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Effects of local handwashing agents on microbial contamination of the hands in a rural setting in Northwest Ethiopia: a cluster randomised controlled trial

Zemichael Gizaw ,[1,2,3] Alemayehu Worku Yalew,[4] Bikes Destaw Bitew,[1] Jiyoung Lee,[5,6] Michael Bisesi[5]

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[1]Department of Environmental and Occupational Health and Safety, University of Gondar, Gondar, Ethiopia
[2]Addis Continental Institute of Public Health, Addis Ababa, Ethiopia
[3]Global One Health Initiative (GOHi), the Ohio State University, Columbus, Ohio, USA
[4]Addis Ababa University, Addis Ababa, Ethiopia
[5]Division of Environmental Health Sciences, The Ohio State University, Columbus, Ohio, USA
[6]Department of Food Science and Technology, The Ohio State University, Columbus, Ohio, USA

Correspondence to
Zemichael Gizaw;
zemichael12@gmail.com

ABSTRACT

Objective To evaluate the effectiveness of handwashing with water and wood ash in reducing faecal contamination of the hands.

Design A cluster randomised controlled trial was employed with two arms: handwashing with water and wood ash versus handwashing with water alone.

Setting Rural households of East Dembiya District, Central Gondar Zone, Amhara National Regional State, Ethiopia.

Participants 440 mothers and caregivers of children younger than 5 years assigned (1:1, 220 in each group) in clusters, with buffer zones between each cluster.

Intervention Health education on effective handwashing was given to the intervention and control groups. Participants in the intervention group used wood ash of the same quantity (ie, one closed palm).

Outcome measures The primary outcome was microbial contamination of the hands, measured by means of *Escherichia coli* counts before and after handwashing.

Results At baseline, 75.9% and 67.7% of the participants in the intervention and control groups, respectively, had dirt on their fingernails, and the hands of all participants in both groups were contaminated with *E. coli*. The mean *E. coli* counts recovered at baseline were 3.07 log10 colony forming unit (CFU)/swab in the intervention group and 3.03 log10 CFU/swab in the control group, while at endline it was 1.4 log10 CFU/swab in the intervention group and 3.02 log10 CFU/swab in the control group. The mean *E.coli* counts was reduced by 1.65 log10 due to the intervention (difference-in-differences: β= −1.65, 95% CI= −1.84 to −1.46).

Conclusion Two-thirds of the swab samples tested positive for *E. coli* after handwashing with water and wood ash, which indicates wood ash is not very effective in terms of completely removing micro-organisms on the hands. However, wood ash was significantly better than water alone in reducing the concentration of faecal coliform organisms on the hands. Local health authorities should primarily promote handwashing with soap. However, in the absence of soap, use of wood ash over water alone might be appropriate.

Trial registration number PACTR202011855730652.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ This study assessed the effect of handwashing with ash on microbial contamination of the hands in rural Northwest Ethiopia as there is limited evidence on such effects in Ethiopian context.

⇒ To control bias, clusters were randomly allocated to control and intervention arms, with a buffer zone of a minimum of 15 min walking distance applied between clusters and with the principal investigators blinded to the randomised group assignment until the primary analysis was completed.

⇒ The adjusted and unadjusted values for the treatment effect were the same, showing that the two trial groups were similar with respect to important confounders and indicating that randomisation was successful in ensuring well-balanced groups.

⇒ Important limitations of this study include the inability to inform on the efficacy of wood ash relative to soap due to lack of handwashing with soap as a comparator and lack of information on the potential harms of wood ash, including data on adverse events and on the pH and microbial quality of the ash.

⇒ Investigating a proxy measure only (hand contamination) rather than disease outcomes and using only a single indicator organism (Escherichia coli) are other important limitations.

INTRODUCTION

The hands are important given that they are the last line of defence within the chain of transmission of enteropathogens, with transmission occurring either directly from the hand to the mouth or indirectly via handling and transfer of contaminated food and water. The hands can also play a role in the transmission of respiratory pathogens through fingers contaminated with respiratory viruses; for example, transmission can occur by rubbing the conjunctiva of the eyes or the nasal mucosa with contaminated fingers. The hands can also play a part in the transmission of skin, wound, eye and other infections.[1 2]

There is a clear causal link between hand hygiene and infection transmission. The hands are among the most important mechanisms that transmit pathogenic micro-organisms that cause infection.[3] Evidence indicates that the hands, along with contact with food and other environmental surfaces, cause 60% of spread of gastrointestinal infections. Contaminated hands are also associated with up to 50% of respiratory tract infections.[4] Promoting improved hand hygiene has been recognised as an important public health measure. Results from intervention studies show that handwashing reduces gastrointestinal and respiratory tract infections to up to 50%–60%.[4] Education about handwashing in the community reduces the number of people who get sick with diarrhoea by 23%–40%,[5–7] reduces diarrhoeal illness in people with weakened immune systems by 58%,[8] reduces respiratory illnesses such as common cold in the general population by 16%–21%,[6 9] and reduces absenteeism due to gastrointestinal illness among schoolchildren by 29%–57%.[10]

