



Associations of hand washing frequency with the incidence of illness: a systematic review and meta-analysis

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Background: Hand hygiene is one of the ways to prevent the spread of diseases. Our aim was to explore the relationship between hand washing frequency and the impact on disease, and give recommendations on the number of times to wash hands.

Methods: We searched seven electronic databases from their inception to April 11, 2020, and reference lists of related reviews for all studies on hand washing frequency and disease prevention. The Review Manager 5.3. software was used to conduct a meta-analysis. We assessed the risk of bias of included studies, and quality of evidence of the main findings.

Results: A total of eight studies were included. The results of the meta-analysis showed that there was no statistical significance between the effect of disease prevention and washing more than 4 times/day compared to not [odds ratio (OR) =0.61, 95% confidence interval (CI): 0.37 to 1.01]. The results of a case-control study showed that compared with hand washing ≤ 4 times/day, hand washing 5–10 times/day (OR =0.75, 95% CI: 0.63 to 0.91) and hand washing >10 times/day (OR =0.65, 95% CI 0.53 to 0.80) could reduce the risk of disease infection. There was no statistical significance advantage to hand washing more than 10 times/day compared to 5–10 times/day (OR =0.86, 95% CI: 0.70 to 1.06). Comparing hand washing ≤ 10 times/day with hand washing >10 times/day, increased hand washing was a protective factor against infection (OR =0.59, 95% CI: 0.36 to 0.97).

Conclusions: The more frequently hands were washed, the lower risk of disease. So far however, there is no high-quality evidence indicating the best range of hand washing frequency for disease prevention.

Keywords: Hand washing; diseases prevention; meta-analysis; systematic review

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Introduction

Dirty hands can be a vector for several gastrointestinal infections, such as diarrhea (1), and respiratory infections, such as influenza, severe acute respiratory syndrome (SARS), and coronavirus disease 2019 (COVID-19) (2-5). These infectious diseases can cause serious complications, especially for people with poor compromised immunity (6-8). Diarrhea and pneumonia, for example, are the top two leading causes of mortality among children under five years of age, causing approximately 1.8 million deaths each year (9). Since December 2016, the main cause of excess all-cause mortality in many European countries, especially among people over 65 years of age, has been the influenza virus (10). Subsequently, hand hygiene is one of the most effective ways to prevent the spread of these infectious diseases and reduce associated morbidity and mortality (11).

Numerous studies have shown that proper hand washing can prevent the transmission of certain diseases (12-15). Specifically, promoting the frequency of hand washing can prevent about 30% of diarrhea-related illnesses (16) and about 20% of respiratory infections (17). Through efforts such as community hand washing education, diarrhea can be reduced by 23–40% (17-19). For people with poor compromised immunity, their proper hand washing can reduce certain infectious diseases by 58% (20), while respiratory diseases such as cold will be reduced by 16–21% (17,18,21). This simple prevention measure can also have lasting impacts. Some research has reported that rates of absenteeism among primary school students due to gastrointestinal diseases can be reduced by 29–57% with the implementation of hand washing (22). For this reason, The World Health Organization (WHO) advises the public to wash their hands frequently as a part of basic protection against COVID-19 (23).

While some studies have shown that increasing the frequency of hand washing can effectively prevent illness, there is no advice regarding the specific frequency of washing hands that is needed. A study by Alba *et al.* showed that for every unit of improvement in hand washing frequency, typhoid infection rates decreased by about 62% (24). The study found that hand washing more than 4 times per day was a protective factor in reducing disease risk (25). However, some studies have reported no statistical difference in the prevention of diseases due to the frequency of hand washing (26,27). Thus, research on the frequency of hand washing and its effect on prevention of disease is not consistent. Hence, the purpose of this

study was to conduct a systematic review to explore the relationships between the frequency of hand washing and the effect of disease prevention, and develop more comprehensive suggestions on the frequency of hand washing. We present the following article in accordance with the PRISMA reporting checklist (available at <http://dx.doi.org/10.21037/atm-20-6005>).

Methods

We pre-registered our meta-analysis at PROSPERO (CRD42020183002).

