et al., 2011). This study investigated bacterial contamination of bath towels used by tertiary students and the washing and drying practices relative to their use. Of the 50 bath towel swab samples, coliforms (23, 46%) including *Escherichia coli* (18, 36%) were isolated by culture methods. *E. coli* contamination

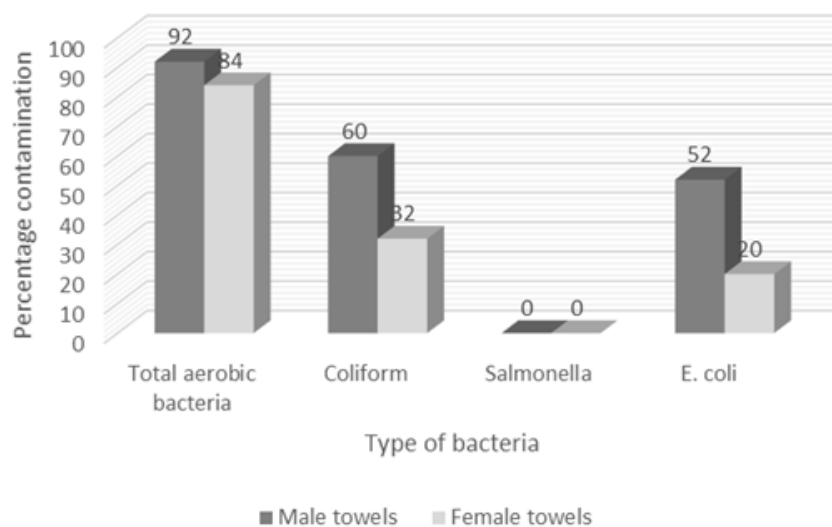


Fig. 1 Proportion of male and female student towels contaminated with bacteria (CFU/100 μ l per 0.96cm² of towel)

TABLE 2

Statistical comparison of bacterial colony counts (CFU/100µl) in male and female towel samples: medians, mean ranks, Mann-Whitney U, and p-values

Type of bacteria	Colony counts in male towels (N=25)		Colony counts in female towels (N=25)		Mann-Whitney U; p-value
	Mean rank	Median	Mean rank	Median	
Total aerobic bacteria	27.10	5	23.90	5	352.50; 0.435
Coliform	29.68	3	21.32	0	417.00; 0.027
Escherichia coli	30.16	3	20.84	0	429.00; 0.008
Salmonella	-	-	-	-	*

N: sample size; CFU/100µl: colony-forming units/100µl; -: no growth; *: not applicable

was higher in male towels (13, 52%) than in female towels (5, 20%) [$\chi^2(1) = 5.44$, $p = 0.0196$], Figure 1. The mean ranks for total aerobic bacterial colony counts in male and female towel samples were not significantly different, implying that the bacterial loads were similarly distributed in both male and female towel samples. However, mean rank colony counts differed for coliforms in male (29.68) vs. female (21.32) towels ($U = 417$, $p = 0.027$) and for *E. coli* in male (30.16) vs. female (20.84) towels ($U = 429$, $p = 0.008$),

Table 2. These findings suggest that male towels have significantly higher coliform and *E. coli* colony counts than female towels, according to the Mann-Whitney U test.

Biochemical tests identified bacteria in seven genera, four coliform species: *E. coli*, *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter* species and three non-coliform species: *Vibrio cholerae*, *Salmonella typhi*, and *Alcaligenes* species (Table 3). The lack of growth on culture media may be explained by the viable, but non-culturable (VBNC)

TABLE 3

Biochemical test results for bacteria isolated from male and female students' towels

Sample ID	TSI TEST				MIU TEST			Species
	Slope	Butt	H2S	Gas	Motility	Indole	Urea	
M 1	R	Y	-	-	+	+	-	<i>Vibrio cholerae</i>
M 1.1	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 1.2	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 2	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 2.1	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 2.2	Y	Y	-	-	+	-	-	<i>Serratia marcescens</i>
M 2.3	Y	Y	+	+	+	-	-	<i>Citrobacter freundii</i>
M 3	Y	Y	-	+	+	-	-	<i>Enterobacter</i> species
M 3.1	Y	Y	-	+	+	-	-	<i>Enterobacter</i> species
M 3.2	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 4	R	R	-	-	+	-	-	<i>Alcaligenes</i> species
M 4.1	R	R	-	-	+	-	-	<i>Alcaligenes</i> species
M 4.2	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 5	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 5.1	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 6	Y	Y	+	+	+	+	-	<i>Escherichia coli</i>
M 6.1	R	Y	+	+	+	-	-	<i>Other Salmonella</i> spp.
M 7	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 7.1	R	Y	+	-	+	-	-	<i>Salmonella typhi</i>
M 8	Y	Y	-	+	+	+	-	<i>Escherichia coli</i>
M 8.1	Y	Y	+	-	+	-	-	<i>Serratia marcescens</i>

Key: Positive (+); Negative (-); Red(R)-Alkaline; Yellow(Y)-Acid; Triple Sugar Iron (TSI); Motility Indole Urea (MIU); Hydrogen Sulphide (H2S); Species (spp.); Identification (ID)

strategy (Zhang et al., 2023; Dong et al., 2020) used by some species of bacteria, such as *Salmonella* spp. (Zhao et al., 2017; Ozçakir, 2007), *Vibrio cholerae* (Ozçakir, 2007; Xu et al., 1982), and *Shigella* spp. (Ozçakir, 2007) to cope with stressful environments. This may be influenced by stressful environmental factors, such as high oxygen levels, nutrient scarcity (Wu et al., 2016), and extreme pH or temperature variations (Shen and Zhang, 2022). The ability of these pathogenic VBNC bacteria to recover both culturability and pathogenicity may pose potential health risk (Zhao et al., 2017).