Both mechanical actions (rubbing and rinsing) and chemical actions (killing or inactivation) and disinfection of clean hands with, for example, alcohol, are generally considered very effective in removing micro-organisms from the hands.[11–13] The effectiveness of rubbing in removing microbes from the hands is determined by the degree to which the microbes are bound to the surface of the skin. The effectiveness of the mechanical and chemical actions of handwashing agents in removing microbial contaminants can be measured by enumerating the density of indicators of faecal contamination or specific pathogens in the samples taken from the hands before and after washing.[14 15] The occurrence and concentration of specific pathogens, instead of using indicators as proxies, are less commonly measured in environmental matrices (water, food, soil, hands and other surfaces), although they may be more representative of the actual health risks associated with specific pathogens.[16] Measuring specific pathogens needs to consider not only the diversity of potential pathogens occurring in environmental matrices but also the relevance of each included pathogen in the health outcomes of interest, which is highly context-specific. The possibility of improved specificity of measuring specific pathogens instead of indicators of faecal contamination may come at a loss of sensitivity since selected pathogens may not be representative of all possible pathogens in the environment.[16] Therefore, use of indicator organisms as a surrogate for evaluating the presence of pathogens in environmental matrices has the advantage of being easier and less expensive to measure compared with multiple specific pathogens and can also be indicative of a range of pathogens.[17] Culturable faecal indicator bacteria such as *Escherichia coli* are indicators of the presence of faecal material from warm-blooded animals and can be used as microbial surrogates for environmental quality monitoring. The existence of *E. coli* in environmental matrices corresponds to the presence of other pathogens such as bacteria, viruses, protozoans and helminths either from human or animal sources.[18]

Rubbing duration and quality of water are also important factors in determining the effectiveness of rubbing agents in removing microbial contaminants from the hands. It has been documented that handwashing for 30 s removes 10 times the amount of bacteria as handwashing for 15 s.[19 20] Moreover, research suggests that extending the duration of washing to 1 min results in a reduction of 2.6–3.23 log *E. coli* counts, but extending the process to more than 1 min did not show further reduction.[4] In addition, hands washed with contaminated water pose a risk of higher levels of hand contamination.[21–23] Poor sanitation also affected the effectiveness of handwashing. Poor sanitation increases the frequency and intensity of hand contamination from human and animal excreta.[1]

The perception of individuals on the role of hands in infection transmission affects the handwashing behaviours since hand hygiene reflects attitudes, behaviours and beliefs. When someone believes their hands can be contaminated by germs and can spread infections, he/she will avoid unnecessary touching of surfaces in close proximity to a source of contaminants and will wash their hands frequently. On the other hand, individuals will not frequently wash hands if they perceive that they have low risk of acquiring infection from the hands and contact surfaces. Furthermore, there might be lack of active participation in promoting hand hygiene if individuals do not believe in the value of hand hygiene in the prevention of infections.[24–26]

Due to its surfactants, the use of soap with water greatly improves elimination of bacteria from the hands compared with that accomplished by washing with water alone. In low-income societies in developing countries, where soap or alcohol-based agents are not usually available due to cost, soil, mud and ash are sometimes used as a zero-cost alternative to soap.[4] For instance, studies conducted in Bangladesh and India found that soil, ash and soap were all effective in removing faecal coliforms from the hands.[27 28] Cleansing using wood ash is achieved by its friction and disinfection (alkaline) properties. Wood ash has been mentioned as a disinfectant in a WHO publication.[29] Wood or kitchen ash can be used to rub away, kill or inactivate pathogens on the hands. Wood ash can also aid in the mechanical removal of loosely adhered debris and microbes. However, depending on the source materials, ash may be contaminated with chemical toxicants, such as metals, since it is a product of combusted wood, coal, leaves and other biomaterials. It may also be contaminated with microbial pathogens if it did not come from fresh sources.[30]

METHODS

Study area and setting

This study was carried out in a rural setting of the East Dembiya District, which is one of the 13 districts of Central Gondar Zone, Amhara National Regional State, Ethiopia. The district is subdivided into 28 rural and 4 urban kebeles (the lowest administrative unit).[31] The

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district health department report in July 2020 showed that East Dembiya District had a total population of 210 761, of whom 192 020 (91%) and 18 741 (9%) were rural and urban residents, respectively.[31] Hygiene-related and sanitation-related communicable diseases were highly prevalent in the area. In June 2017, intestinal parasitic infections and diarrhoeal disease were the top 4 and 5 prevalent diseases, accounting for 5161 (9.97%) and 4981 (9.62%), respectively.[32]

