Prevalence of Bacterial Contamination on Handkerchiefs Used by Pre-School Children in Bungoma County, Kenya.

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ABSTRACT

INTRODUCTION; In Kenya, and specifically Bungoma County, sanitation challenges and limited hygiene awareness contribute to bacterial transmission through reused handkerchiefs, elevating infection risks among schoolchildren. WASH programmes have been reinforced but little effort has been made to identify the types and prevalence of bacteria on used handkerchiefs of pre-primary school children**.** This study examined bacterial contamination of used handkerchiefs among pre-school children in Bungoma County, Kenya, using a cross-sectional quantitative design.

METHODS; From Cochrans'formular, a sample of 248 children was selected using stratified random sampling. Swabbing of used handkerchiefs focusing on high-contact nasal and oral regions was done. Samples were transported under cold chain conditions to Kibabii University Microbiology Laboratory. Laboratory analysis included serial dilution up to 10^-3 and culture on appropriate media. Incubation was done at 37°C for 24 hours. Colony forming units (CFU/mL) were quantified to determine bacterial load. Data was analyzed using SPSS version 22.

RESULTS; Results showed universal contamination (100%) of all sampled handkerchiefs. The bacterial profile included both Gram-negative and Gram-positive organisms, suggesting multiple contamination sources. The mean bacterial load was 57,370.97 CFU/mL, reflecting consistently high microbial burden across samples.

CONCLUSION; These findings indicate that poor hand and handkerchief hygiene practices contribute to microbial proliferation and contamination among young children It is recommended that schools and parents strengthen personal hygiene education practices among prescholars.

Keywords: Hygiene practices, Pre-school children, Handwashing, Handkerchief use, Bungoma County, Kenya.

INTRODUCTION

Personal hygiene especially hand hygiene is expansively identified as one of the most cost-effective interventions for preventing respiratory infectious diseases(Seguin & Niño Zarazúa, 2015). pre-school children are highly vulnerable due to underdeveloped immune system, hygiene behaviors, frequent environmental exposure compounded with limited knowledge and practice of self-care capacity(Iyevhobu et al., 2022). Globally, infectious diseases such as diarrheal diseases and respiratory tract infections remain leading causes of morbidity and mortality among young children (Mulatya & Ochieng, 2020). Poor hygiene has been identified

as a key driver of diarrheal transmission. Fomites such as handkerchiefs play an important but often overlooked role in disease transmission (Oshilim & Orogu, 2020). These materials are frequently used to wipe nasal and oral secretions, which are known reservoirs of pathogenic microorganisms. Repeated use without adequate cleaning creates favorable conditions for bacterial survival and multiplication due to moisture, organic matter, and constant handling(Oshilim & Orogu, 2020) . Evidence from microbiological studies shows that personal items commonly harbor organisms such *as Escherichia coli, Staphylococcus aureus, Klebsiella spp., Enterococcus spp., and Pseudomonas aeruginosa* (Fijan et al., 2017). These organisms are associated with gastrointestinal, respiratory, and opportunistic infections. *Escherichia coli*, which indicates fecal contamination and poor sanitation practices. In low- and middle-income countries, hygiene-related disease burden is intensified by inadequate access to clean water, poor sanitation infrastructure, overcrowded learning environments, and limited hygiene education (Prüss-Ustün et al., 2019). Sub-Saharan Africa continues to experience significant gaps in Water, Sanitation, and Hygiene (WASH) implementation, particularly in early childhood settings where supervision is inconsistent. Hand hygiene plays a vital role in preventing infectious diseases among young children, with contaminated hands and personal items like handkerchiefs serving as key transmission routes for pathogenic bacteria (Ray et al., 2011). In sub-Saharan Africa, inadequate access to water, soap, and hygiene education exacerbates microbial contamination in schools (Okesanya et al., 2024), with studies in Nigeria and South Africa confirming high bacterial loads on children's personal items, particularly among low-income populations (Ataiyero et al., 2019; Blackmon et al., 2024). In Kenya, and specifically Bungoma County, sanitation challenges and limited hygiene awareness further contribute to bacterial transmission through reused handkerchiefs, elevating infection risks among schoolchildren (Greene et al., 2012; KDHS, 2022). Although studies have examined contamination of hands and environmental surfaces, limited attention has been given to handkerchiefs used by pre-school children. This represents a critical gap because these items are frequently used, repeatedly exposed to biological fluids, and rarely disinfected. School environments further contribute to transmission due to close interaction among children and inconsistent hygiene supervision. Individual behavioral factors such as hand hygiene practices and sex-based differences may also influence contamination levels. This study therefore investigated the prevalence and level of bacterial contamination on used handkerchiefs among pre-school children in Bungoma County, Kenya, and examined differences across schools and participant characteristics. It aligns with global health goals to combat antimicrobial resistance and prevent infections through behavior change and hygiene education (Ahmed et al., 2024). Moreover, the findings will support public health strategies, influence hygiene policy, and equip caregivers and educators with data to improve early childhood health

METHODOLOGY

Study Design and Population

This was a cross-sectional study conducted among 248 pre-school children from ten schools in Ndivisi zone of Webuye East, Bungoma County, Kenya. Used handkerchiefs were swabbed using sterile swabs between 10am and 11 am. The swabs were put in amine solution to enhance bacteria viability. The swabs were then labelled with child identifiers and put in a cool box to prevent bacteria degeneration and then transported to Kibabii university Microbiological lab within 4 hours.