Search strategy

We searched the following electronic databases from their inception to April 11, 2020: Cochrane library, MEDLINE (via PubMed), Embase, Web of Science, China Biology Medicine disc (CBM), China National Knowledge Infrastructure (CNKI), and Wanfang Data. The WHO Clinical Trials Registry Platform, US National Institutes of Health Trials Register, the International Standard Randomized Controlled Trial Number [ISRCTN] Register, the official websites of the WHO and Centers for Disease Control (CDC), Google Scholar (first 100 records), and reference lists of relevant reviews were also scanned. Mesh terms included prevent*, safeguard, precaution, defense, prophylaxis, “prevention and control”, “preventive therapy”, “preventive measures”, handwashing*, “hand washing*”, hand-washing, hand-cleaning, “hand hygiene”, “wash* hand*”, “clean hand*”, frequency, times, seldom, always, and often. A full list of search terms can be found in [Appendix 1](#).

Eligibility criteria

A number of inclusion criteria were considered, including (I) randomized controlled trial (RCT), cohort study, case-control study, cross-sectional study; (II) participants were not limited by age, gender, race, or type of disease; (III) studies on hand washing frequency of participants and effect on disease prevention of participants. Studies were excluded based on the following criteria: (I) no clear frequency of hand washing; (II) duplicate studies; or (III) articles were written in languages other than English or Chinese; or (IV) abstracts, comment letters, or reviews.

The primary interest was the impact of frequency of hand washing on disease risk (defined as disease infection

risk, reinfection risk, or episodes risk). The secondary outcome of interest was the symptoms of the disease, or adverse effects.

Study selection

After removing those studies which were duplicates, two researchers independently selected the studies using Endnote. The first stage of screening was based on article titles and abstracts. Second, for articles with incomplete information and ambiguous titles, the full-text was downloaded and reviewed in order to determine inclusion. Inconsistent results were resolved through discussion or a third researcher was consulted if necessary. The detailed literature screening process is shown in *Figure 1*.

Data extraction

Two researchers extracted data independently with a pre-designed data collection form, which was reviewed, and disagreements were resolved by consensus or consulting a third researcher. Information extracted from the studies included: (I) basic information (title, first author, publication year, type of study, single-center or multi-center study, sample size, and disease); (II) participants (age and gender); (III) intervention (specific hand washing frequency); and (IV) results (the total number, mean, and standard deviation of disease cases or symptom occurrence).

Assessment of risk of bias

Two researchers independently assessed the potential bias in each included study. Discrepancies were resolved by discussion or with consensus from a third researcher. RCTs were assessed for bias according to the Cochrane risk-of-bias tool (RoB) (28) consisting of seven domains: random sequence generation, allocation concealment, blinding of participants and personnel, blinding of outcome assessment, incomplete outcome data, selective outcome reporting, and other bias. Each potential source of bias as was graded “Low”, “Unclear” or “High”. High risk of bias means the study is judged to be at high risk of bias in at least one domain for this result, or the study is judged to have some concerns for multiple domains in a way that substantially lowers confidence in the result. Cohort and case-control studies were assessed for the risk of design-specific bias using the relevant Newcastle-Ottawa Scale (NOS) (29), which consists of three aspects: the selection of exposure,

comparability, and assessment of outcome. The maximum score was nine, and scores of seven or more were graded as high quality while scores of less than seven were defined as low quality. For cross-sectional studies, the Agency for Healthcare Research and Quality Scale (AHQR) (30) was used to assess for bias. It consists of 11 items, that are answered with “Yes”, “No” or “Unclear”.