Study design

A two-arm, cluster randomised controlled trial was employed among mothers and caregivers of under-5 children in rural Northwest Ethiopia to examine the effect of using wood ash, a local handwashing agent, on faecal contamination of the hands. The protocol of the trial was registered with the Pan African Clinical Trial Registry (PACTR202011855730652) on 6 November 2020 (http://www.pactr.org) and published in *BMJ Open*.[33]

Participant eligibility and enrolment

This trial was implemented among mothers and caregivers of children under the age of 5 in the East Dembiya District, Northwest Ethiopia, where no similar interventions were operating or planned during the intervention period. Participants were recruited based on the following inclusion criteria: (1) presence of under-5 children in the household; (2) mothers who volunteered to be part of the study; (3) mothers who were not using soap to wash hands; and (4) geographical accessibility. One mother/caregiver with chronic skin lesions was excluded. Written informed consent was obtained from the study participants.

Description of interventions

We provided health education on effective handwashing procedures to both arms, and provided local rubbing agents such as wood ash to the intervention group. All participants in the intervention group used the same ash, that is, wood or kitchen ash, regardless of the type of wood that each household used as a cooking energy source, with the same quantity of ash used (ie, one closed palm). Since ash from municipal solid wastes contains toxic materials and accumulated ash at or near homes may be contaminated with pathogens from human or animal faeces, we only used ash freshly produced from burning of wood since this ash is sterile. The control arm did not use wood ash and continued with their usual handwashing practices.

Sample size determination

Stata V.14 was used to calculate the number of clusters, with the following assumptions: log10 mean (±SD) bacterial count before handwashing (dominant hand)=3.17±0.71; log10 mean (±SD) bacterial count after handwashing=3.41 ± 0.61[34]; equal cluster size with average size (m)=20; intracluster correlation coefficient (ICC)=0.02, as recommended by Hayes and Bennett[35]; 80% power; and 95% CI with 5% alpha level and a design effect of 1.38, that is, [1+(m−1) ICC]. With these assumptions, the number of clusters per arm is 10. Assuming one extra cluster for loss of follow-up, the total cluster becomes 11 per arm. Therefore, a total of 220 mothers and caregivers received the intervention and 220 mothers and caregivers were taken as the control group.

Sampling procedures

Clusters were selected and formed based on simple random sampling method. Clusters were defined by the presence of adequate households with under-5 children eligible for enrolment and by geographical accessibility. We conducted a pilot survey to determine the size of clusters. As indicated in the Sample size determination section, the geographical area where we got 20 households with children under the age of 5 was taken as a cluster. A buffer zone of a minimum of 15 min walking distance was used before enrolling the next cluster to minimise information contamination between the enrolled clusters. All households with children under the age of 5 in the selected clusters and who fulfilled the inclusion criteria were entered in the cluster randomised controlled trial schemes.

Randomisation and blinding

Clusters were randomly allocated in each arm. Random sequence generation and allocation were conducted by the coordinating team. Due to the nature of the intervention and the study purpose, participants were not blinded to their intervention assignment. Researchers remained blinded to the randomised group assignments until the primary analysis was completed. They also did not participate in the data collection. The supporting teams generated the random allocation sequence, enrolled the participants and assigned the participants to the intervention.

Outcome measures

The primary outcome of this study was microbial contamination of the hands, measured by the mean concentration of the indicator organism for faecal contamination, that is, *E. coli*, in colony forming unit (CFU) before and after handwashing with plain water alone and with water and wood ash. We adopted a laboratory procedure to recover *E. coli* from the standard methods described in the WHO guideline.[36] At the time of trial registration, we had intended to include additional outcome components (detection of viruses, parasites and other bacteria), but during study implementation resource constraints limited us to detecting *E. coli* as the only indicator organism.

Baseline data collection tools and procedures

Baseline data were collected using an interviewer-administered questionnaire with a structured observational checklist to assess the baseline characteristics of the study participants. We prepared the tool based on a review of relevant literature. The tool was first prepared in the English language and translated to the local Amharic language by two native Amharic speakers fluent in English, and then back-translated to English by two independent English language experts fluent in Amharic to check for consistency. After translation, we conducted face validity, content validity and internal consistency studies, and finally prepared a valid and reliable questionnaire (content validity ratio=0.93; item-level content validity index=0.96; universal agreement scale-level content validity index=0.95; modified kappa=0.96; Cronbach's alpha for internal consistency=0.80).