Data Collection

Data collection was carried out in a structured but practical way within the selected pre-school schools in Ndivisi zone, Bungoma County. After obtaining permission from school administration, a list of eligible PP1 and PP2 learners was compiled with the help of class teachers. From this list, participants were selected using simple random sampling after ensuring that written consent had been obtained from parents or guardians and assent from the children. For the children, no formal interviews were conducted due to their age. Instead, information was gathered through observation of routine hygiene practices during normal school activities. This included how children handled and stored their handkerchiefs and general cleanliness behavior during school time. The aim was to capture natural behavior rather than responses influenced by questioning. Teachers played an important role in providing contextual information. Short guided discussions were held with class teachers focusing on pupils' hygiene routines, handwashing practices, and how personal items such as handkerchiefs were managed within the school setting. These discussions helped to validate what was observed

in class. Parents and guardians were engaged indirectly through the consent process and through brief follow-up questions. These focused on home hygiene practices, especially how often children's handkerchiefs were washed and general sanitation habits at home. Most responses were obtained from mothers or primary caregivers. After field procedures, each handkerchief was collected under supervision. Sterile swabs were used to swab high-contact areas of each handkerchief. Each sample was coded to maintain confidentiality, placed in sterile transport media, and stored in cool boxes at 4–8°C. All samples were transported to Kibabii University Microbiology Laboratory within four hours for further analysis. Care was taken throughout to avoid contamination and ensure sample integrity.

Laboratory Procedure

In the laboratory, serial dilution was done using sterile normal saline to a factor of 1;1000 before inoculation onto the agar media that were present. Spread plate method was employed to ensure formation of colony forming units that could easily be counted. Nutrient agar served as a general-purpose media for showing bacteria viability. Blood agar was for growth of bacteria that show hemolysis patterns and fastidious organisms. MacConkey agar was used to show a difference between gram negative enteric bacteria that could point to potential fecal contamination. All these laboratory works was carried out under strict aseptic conditions including sterilized tools, disposable gloves and lab coats to prevent contamination during processing. Some used materials were autoclaved and others incinerated after analysis. After inoculation, plates were aerobically incubated at 37^{o}C for 24 hours. Colonies were then examined for growth characteristics like size, shape, color and hemolysis patterns

Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 22. Descriptive statistics such as mean, median, standard deviation, and interquartile range were used to summarize bacterial load distribution. Logarithmic transformation (log10 CFU/mL) was applied to normalize skewed data. Inferential analysis included chi-square tests to determine associations between categorical variables such as school, sex, and contamination level. Statistical significance was set at $p < 0.05$.

ETHICAL CONSIDERATIONS

Ethical approval was obtained from the Institutional review and ethics committee of Masinde Muliro University of science and Technology and research clearance was granted by the National Commission for Science, Technology and Innovation (NACOSTI), Kenya. Informed consent was obtained from parents or guardians, and assent was obtained from children in an age-appropriate manner. Participation was voluntary and confidentiality was maintained using coded identifiers. This study involved minors, therefore, additional safeguarding measures were adhered to. No formal interviews were conducted due to their age. Interactions were carried out in secure monitored environments, primarily within the school setting with a designated school representative. Interview questions were carefully designed to avoid psychological distress, coercion or discomfort. No sensitive personal disclosures beyond the scope of the study were requested. Confidentiality of the minors and anonymity were maintained through the use of coded identifiers only. All the collected data was securely stored with restricted access limited to the researcher alone. No identifying information was included in any report or publication. Biosafety and laboratory protocols were strictly followed. All laboratory waste was autoclaved. Disposal was done in line with Biosafety level 2 procedures

RESULTS

A total of 248 respondents were included in the study. Participants were drawn from ten selected schools within the study area. The highest proportion of children was from Lutacho Primary School (13.31%), followed by Silungai (12.50%) and Sinoko (11.69%), while Precious Academy (6.85%) contributed the least number of participants. In terms of child characteristics, slightly more than half were male (52.42%), while 47.58% were female. The distribution by class was nearly equal, with 51.21% in PP2 and 48.79% in PP1 as shown in table 1

Table 1: Participant characteristics

Variable	Category	Frequency (n)	Percent (%)
School Name	Lukusi	23	9.27
	Lutacho	33	13.31
	Magemo	19	7.66
	Malomonye	25	10.08
	Musa	25	10.08
	Ndivisi	22	8.87
	Precious	17	6.85
	Silungai	31	12.50
	Sinoko	29	11.69
	Wandabwa	24	9.68
Child Sex	Female	118	47.58
	Male	130	52.42
Class	PP1	121	48.79
	PP2	127	51.21

The prevalence of bacterial contamination on handkerchiefs used by pre- school children

Prevalence by bacterial strains.