Coliform bacteria (Arora, 2003) are a diverse group found in the environment, faeces of warm-blooded animals and humans, and drinking water (Leclerc et al., 2001; Xu et al., 2022). Their presence in bath towels, specifically *Citrobacter* and *Enterobacter* genera, suggests environmental contamination. Faecal coliforms from wastewater, livestock, and the alimentary canals of warm-blooded animals, including humans (Ahmed et al., 2015), pollute environments and water bodies, posing health risks (Florini et al., 2020; Berounsky et al., 2018; Syafrudin et al., 2017). These bacteria indicate faecal contamination and the potential presence of waterborne pathogens (Niyoyitungiye et al., 2020; Hunter et al., 2004). The presence of faecal coliform, specifically *Escherichia coli*, was higher in towels used by males (13, 52%) than females (5, 20%) [$\chi^2(1) = 5.44$; $p = 0.019$], indicating recent faecal contamination and a higher risk of pathogens (Sala-Comorera et al., 2021; Khan and Gupta, 2020; Foster et al., 2019). Although *E. coli* is generally part of the normal enteric flora, certain strains are enterovirulent and pose health risks. Enterotoxigenic *E. coli* (ETEC) is a significant cause of global diarrhea mortality (Khalil et al., 2018), while *E. coli* O157:H7 can cause severe diarrhea, hemolytic uremic syndrome, and even death (Berry and Wells, 2010). These findings highlight concerns about student towel hygiene.

The moist and warm environment in used bath towels offer microbes favorable place to grow and survive (Hadi et al., 2021). In

this study, male students (7, 28%) and female students (16, 72%) reported washing their towels within two weeks of use. More males (16, 64%) than females (8, 32%) washed towels one to two months after use (Table 1), contrary to appropriate towel washing practices. Appropriate hygiene practices for bath towels include washing them every two to three (Migala, 2023) or three to five normal (CI, 2022) uses as long as they are fully dry in between, translating to about twice a week. Overwhelming majority of the students (41, 82%) wash their towels with cold water and soap or detergent without using hot water required for pathogen inactivation. The students also reported drying their towels in rooms (3, 6%) and balconies (28, 56%), which may create favorable conditions for bacterial growth, as well as dry lines in the sun (15, 30%). Temperature plays the most important role in pathogen control, requiring $> 40^{\circ}\text{C}$ to 60°C for proper inactivation, while detergents release microbes attached to fabrics and inactivate those sensitive to them, thereby reducing microbial load (Abney et al., 2021). Washing and drying temperatures and the length of drying time are factors influencing the occurrence of microbes, such as bacteria in laundry. Microbes have greater chance of survival at lower wash (Gerba et al., 2007) and drying (Fijan et al., 2007; Gerba et al., 2007) temperatures as well as shorter length of drying time (Fijan et al., 2007; Gerba et al., 2007) for laundry. Air-drying of laundry under humid outdoor conditions may increase the number of bacteria (Amichai et al., 2013). Interestingly, towel washing and drying practices reported in this study do not conform to the required practices, raising concern about the potential risk of exposure to harmful bacteria. These findings highlight the importance of educational interventions on appropriate towel hygiene practices among students.

Conclusion

The study reveals bacterial contamination of the students' bath towels with coliforms (23, 46%) including *Escherichia coli* (18, 36%)

isolated by culture methods. Male students had a higher rate of coliform contamination on towels (15, 60%) than female students (8, 32%) [$\chi^2(1) = 3.87$, $p = 0.049$]. Similarly, *E. coli* contamination was also more common in males (13, 52%) than females (5, 20%) [$\chi^2(1) = 5.44$, $p = 0.019$]. Mean rank colony counts differed for coliforms in male (29.68) vs. female (21.32) towels ($U = 417$, $p = 0.027$) and for *E. coli* in male (30.16) vs. female (20.84) towels ($U = 429$, $p = 0.008$). Biochemical tests identified bacteria in seven genera, four coliform species: *Escherichia coli*, *Serratia marcescens*, *Citrobacter freundii*, *Enterobacter* species and three non-coliform species: *Vibrio cholerae*, *Salmonella typhi*, and *Alcaligenes* species. Seven male students (28%) and 16 female students (72%) reported washing their towels within two weeks of use. More males (16, 64%) than females (8, 32%) washed towels one to two months after use. These findings raise concern about the risk of exposure to potentially harmful bacteria and calls for appropriate towel hygiene practices by students.

Acknowledgements

The authors wish to thank the Departments of Animal Biology and Conservation Science (DABCS) and Animal Science, University of Ghana, for the logistical support.

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