Statistical analysis

A meta-analysis of outcomes for the compatible data was conducted using Review Manager 5.3 software (a software was recommended by Cochrane Handbook for developing systematic reviews) (31), which means the frequency of hand washing included in the same range can be conducted meta-analysis. A descriptive analysis of outcome indicators that could not be combined quantitatively was also conducted. Meta-analyses of binary variables were performed using the Mantel-Haenszel method and continuous variables using the inverse variance method. Dichotomous data were evaluated using odds ratio (OR) or risk ratio (RR) with 95% confidence intervals (CI). Continuous data were analyzed using the weighted mean difference (WMD) with 95% CI. Two-sided P values of <0.05 were considered statistically significant. We assessed evidence of heterogeneity using the χ^2 test and I^2 statistic. $P < 0.10$ was considered to be consistent with statistically significant heterogeneity. A random-effects model was used for $I^2 > 50\%$ and a fixed effect model was used for $I^2 \leq 50\%$. We conducted a subgroup analysis according to the study design to explore the reasons for substantial heterogeneity. Publication bias was assessed by the Egger test.

Quality of evidence assessment

Two reviewers assessed the quality of main evidence independently using the Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool. A “Summary of Findings” table using the GRADEpro software (32) was created, which shows the overall grading of the body of evidence for each pre-specified outcome that was accounted for in the meta-analysis. Direct evidence from RCTs starts at high quality, and evidence from observational studies at low quality. The quality can be downgraded for five different reasons (study limitations, consistency of effect, imprecision, indirectness, and publication bias) and upgraded for three reasons (large magnitude of effect, dose-response relation, and plausible

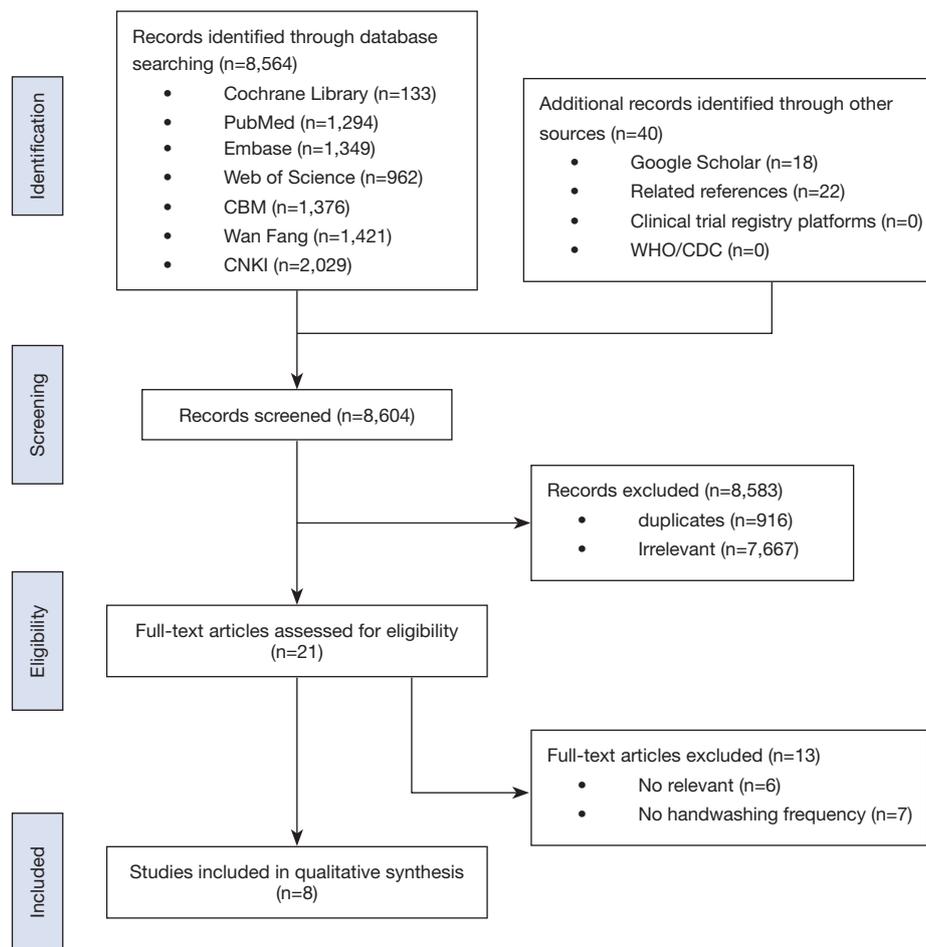


Figure 1 Flow diagram of the study selection process.

confounders or biases) (33-36). The quality of evidence for each outcome is classified as “high”, “moderate”, “low”, or “very low” reflecting to what extent we can be confident that the effect estimates are correct.