Table 2 presents the distribution of bacterial load across different bacterial species isolated from handkerchief samples, expressed as CFU/mL and log-transformed values. The mean *Escherichia coli* load was 15,012.10 ± 3,944.43 CFU/mL (95% CI: 14,518.76–15,505.43), with a median of 1.55×10^4 CFU/mL (IQR: 1.30×10^4–1.80×10^4). *Staphylococcus* showed a slightly lower mean load of 11,560.48 ± 2,811.98 CFU/mL, with a median of 1.10×10^4 CFU/mL (IQR: 1.00×10^4–1.30×10^4). Lower bacterial counts were observed for *Salmonella*, which had a mean of 1,483.87 ± 2,061.73 CFU/mL and a median of 1.00×10^3 CFU/mL (IQR: 0–3.00×10^3), indicating substantial variability and the presence of zero counts in some samples. Similarly, *Pseudomonas* and *Bacillus* exhibited relatively lower median loads of 3.00×10^3 CFU/mL, with wide interquartile ranges, suggesting heterogenesous distribution across samples. In contrast, *Enterococcus*, *Klebsiella*, and *Micrococcus* demonstrated moderate bacterial loads, with median values ranging from 6.00×10^3 to 1.00×10^4 CFU/mL. Among all isolates, the overall level of colonization showed the highest bacterial burden, with a mean of 57,370.97 ± 15,114.66 CFU/mL (95% CI: 55,480.57–59,261.37) and a median of 5.70×10^4 CFU/mL (IQR: 5.00×10^4–6.60×10^4), indicating a high cumulative microbial load on handkerchiefs. Following $\log_{10}$ transformation, the bacterial loads demonstrated reduced variability and improved distribution. The mean log-transformed total bacterial load was 4.74 ± 0.13 $\log_{10}$ CFU/mL (95% CI: 4.73–4.76), with a median of 4.76 (IQR: 4.70–4.82). Among individual organisms, *E. coli* and *Staphylococcus* maintained relatively higher log means of 4.16 ± 0.13 and 4.05 ± 0.12, respectively, while *Salmonella* showed the lowest and most variable log values (1.70 ± 1.70), reflecting the presence of zero and low counts.The findings indicate that while all handkerchiefs harbored bacterial contamination, the magnitude of contamination varied considerably across bacterial species, with total bacterial load demonstrating consistently high levels across samples.

Table 1: Distribution of Bacterial Load (CFU/mL and Log CFU)

Variable	**Mean ± SD (95% CI)**	**Median (IQR)**	**Minimum**	**Maximum**
E. coli CFU/mL	15012.10 ± 3944.43 (14518.76–15505.43)	1.55×10^4 (1.30×10^4 – 1.80×10^4)	4.00×10^3	2.80×10^4
Staphylococcus CFU/mL	11560.48 ± 2811.98 (11208.79–11912.18)	1.10×10^4 (1.00×10^4 – 1.30×10^4)	3.00×10^3	1.90×10^4
Salmonella CFU/mL	1483.87 ± 2061.73 (1226.01–1741.73)	1.00×10^3 (0 – 3.00×10^3)	0	8.00×10^3