Swab sample collection

Transferring peptone-buffered water (PBW, 0.1%) was prepared in each test tube (5 mL) with autoclaving. The prepared PBW tubes were transported in a cooler to the sampling sites. After getting consent, the fieldworkers asked the study subjects to do their usual activities that heavily contaminate their hands. The fieldworkers then took swab samples from the dominant hand before washing. After swabbing, the fieldworkers instructed the participants to wash their hands either with plain water alone or with water and wood ash and to rub for at least 20 s. The fieldworkers demonstrated proper handwashing techniques to all mothers in the control and intervention groups, observed while they were washing and coached them when they missed the procedures, to ensure similar handwashing procedures between the two groups. The fieldworkers informed the mothers and caregivers to dry their hands in the air after washing and they then took swab samples from the dominant hand. The background level of *E. coli* in the water that was used for handwashing was in the range of 0–1613 CFU.

In order to collect swabs, the fieldworkers first inserted the swab in to the test tube to wet it by holding over the notch. After removing the excess buffer from the swab, they gently rubbed the swab all over the surface of the palm, between the fingers and on the tip of the fingers of the dominant hand. They first swabbed in horizontal direction by gently moving and rolling over back and forth and keeping the entire swab flat. They then swabbed in a perpendicular direction using the same technique. The head of the swab was then inserted into the test tube, the notch placed on the edge of the test tube, the swab handle pushed down until the head of the swab fell into the test tube, and then the cap carefully placed over the tube. The fieldworkers repeated the same procedure until the required 880 swab samples had been collected. The collected samples were transported in a cooler to the laboratory of the Department of Biology at University of Gondar for analysis.

E. coli measurement

We transferred the collected swab samples to a test tube containing 100 mL of sterile peptone buffer followed by vigorous manual shaking of the tube to release the *E. coli* cells from the swab into the buffer solution. The head of the swab was then aseptically wrung out inside the test tube to release the buffer solution, after which the head of the swab was removed. The entire 100 mL solution was then filtered through 47 mm diameter, 0.45 µm pore-sized filter membrane (Millipore, Burlington, Massachusetts, USA) and cultured on a membrane lauryl sulfate broth before pouring them into an absorbent pad (Oxoid, Basingstoke, UK). The prepared samples were incubated at 44.5°C for a 24-hour growth period before counting the number of CFU. This method followed the standard methods described in the WHO guideline.[36] The laboratory personnel were blinded to the handwashing methods. The filtration apparatus was washed with distilled water and flamed between analyses of consecutive samples and was sterilised at intervals. The number of colony was counted and the result was expressed as CFU/swab. One field blank per sample collector per week and one laboratory blank per laboratory assistant per day were processed for quality control.

Statistical analysis

We used Stata V.14 for data analysis. χ^2 test was applied to check for similarity of baseline data between the intervention and the control group. An intention-to-treat analysis was used to compare the mean *E. coli* count between the intervention and the control group. In our analysis, we compared the mean *E. coli* counts among the group doing handwashing with water alone and the mean *E. coli* counts among the group doing handwashing with water and wood ash.

A difference-in-differences (DID) model was used to estimate the average treatment effect of washing hands with wood ash in the intervention group by comparing the difference between before and after handwashing and the difference in mean *E. coli* counts between the control and treatment groups. We created two dummy variables for time: time=0 before handwashing and time=1 after handwashing. We also created two dummy variables for programme: programme=0 for the control group and programme=1 for the intervention group. We then created an interaction between time and programme. We entered in the final model the above three variables and the covariates which are associated with the level of *E. coli* with p<0.25 in the bivariate analysis and well-known confounders from the literature. The regression coefficient of the interaction term is the effect of handwashing with water and wood ash. We used Stata's *xtreg* command to fit the model. We controlled for clustering effect by having option cluster (cluster identification) in the model. Statistically significant variables were identified on the basis of adjusted betas with 95% CI and p<0.05.

Patient and public involvement

No patients were involved.

RESULTS

Characteristics of the study participants

A total of 220 mothers and caregivers in 11 clusters in the intervention group and 220 mothers or caregivers in 11 clusters in the control group were enrolled. The size of each cluster was 20 mothers and caregivers. All study subjects completed the short trial, with no loss to