Enterococcus CFU/mL	9362.90 ± 3178.46 (8965.37–9760.43)	1.00×10^4 (8.00×10^3 – 1.10×10^4)	1.00×10^3	1.80×10^4
Pseudomonas CFU/mL	3592.74 ± 2862.48 (3234.73–3950.75)	3.00×10^3 (1.00×10^3 – 5.00×10^3)	0	1.40×10^4
Klebsiella CFU/mL	6334.68 ± 2979.84 (5961.99–6707.37)	6.00×10^3 (4.00×10^3 – 8.00×10^3)	1.00×10^3	1.70×10^4
Micrococcus CFU/mL	7137.10 ± 3592.24 (6687.81–7586.38)	7.00×10^3 (4.00×10^3 – 1.00×10^4)	1.00×10^3	1.70×10^4
Bacillus CFU/mL	2887.10 ± 1674.82 (2677.63–3096.57)	3.00×10^3 (1.00×10^3 – 4.00×10^3)	0	9.00×10^3
Level of colonization CFU/mL	57370.97 ± 15114.66 (55480.57–59261.37)	5.70×10^4 (5.00×10^4 – 6.60×10^4)	1.90×10^4	1.10×10^5
E. coli Log CFU	4.16 ± 0.13 (4.14–4.18)	4.19 (4.11–4.26)	3.6	4.45
Staphylococcus Log CFU	4.05 ± 0.12 (4.03–4.06)	4.04 (4.00–4.11)	3.48	4.28
Salmonella Log CFU	1.70 ± 1.70 (1.49–1.92)	3.00 (0–3.48)	0	3.9
Enterococcus Log CFU	3.93 ± 0.20 (3.91–3.96)	4.00 (3.90–4.04)	3	4.26
Pseudomonas Log CFU	3.21 ± 0.98 (3.09–3.33)	3.48 (3.00–3.70)	0	4.15
Klebsiella Log CFU	3.75 ± 0.21 (3.73–3.78)	3.78 (3.60–3.90)	3	4.23
Micrococcus Log CFU	3.79 ± 0.24 (3.76–3.82)	3.85 (3.60–4.00)	3	4.23
Bacillus Log CFU	3.35 ± 0.46 (3.29–3.41)	3.48 (3.00–3.60)	0	3.95
Level of colonization Log CFU	4.74 ± 0.13 (4.73–4.76)	4.76 (4.70–4.82)	4.28	5.04

Values are represented as mean ±s Standard deviation (SD) with 95% confidence interval for normally distributed data and as median with interquartile range (IQR: 25th–75th percentile) for skewed data. Minimum and maximum values indicate the observed range. Bacterial counts are expressed as colony forming units per milliliter (CFU/mL). Median, minimum, and maximum values for CFU/mL are presented in scientific notation ($x.xx \times 10^n$) for clarity.

Log-transformed values represent $\log_{10}$ (CFU/mL + 1) to normalize data distribution and stabilize variance. The variable "level of colonization" represents the total bacterial load (sum of all bacterial counts per sample).

Zero values indicate no detectable bacterial growth in the respective samples

Level of bacterial contamination

Table 3 presents the distribution of bacterial contamination levels across different bacterial species based on median $\log_{10}$ CFU thresholds. For *Escherichia coli*, contamination was evenly distributed, with 50% of samples classified as low and 50% as high contamination, using a threshold of 4.19 $\log_{10}$ CFU. A similar near-equal distribution was observed for *Salmonella* and overall bacterial load, with 50.8% of samples categorized as high contamination for both, based on thresholds of 3.00 $\log_{10}$ CFU and 4.76 $\log_{10}$ CFU, respectively. Higher proportions of high contamination were observed for *Staphylococcus* and *Enterococcus*, where 69% and 59.3% of samples, respectively, were above the median thresholds (4.04 $\log_{10}$ CFU for *Staphylococcus* and 4.00 $\log_{10}$ CFU for *Enterococcus*). This suggests relatively greater bacterial burden for these organisms among the sampled handkerchiefs. In contrast, the majority of samples were classified as low contamination for *Pseudomonas* (53.2%), *Klebsiella* (55.2%), *Micrococcus* (57.3%), and *Bacillus* (56.9%), indicating comparatively lower bacterial loads relative to their respective median thresholds. Overall, the findings demonstrate variability in contamination patterns across bacterial species, with some organisms showing a predominance of high contamination while others were largely within lower contamination levels, reflecting differences in distribution and potential transmission dynamics.

Table 2: Level of bacterial contamination

Bacteria	Level of Contamination	Frequency (n)	Percent (%)	Median $\log_{10}$ CFU
E. coli	Low	124	50	4.19
	High	124	50	
Staphylococcus	Low	77	31	4.04
	High	171	69	
Salmonella	Low	122	49.2	3
	High	126	50.8	
Enterococcus	Low	101	40.7	4
	High	147	59.3	
Pseudomonas	Low	132	53.2	3.48
	High	116	46.8	
Klebsiella	Low	137	55.2	3.78
	High	111	44.8	
Micrococcus	Low	142	57.3	3.85
	High	106	42.7	
Bacillus	Low	141	56.9	3.48
	High	107	43.1	
Overall (Total Load)	Low	122	49.2	4.76
	High	126	50.8	

Contamination patterns by characteristics

Table 4. presents the association between school attended and participant characteristics with levels of bacterial contamination of handkerchiefs, where each school (Yes) is compared against all other schools combined (No). Significant variations in contamination levels were observed across schools. Handkerchiefs from Lutacho were significantly more likely to have high contamination compared to those from other schools (OR = 2.5; 95% CI: 1.14–5.51; p = 0.024), with 69.7% of samples classified as highly contaminated compared to 47.9% among other schools. Similarly, handkerchiefs from Ndivisi had higher odds of high contamination (OR = 3.65; 95% CI: 1.30–10.23; p = 0.013), with 77.27% categorized as highly contaminated compared to 48.23% in other schools. In contrast, significantly lower odds of contamination were observed in several schools. Handkerchiefs from Magemo were markedly less likely to have high contamination (OR = 0.10; 95% CI: 0.02–0.44; p < 0.001), with only 10.53% classified as highly contaminated compared to 54.15% in other schools. Similar protective patterns were observed in Silungai (OR = 0.29; 95% CI: 0.13–0.68; p = 0.004) and Wandabwa (OR = 0.12; 95% CI: 0.03–0.40; p < 0.001), where the proportion of highly contaminated handkerchiefs was substantially lower than in other schools. For Lukusi and Malomonye, all handkerchiefs in the "Yes" category were classified as highly contaminated (100% high contamination), whereas in Precious, none of the handkerchiefs in the "Yes" category had high contamination (0% high contamination). Although these findings were statistically significant (p < 0.001), the extreme distributions limit the stability and interpretability of the corresponding odds ratios. No statistically significant differences were observed for Musa (OR = 0.88; 95% CI: 0.39–2.02; p = 0.835) and Sinoko (OR = 0.76; 95% CI: 0.35–1.66; p = 0.556), indicating that contamination levels in these schools were comparable to those in other schools. With regard to participant characteristics, sex was significantly associated with contamination levels. Handkerchiefs from male children were more likely to have high contamination compared to those from female children (OR = 1.70; 95% CI: 1.30–2.21; p < 0.001), with 63.08% of samples from males classified as highly contaminated compared to 37.29% among females. However, class level was not significantly associated with contamination (OR = 1.16; 95% CI: 0.90–1.49; p = 0.309), as similar proportions of high contamination were observed between PP1 (47.11%) and PP2 (54.33%). The findings indicate that both school environment and child sex are important determinants of bacterial contamination of handkerchiefs, with certain schools associated with increased risk while others demonstrate comparatively lower contamination levels

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Table 4. Level of contamination by school and participant characteristics

School/ Participant Characteristics		Level of Contamination		OR	95% CI	P Value
		Low	High			
Lukusi	No	122 (54.22)	103 (45.78)	0.46	0.4 - 0.53	<0.001
	Yes	0 (0.00)	23 (100.00)			
Lutacho	No	112 (52.09)	103 (47.91)	2.5	1.14 - 5.51	0.024
	Yes	10 (30.30)	23 (69.70)			
Magemo	No	105 (45.85)	124 (54.15)	0.1	0.02 - 0.44	<0.001
	Yes	17 (89.47)	2 (10.53)			
Malomonye	No	122 (54.71)	101 (45.29)	0.45	0.39 - 0.52	<0.001
	Yes	0 (0.00)	25 (100.00)			
Musa	No	109 (48.88)	114 (51.12)	0.88	0.39 - 2.02	0.835
	Yes	13 (52)	12 (48)			
Ndivisi	No	117 (51.77)	109 (48.23)	3.65	1.3 - 10.23	0.013
	Yes	5 (22.73)	17 (77.27)			
Precious	No	105 (45.45)	126 (54.55)	0.45	0.39 - 0.52	<0.001
	Yes	17 (100.00)	0 (0.00)			
Silungai	No	99 (45.62)	118 (54.38)	0.29	0.13 - 0.68	0.004
	Yes	23 (74.19)	8 (25.81)			
Sinoko	No	106 (48.40)	113 (51.60)	0.76	0.35 - 1.66	0.556
	Yes	16 (55.17)	13 (44.83)			
Wandabwa	No	101 (45.09)	123 (54.91)	0.12	0.03 - 0.4	<0.001
	Yes	21 (87.50)	3 (12.50)			
Child Sex	Female	74 (62.71)	44 (37.29)	1.7	1.3 - 2.21	<0.001
	Male	48 (36.92)	82 (63.08)			
Class	PP1	64 (52.89)	57 (47.11)	1.16	0.9 - 1.49	0.309
	PP2"	58 (45.67)	69 (54.33)			

DISCUSSION

This study examined bacterial contamination on handkerchiefs used by pre-school children in Bungoma County, Kenya, and found a consistently high level of contamination across all sampled learners. The findings show that handkerchiefs used by young children are frequently exposed to bacteria from different sources and may act as carriers of microorganisms within the school environment (Sanders et al., 2021). The results are important because they highlight everyday hygiene practices that are often overlooked but have direct implications on child health and school sanitation (WHO, 2023). One of the key findings is that contamination was present in all samples regardless of class level. Both PP1 and PP2 learners recorded bacterial growth, indicating that exposure begins early and continues as children progress through pre-school (Mwapasa et al., 2023)**.** While PP2 learners showed slightly higher contamination in some cases, the difference was not very large. This may be explained by the fact that older pre-school children tend to handle their personal items more independently, sometimes without close supervision. On the other hand, PP1 learners, though younger, are often more closely guided by teachers, which may slightly reduce improper handling of handkerchiefs. Both class levels face similar hygiene challenges, suggesting that interventions should target all pre-school children rather than focusing on a specific class (Mwapasa et al., 2023).