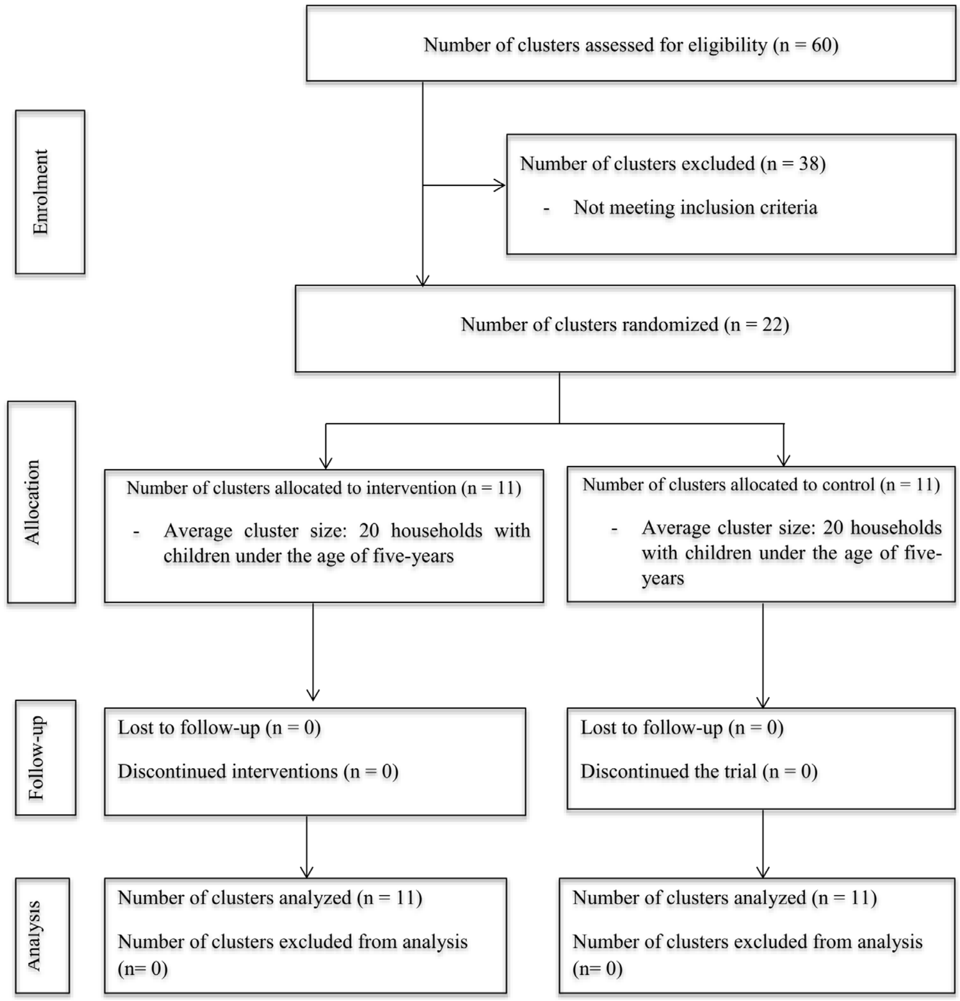

Figure 1 Trial flow diagram.

follow-up or missing outcome data (figure 1). Before implementing the intervention, we collected baseline data from the intervention and control groups. The baseline sociodemographic characteristics (table 1) and hygiene or sanitation characteristics (table 2) were well balanced between the study groups.

Perception of mothers and caregivers about handwashing practices

Of the mothers and caregivers, 17 (7.7%) and 15 (6.8%) in the intervention and control groups, respectively, did not perceive that human faeces contains disease-causing pathogens. Similarly, 63 (28.6%) and 56 (25.5%) mothers and caregivers in the intervention and control groups, respectively, did not recognise that animal excreta contains disease-causing pathogens. In the intervention and control groups, 93 (42.3%) and 96 (43.6%) mothers and caregivers believed that germs are found on dirty hands only. Of the mothers and caregivers, 60 (27.3%) and 53 (24.1%) in the intervention and control groups, respectively, did not think that they always have to wash hands after visiting the toilet. Of the mothers and caregivers, 94 (42.7%) and 93 (42.3%) in the intervention and control groups, respectively, perceived that washing

Table 1 Sociodemographic characteristics of the study participants in the intervention (n=220) and control (n=220) arms in rural Northwest Ethiopia, May 2021

Sociodemographic characteristics	Intervention, n (%)	Control, n (%)
Age		
20–25	42 (19.1)	47 (21.4)
26–30	62 (28.2)	58 (26.4)
31–35	35 (15.9)	30 (13.6)
36–40	45 (20.5)	43 (19.5)
40–45	36 (16.4)	42 (19.1)
Education status		
No formal education	143 (65.0)	144 (65.5)
Primary education	48 (21.8)	44 (20.0)
Secondary education	29 (13.2)	32 (14.5)
Household has livestock		
Yes	163 (74.1)	159 (72.3)
No	57 (25.9)	61 (27.7)

Table 2 Personal hygiene and sanitation of the living environment among study participants in the intervention (n=220) and control (n=220) arms in rural Northwest Ethiopia, May 2021