Gender differences were also considered in the study. Both boys and girls showed bacterial contamination on their handkerchiefs, with only small variations between the two groups. Boys appeared to have slightly higher contamination levels in some bacterial types, which may be linked to more active play, frequent contact with soil or shared surfaces, and less careful handling of personal items (Hokunan et al., 2016). Girls also showed contamination, but in some cases at slightly lower levels. However, the general pattern shows that gender does not strongly determine contamination levels. This suggests that hygiene behaviors, rather than biological or gender-based factors, plays a more important role in explaining contamination patterns. The bacteria identified

in the study included *Escherichia coli, Staphylococcus species, Klebsiella, Pseudomonas, Enterococcus, Bacillus, and Micrococcus*. The presence of *Escherichia coli* is important because it is usually linked to contamination from human waste, which points to gaps in handwashing practices after using the toilet or handling dirty materials (Vishwanath et al., 2019). .*Staphylococcus species* are commonly found on human skin and in the nose, and their presence reflects frequent wiping of nasal secretions and repeated handling of handkerchiefs without cleaning. Together, these two organisms suggest that contamination is coming from both fecal and respiratory sources.

Other organisms such as *Klebsiella, Pseudomonas, Enterococcus,* and *Bacillus* show that the contamination is not from a single source but from a mix of environmental and human contact. These bacteria are commonly found in soil, water, dust, and on human skin (Abney et al., 2021). Their presence suggests that handkerchiefs are exposed to multiple environments during the school day and at home. In practical terms, this means that once a handkerchief becomes contaminated, it continues to collect more organisms over time, especially when it is reused without proper washing or drying. The overall bacterial load was high across all samples. This indicates repeated use of handkerchiefs without adequate cleaning. In many cases, children reuse the same handkerchief throughout the day or over several days. Because handkerchiefs are often kept in pockets, bags, or shared spaces, they remain slightly moist and are exposed to warmth, which supports bacterial survival (Reynolds et al., 2021).Over time, this creates a cycle where bacteria continue to multiply and are repeatedly transferred back to the child's hands, nose, and mouth. This cycle increases the risk of common infections such as diarrhea, coughs, and respiratory illnesses.

Differences between schools were also observed. Some schools recorded higher levels of contamination compared to others. The results show strong institutional effects on contamination. Lutacho and Ndivisi show significantly higher odds of contamination, suggesting weaker hygiene infrastructure or enforcement. Magemo, Silungai, and Wandabwa show protective effects, indicating better WASH conditions These differences may be linked to variations in hygiene supervision, availability of clean water, and how strongly hygiene routines are enforced. In schools where handwashing is regularly encouraged and monitored, contamination levels appeared relatively lower. In schools where such practices are less consistent, higher contamination was observed. This shows that the school environment plays an important role in shaping hygiene outcomes among children (Vishwanath et al., 2019) . Behavioral factors also contribute significantly to the findings. Children at this age are still developing self-care habits, and many do not fully understand proper hygiene practices. Handkerchiefs are often handled casually, stored in unclean places, or shared between children in some cases. At home, caregivers may not consistently wash or replace handkerchiefs daily. These combined factors increase the likelihood of bacterial growth and transfer. The findings therefore reflect not only school hygiene conditions but also household practices.

From a public health perspective, the results suggest that handkerchiefs can easily become hidden sources of infection among young children. They are frequently used on the nose and mouth and so directly interact with areas where pathogens can enter the body. If not properly cleaned, they can contribute to repeated exposure to bacteria within the same child and also spread organisms to other children through indirect contact (Reynolds et al., 2021). The study highlights the need for simple and practical hygiene interventions in early childhood settings. Regular handwashing with soap, daily cleaning of handkerchiefs, and proper storage of personal items should be encouraged both at school and at home. Teachers and caregivers play a central role in reinforcing these habits. In addition, schools may consider promoting safer alternatives such as disposable tissues where feasible, especially during periods of illness outbreaks.

Although the study provides clear findings, it is important to note some limitations. The design captured contamination at one point in time and may not reflect changes over longer periods. Also, laboratory methods used standard culture techniques, which may not detect all types of microorganisms present. Despite these limitations, the findings still give a realistic picture of hygiene challenges faced by pre-school children in everyday school life. In conclusion, the study shows that handkerchiefs used by pre-school children in Bungoma County are commonly contaminated with bacteria, regardless of class level or gender. The main influencing factors appear to be hygiene practices at school and at home rather than biological differences among children. This points to the need for stronger hygiene education and consistent supervision to reduce preventable infections in early childhood settings.

LIMITATION.

The study was limited to selected pre-school children in public and private schools within Ndivisi zone, Bungoma County, which may restrict generalizability to other regions with different environmental and socio-economic conditions. The cross-sectional design captured data at a single point in time, limiting the ability to assess temporal changes or establish causality between hygiene practices and bacterial contamination. Data collected through structured questionnaires may also be subject to response bias from caregivers and teachers. Additionally, the study focused only on culturable bacteria, which may underestimate the full microbial diversity present on handkerchiefs

CONCLUSION

Pre-school children's handkerchiefs in Bungoma County are universally contaminated with high bacterial loads. This represents a significant public health risk requiring targeted hygiene interventions in early childhood settings. highly prevalent, with all sampled handkerchiefs showing evidence of microbial presence. The overall bacterial load was substantial, indicating that handkerchiefs serve as significant reservoirs of contamination. In terms of bacterial strains, *Escherichia coli* and *Staphylococcus* species were predominant, suggesting the presence of both fecal and skin-related contamination pathways. Other organisms, such as *Salmonella*, showed variable occurrence, reflecting multiple and context-dependent sources of contamination. Contamination patterns varied across participant characteristics. Notably, male children exhibited higher levels of contamination, and children under guardian care were more likely to have highly contaminated handkerchiefs compared to those under parental care. Additionally, significant differences across schools indicate that environmental and institutional factors play a critical role in shaping contamination risk.

RECOMMENDATIONS

- Based on the finding of universal bacterial contamination (100%) and high loads of *Escherichia coli* and *Staphylococcus* species, Water, Sanitation and Hygiene (WASH) interventions in pre-primary schools should be strengthened. emphasis should be on provision of running water, soap, and structured hand hygiene practices, especially after handkerchief use.
- Schools with higher contamination levels such as Lutacho and Ndivisi should be prioritized for targeted hygiene monitoring and behavior change interventions.
- On contamination across schools and higher contamination among male learners, hygiene education should be reinforced through teacher supervision and consistent behavioral reinforcement.
- Future research should incorporate biochemical or molecular methods to improve species-level identification and better assess pathogenic risk.
- A longitudinal study design is recommended to assess changes in bacterial contamination over time and evaluate effectiveness of hygiene interventions.

ABBREVIATIONS

IERC	Institutional Ethics Review Committee
MMUST	Masinde Muliro University of Science and Technology
NACOSTI	National commission for Science Technology and Innovation
SPSS	Statistical package for social sciences
WASH	water, sanitation and hygiene
WHO	World Health Organization