Variables	Intervention, n (%)	Control, n (%)
Mothers and caregivers kept their fingernails short		
Yes	53 (24.1)	71 (32.3)
No	167 (75.9)	149 (67.7)
Mothers' and caregivers' fingernails have visible dirt		
Yes	142 (64.5)	140 (63.6)
No	78 (35.5)	80 (36.4)
Mothers' and caregivers' palms have visible dirt		
Yes	139 (63.2)	141 (64.1)
No	81 (36.8)	79 (35.9)
Mothers and caregivers always washed hands		
After visiting toilet	173 (78.6)	179 (81.4)
Before food preparation	194 (88.2)	190 (86.4)
Before eating	197 (89.5)	193 (87.7)
After defecating a child	155 (70.5)	157 (71.4)
After cleaning animal barns	180 (81.8)	177 (80.5)
Whenever hands have dirt	131 (59.5)	135 (61.4)
What mothers and caregivers usually use to wash hands		
Water only	184 (83.6)	187 (85.0)
Water and soap	19 (8.6)	20 (9.1)
Water and ash	17 (7.7)	13 (5.9)
Handwashing practices of mothers and caregivers (results from the observation)		
Used rubbing agent	46 (20.9)	45 (20.5)
Rubbed all parts of the hands well for at least 20 s	52 (23.6)	57 (25.9)
Wiped on their clothes to dry after washing	49 (22.3)	44 (20.0)
Dry in the air after washing	171 (77.7)	176 (80.0)
Family practised open defecation		
Yes	99 (45)	119 (54.1)
No	121 (55)	101 (45.9)
Living environment has animal excreta		
Yes	83 (39.5)	107 (48.6)
No	137 (60.5)	113 (51.4)

hands with water alone can remove germs. Of the study participants, 141 (64.1%) and 138 (62.7%) in the intervention and control groups, respectively, perceived that ash can remove germs as effectively as soap, and 81 (36.8%) and 79 (35.9%) mothers and caregivers believed that they only need to wash hands with soap when their hands are heavily dirty. Of the mothers, 183 (83.2%) and 181 (82.3%) in the intervention and control groups, respectively, perceived that germs are acquired when touching doors, chairs, tables, animals and other things. An overwhelming majority of mothers and caregivers, 208 (94.5%) and 205 (93.2%) in the intervention and control groups, respectively, perceived that dirty hands transmit infections (table 3).

Microbial quality of the hands before and after handwashing

At baseline, the hand swabs collected from the intervention and control groups were positive for *E. coli* (p>0.05), while at endline 142 (64.5%) swabs in the intervention group tested positive, while all samples in the control group tested positive (p<0.001). At baseline, the mean (±SD) *E. coli* counts recovered in the intervention group were 3.07 log10 CFU/swab (±0.22) and in the control group 3.03 log10 CFU (±0.22). The pretest *E. coli* counts in the intervention and control groups were not significantly different (p>0.05). At endline, it was 1.4 log10 CFU/swab (±0.12) in the intervention group and 3.02 log10 CFU/swab (±0.22) in the control group. The mean *E. coli* counts in the control group before and after handwashing were not significantly different (p>0.05), and the mean *E. coli* counts in the intervention group before and after handwashing were significantly different (p<0.001; table 4).

With respect to the primary outcome, the mean *E. coli* counts reduced by 1.65 log10 due to the intervention (DID result: β= −1.65, 95% CI =1.84 to −1.46), after adjusting the model for baseline *E. coli* level of the water used to wash hands, condition of the fingernails and sanitation conditions. The intervention (ie, washing hands with water and wood ash) is more effective in reducing *E. coli* counts compared with the control (ie, washing hands with plain water alone) (p<0.001; table 5).

DISCUSSION

This is a two-arm, cluster randomised controlled trial conducted in rural Northwest Ethiopia to assess the effect of handwashing with water and wood ash on microbial contamination of hands. It was found that handwashing with water and wood ash is significantly better in reducing the concentration of *E. coli* than washing hands with plain water alone. This suggests that using locally available handwashing agents such as wood ash can help to reduce hand contamination with faecal coliforms. However, a significant proportion of the samples from the group doing handwashing with water and wood ash still tested positive for *E. coli*, which indicates that washing hands with water and wood ash is not very effective in completely removing micro-organisms. Similarly, other scientific studies conducted in Bangladesh[27 37] and India[28] reported that washing hands with wood ash and other locally available rubbing agents such as mud reduced the concentration of faecal coliform bacteria on the hands.

Cleansing using wood ash is achieved by its friction and disinfection (alkaline) properties. Wood ash has been mentioned as a disinfectant in a WHO publication.[29] As discussed by Howard and Bogh,[29] wood or kitchen ash can be used to rub away, kill or inactivate pathogens on

Table 3 Perception of study participants about handwashing practices and their benefits in the intervention (n=220) and control (n=220) arms in rural Northwest Ethiopia, May 2021