REFERENCES

1. Ahmed, S. K., Hussein, S., Qurbani, K., Ibrahim, R. H., Fareeq, A., Mahmood, K. A., & Mohamed, M. G. (2024). Antimicrobial resistance: Impacts, challenges, and future prospects. *Journal of Medicine, Surgery, and Public Health*, *2*, 100081. https://doi.org/10.1016/J.GLMEDI.2024.100081
2. Ataiyero, Y., Dyson, J., & Graham, M. (2019). Barriers to hand hygiene practices among health care workers in sub-Saharan African countries: A narrative review. *American Journal of Infection Control*, *47*(5), 565–573. https://doi.org/10.1016/j.ajic.2018.09.014
3. Bennett, R. W., Hait, J. M., & Tallent, S. M. (2025). Staphylococcus aureus Infection. *Guide to Foodborne Pathogens, Second Edition*, 26–44. https://doi.org/10.1002/9781118684856.ch2
4. Blackmon, S., Avendano, E., Nirmala, N., Chan, C. W., Morin, R. A., Balaji, S., McNulty, L., Alemu Argaw, S., Doron, S., & Nadimpalli, M. L. (2024). Socioeconomic status and the risk for colonization or infection with priority bacterial pathogens: a global evidence map. *MedRxiv*, 2024.04.24.24306293. https://doi.org/10.1101/2024.04.24.24306293
5. Enkhbat, M., Togoobaatar, G., Erdenee, O., & Katsumata, A. T. (2022). Handwashing Practice among Elementary Schoolchildren in Urban Setting, Mongolia: A School-Based Cross-Sectional Survey. *Journal of Environmental and Public Health*, *2022*, 3103241. https://doi.org/10.1155/2022/3103241
6. Fijan, S., Pahor, D., & Šostar Turk, S. (2017). Survival of Enterococcus faecium, Staphylococcus aureus and Pseudomonas aeruginosa on cotton. *Textile Research Journal*, *87*(14), 1711–1721. https://doi.org/10.1177/0040517516658514
7. Greene, L. E., Freeman, M. C., Akoko, D., Saboori, S., Moe, C., & Rheingans, R. (2012). Impact of a school-based hygiene promotion and sanitation intervention on pupil hand contamination in Western Kenya: A cluster randomized trial. *American Journal of Tropical Medicine and Hygiene*, *87*(3), 385–393. https://doi.org/10.4269/AJTMH.2012.11-0633
8. Hokunan, H., Koyama, K., Hasegawa, M., Kawamura, S., & Koseki, S. (2016). Survival kinetics of Salmonella enterica and enterohemorrhagic Escherichia coli on a plastic surface at low relative humidity and on low-water activity foods. *Journal of Food Protection*, *79*(10), 1680–1692. https://doi.org/10.4315/0362-028X.JFP-16-081
9. Iyevhobu, K. O., Omolumen, L. E., Ken-Iyevhobu, B. A., & Oseni, D. I. (2022). Isolation of bacteria from used handkerchiefs among students. *Journal of Advances in Microbiology Research*, *3*(2), 100–106. https://www.microbiojournal.com/article/56/3-2-19-439.pdf
10. Kanwar, A., Thakur, M., Wazzan, M., Satyavada, S., Cadnum, J. L., Jencson, A. L., & Donskey, C. J. (2019). Clothing and shoes of personnel as potential vectors for transfer of health care–associated pathogens to the community. *American Journal of Infection Control*, *47*(5), 577–579. https://doi.org/10.1016/J.AJIC.2019.01.028
11. KDHS. (2022). *Kenya Demographic and Health Survey - 2022 - Kenya National Bureau of Statistics*. https://www.knbs.or.ke/reports/kdhs-2022/
12. Knobbe, R. B., Diallo, A., Fall, A., Gueye, A. D., Dieng, A., Immerzeel, T. D. van, Ba, A., Diop, A., Diop, A., Niang, M., & Boye, C. S. (2019). Pathogens Causing Respiratory Tract Infections in Children Less Than 5 Years of Age in Senegal. *Microbiology Insights*, *12*, 1178636119890885. https://doi.org/10.1177/1178636119890885
13. Mulatya, D. M., & Ochieng, C. (2020). Disease burden and risk factors of diarrhoea in children under five years: Evidence from Kenya's demographic health survey 2014. *International Journal of Infectious Diseases*, *93*, 359–366. https://doi.org/10.1016/J.IJID.2020.02.003
14. Mwapasa, T., Chidziwisano, K., Lally, D., & Morse, T. (2023). Hygiene in early childhood development centres in low-income areas of Blantyre, Malawi. *International Journal of Environmental Health Research*, *33*(8), 751–767. https://doi.org/10.1080/09603123.2022.2048802
15. Okesanya, O. J., Eshun, G., Ukoaka, B. M., Manirambona, E., Olabode, O. N., Adesola, R. O., Okon, I. I., Jamil, S., Singh, A., Lucero-Prisno, D. E., Ali, H. M., & Chowdhury, A. B. M. A. (2024). Water, sanitation, and hygiene (WASH) practices in Africa: exploring the effects on public health and sustainable development plans. *Tropical Medicine and Health*, *52*(1), 1–9. https://doi.org/10.1186/S41182-024-00614-3/FIGURES/2
16. Oshilim, A., & Orogu, J. (2020). Microbiological evaluation of daily use handkerchief. *The Pharma Innovation Journal*, *9*(9), 08–11.

https://www.thepharmajournal.com/archives/?year=2020&vol=9&issue=9&ArticleId=5120

17. Prüss-Ustün, A., Wolf, J., Bartram, J., Clasen, T., Cumming, O., Freeman, M. C., Gordon, B., Hunter, P. R., Medlicott, K., & Johnston, R. (2019). Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: An updated analysis with a focus on low- and middle-income countries. *International Journal of Hygiene and Environmental Health*, *222*(5), 765–777. https://doi.org/10.1016/J.IJHEH.2019.05.004
18. Ray, S., Amarchand, R., Srikanth, J., & Majumdar, K. (2011). A study on prevalence of bacteria in the hands of children and their perception on hand washing in two schools of Bangalore and Kolkata. *Indian Journal of Public Health*, *55*(4), 293. https://doi.org/10.4103/0019-557x.92408
19. Seguin, M., & Niño Zarazúa, M. (2015). Non-clinical interventions for acute respiratory infections and diarrhoeal diseases among young children in developing countries. *Tropical Medicine & International Health*, *20*(2), 146–169. https://doi.org/10.1111/TMI.12423
20. Vishwanath, R., Selvabai, A. P., & Shanmugam, P. (2019). Detection of bacterial pathogens in the hands of rural school children across different age groups and emphasizing the importance of hand wash. *Journal of Preventive Medicine and Hygiene*, *60*(2), E103. https://doi.org/10.15167/2421-4248/JPMH2019.60.2.1186
21. Wang, J., Ma, W., & Wang, X. (2021). Insights into the structure of Escherichia coli outer membrane as the target for engineering microbial cell factories. *Microbial Cell Factories*, *20*(1). https://doi.org/10.1186/S12934-021-01565-8