Variables	Intervention, n (%)	Control, n (%)
Do you believe that human faeces contains disease-causing pathogens?		
Yes	197 (89.5)	196 (89.1)
No	17 (7.7)	15 (6.8)
Don't know	6 (2.7)	9 (4.1)
Do you believe that animal excreta contains disease-causing pathogens?		
Yes	144 (65.5)	148 (67.3)
No	63 (28.6)	56 (25.5)
Don't know	13 (5.9)	16 (7.3)
Do you believe that germs are present on dirty hands only?		
Yes	93 (42.3)	96 (43.6)
No	111 (50.5)	116 (52.7)
Don't know	16 (7.3)	8 (3.6)
I don't think I have to always wash hands after visiting the toilet.		
Yes	60 (27.3)	53 (24.1)
No	60 (27.3)	53 (24.1)
Do you perceive that washing hands with water alone can remove germs?		
Yes	94 (42.7)	93 (42.3)
No	111 (50.5)	118 (53.6)
Don't know	15 (6.8)	9 (4.1)
Do you perceive that washing hands with ash can remove germs like that of soap?		
Yes	141 (64.1)	138 (62.7)
No	59 (26.8)	60 (27.3)
Don't know	20 (9.1)	22 (10.0)
We only need to wash hands with soap when hands are heavily dirty.		
Yes	81 (36.8)	79 (35.9)
No	139 (63.2)	141 (64.1)
Germs are acquired when we touch doors, chairs, tables, animals and other things.		
Yes	183 (83.2)	181 (82.3)
No	30 (13.6)	28 (12.7)
Don't know	7 (3.2)	11 (5.0)
Unwashed hands transmit infections.		
Yes	208 (94.5)	205 (93.2)
No	12 (5.5)	15 (6.8)

the hands. Wood ash also aids in the mechanical removal of loosely adhered debris and microbes. According to research, the mechanical action of rubbing and rinsing removes organisms acquired by the hands. Hand rubbing with rubbing agents loosens the germs from the skin and rinsing the hands removes the germs.[11 12] However, despite the fact that our study found that using freshly produced wood ash as a rubbing agent lowered the concentration of faecal indicator bacteria on the hands, there is no conclusive evidence that washing hands with wood ash reduces the risk of infection.[38] Furthermore, depending on the source materials, ash may be contaminated with potential toxic heavy metals such as arsenic, lead and chromium, as well as pesticides. Although this could represent a toxic hazard when used for handwashing, there are no epidemiological or risk assessment data on the magnitude of risk, but again this is likely to be small relative to other routes of exposure (ie, drinking contaminated water).[30] Ash may also be contaminated by microbial pathogens if it did not come from fresh sources.[30] Although there is microbiological evidence indicating the potential for transmission of infections with use of contaminated ash for handwashing, there is little or no epidemiological evidence to suggest to what extent this represents a risk.

The water used for handwashing contributes to hand hygiene. This study found that the mean *E. coli* counts were high among mothers who used water contaminated by *E. coli* for handwashing. Other studies also reported that *E. coli* measured on hands after handwashing has been found to have significant association with the microbial quality of the water used for washing hands.[21–23] This is due to the fact that hands washed with contaminated water pose a risk of higher levels of hand contamination.

Rubbing time is an important factor that determines the effectiveness of rubbing agents in removing microbial contaminants from the hands. This study revealed that rubbing the entire hands with ash for at least 20 s reduced the mean *E. coli* counts. Similarly, other studies have discussed that rubbing the hands for 30 s significantly reduced the amount of bacteria.[19 39] Moreover, research has shown that extending washing time to 1 min results in a considerable reduction, but extending the process to more than 1 min did not show further reduction.[4]

This research depicted that reduction in the log mean *E. coli* counts was associated with the length of the fingernails. In the studied region, the log mean *E. coli* counts were found to be low among mothers who kept their fingernails short compared with mothers with long fingernails. This is because the area beneath the fingernails harbours the highest concentrations of micro-organisms and is the most difficult to clean. The risk of infection from using soil or ash for handwashing is likely to increase when soil or ash particles remain under the fingernails and are not removed by rinsing the hands.[4 40 41]

The current study pointed out that the mean *E. coli* counts were found to be low in households that managed human and animal faeces hygienically. The likelihood of hand contamination is higher in areas where human and animal excreta are disposed in an unhygienic way. Exposure to human and animal faeces is of particular concern in developing countries, where animal husbandry within the household environment is common. Evidence

Table 4 Summary of *Escherichia coli* counts recovered before and after handwashing in both groups in East Dembiya District, Northwest Ethiopia, May 2021

	Before handwashing						After handwashing					
	Intervention			Control			Intervention			Control		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
E. coli counts in CFU/swab in log10	2.62	3.69	3.07	2.48	3.64	3.03	1.96	2.48	1.4	2.36	3.63	3.02

CFU, colony forming units.

suggests that limited access to sanitation leads to higher rates of hand contamination from human and animal excreta.[1 39 42 43] Due to the potential danger posed by animal faeces, a broader range of sanitation interventions have been proposed, such as combining animal faecal management (eg, separating animals from living spaces, removing animal faeces from the household environment with tools and reducing animal movement) with human faecal management (eg, latrines).[44–47]

Moreover, this study revealed that a significant proportion of mothers and caregivers did not think that they always have to wash hands after visiting the toilet. A considerable number of mothers and caregivers also did not perceive that dirty hands transmit infections. A significant proportion of mothers and caregivers perceived that washing hands with water alone can remove germs, and more than one-third of mothers and caregivers believed that they only need to wash hands with soap when their hands are heavily dirty. Such perceptions on sources of hand contamination and the role of hands in infection transmission affect handwashing practices in the studied region. As documented in the literature, if someone believes that his or her hands play a role in infection transmission and that washing hands can lessen the rate of transmission, he or she will wash their hands frequently following effective handwashing practices.[24 26]

Overall, the findings of this study could be applicable to other situations and settings with similar hygiene and sanitation conditions as the population of the current study, such as rural settings in developing countries where the population has no access to soap and has limited access to hygiene and sanitation facilities. In this case, the level of contamination may not vary in different settings.

Table 5 Effects of washing hands with water and wood ash on microbial contamination of hands in East Dembiya District, Northwest Ethiopia, May 2021

Variables	Unadjusted β (95% CI)	Adjusted β (95% CI)
Treatment group		
Control	Reference	Reference
Intervention	−1.65 (−1.84 to −1.46)	−1.65 (−1.84 to −1.46)*
Escherichia coli found in water used to wash hands		
Yes	0.73 (0.28 to 1.18)	0.51 (0.30 to 0 .72)*
No	Reference	Reference
Activities mothers and caregivers performed before washing hands		
Visiting the toilet	−0.08 (−0.24 to 0.08)	−0.01 (−0.22 to 0.20)
Cleaning the house/yard	−0.06 (−0.19 to 0.08)	−0.03 (−0.23 to 0.16)
Cleaning animal barns	Reference	Reference
Mothers and caregivers kept their fingernails short		
No	Reference	Reference
Yes	−0.35 (−0.54 to −0.17)	−0.09 (−0.16 to −0.02)†
Household practised open defecation		
Yes	Reference	Reference
No	−0.51 (−0.76 to −0.25)	−0.33 (−0.45 to −0.21)*
Animal excreta in the living environment		
Yes	Reference	Reference
No	−0.41(−0.61 to −0.21)	−0.16 (−0.25 to −0.07)*

*Statistically significant variables at p<0.001.
†Statistically significant variable at p<0.01.

Our study has several important limitations. We did not assess the pH and microbial quality of the ash used in the trial. Relatedly, lack of data on the adverse effects of using wood ash, such as skin damage, is a limitation of this study. However, we used freshly produced ash to reduce cross contamination because ash freshly produced from burning of wood is sterile. Inability to inform on the efficacy of wood ash relative to soap due to lack of a soap comparator is another important limitation. Moreover, the magnitude of reduction of actual diseases due to handwashing with water and wood ash is unknown given that the assessment was done with only a proxy outcome and using a single indicator organism.

CONCLUSION

Nearly two-thirds of the swab samples from the group doing handwashing with water and wood ash still tested positive for *E. coli*, which indicates washing hands with water and wood ash is not very effective in completely removing micro-organisms. However, washing hands with water and wood ash for at least 20 s significantly reduced the concentration of faecal coliforms, which indicates washing hands with water and wood ash can improve the microbial quality of the hands compared with plain water alone. Local health authorities should, therefore, primarily promote handwashing with soap. However, in the absence of soap, the use of freshly produced wood ash as a handwashing agent can be recommended following the results of this trial showing that washing hands with water and wood ash considerably reduced faecal coliforms compared with washing hands with plain water alone. It is important to consider the materials in which ash is resulting from to minimise its toxic effect. We must also avoid at all costs using ash from municipal wastes as a rubbing agent because ash from burning of municipal solid wastes contains toxic materials.[48 49] Furthermore, attention should be taken to the type of ash used as a rubbing agent. Ash itself may be contaminated with pathogens from human or animal faeces if it is allowed to accumulate at or near homes. However, microbial contamination may not be an issue if the ash is fresh. Freshly produced ash from burning of woods is sterile. Damage to the skin when individuals with sensitive skin are exposed to ash for a long time is another concern. In regard to this, the benefit–harm balance of using wood ash is currently unknown as we do not have robust information on its harmful effects. Above all, promoting environmental sanitation and hygienic behaviours is useful to improve effectiveness of wood ash in reducing microorganisms from hands.

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Patient consent for publication Not required.

Ethics approval This study involves human participants. The ethical and methodological issues of this protocol were approved by the Institutional Review Board (IRB) of the University of Gondar (reference number: V/P/RCS/05/1933/2020). Participants gave informed consent to participate in the study before taking part.

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ORCID iD

Zemichael Gizaw http://orcid.org/0000-0002-6713-1975

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