Results

Study selection and characteristics

Figure 1 shows the literature retrieval and screening process. We identified 8,604 studies, 916 duplicated studies and 7,667 irrelevant studies were excluded, leaving 21 potentially eligible studies to be reviewed. After reading the full-text, six studies with no relevant outcome indicators and seven studies that did not report hand-washing frequency were excluded. Finally, a total of eight studies were included in the meta-analysis (14,15,20,25-27,37,38). Table 1 shows the

included study characteristics of two RCTs (20,27), one cohort study (37), four case-control studies (14,15,25,26), and one cross-sectional study (38). Four studies (14,25-27) used hand washing 4 times per day as the cut-off value and two studies (14,15) used hand washing 10 times per day as the cut-off value. The study population in five of the studies was patients (14,15,20,25,26). The basic characteristics of included studies and baseline characteristics of participants of each included study is summarized in Tables 1 and 2.

The quality of included studies was assessed using the ROB, NOS and AHRQ (see Tables S1-S3). Overall, two and three items of the two RCTs were evaluated as “Low”, while the rest were “Unclear”. Two of the case-control studies were graded as eight stars, while two were seven stars, and one cohort study was also seven stars. Six items from the cross-sectional study were evaluated as “Yes”, two items were “Unclear”, and three items were classified

Table 1 Basic characteristics of the included studies

Study (publication year)	Study design	Country	Single/multi-center study	Sample	Hand washing frequency	Disease
Godoy 2012, (14)	Case-control	Spain	Multi-center	3,087	1–4, 5–10, >10 times/day	Influenza A
Lau 2004, (15)	Case-control	China	Single-center	990	1–10, >10 times/day	Acute respiratory tract infection, influenza-like illness
Merk 2014, (37)	Cohort	Sweden	Single-center	2,864	2–4, 5–9, 10–19, ≥20 times/day	SARS
Huang 2007, (20)	RCT	America	Single-center	148	4, 7 times/day	Diarrhea
Mangklakeree 2014, (27)	RCT	Thailand	Single-center	454	≤3, >3 times/day	Influenza-like illness
Torner 2015, (26)	Case-control	Spain	Multi-center	478	1–4, ≥5 times/day	Influenza
Thumma 2009, (38)	Cross-sectional	America	Single-center	462	3, 3–6, >6 times/day	Upper respiratory, gastrointestinal symptoms
Chen 2011, (25)	Case-control	China	Single-center	132	<4, ≥4 times/day	Influenza A

RCT, randomized controlled trial; SARS, severe acute respiratory syndrome.

Table 2 Baseline characteristics of participants

Study	Participants	Gender (male/female)	Age of test/control (years), (range or mean ± SD)	Test group (n)	Control group (n)
Godoy 2012, (14)	Patients and matching controls	1,507/1,580	38.48±22.79/39.13±22.68	813	2,274
Lau 2004, (15)	Patients and matching controls	475/515	≥16	330	660
Merk 2014, (37)	All	1,053/1,811	56.40/46.30	2,513	351
Huang 2007, (20)	AIDS patients	111/37	43.70±11.00/41.90±11.00	75	73
Mangklakeree 2014, (27)	Elementary school student	216/238	–	175	279
Torner 2015, (26)	Patients and matching controls	269/209	5.40±4.50/5.30±4.60	239	239
Thumma 2009, (38)	Freshman students	215/243	18.50	143	319
Chen 2011, (25)	All	–	–	35	97

SD, standard deviation; AIDS, acquired immune deficiency syndrome; –, unavailable.

as “No”. [Table S4](#) provides a summary of the quality of evidence for each outcome.

The results of hand washing frequency and disease prevention >4 times per day versus ≤4 times per day

Four studies (14,25–27) specifically considered the impact of washing your hands less than 4 times per day *vs.* washing

them more than 4 times per day (*Figure 2*). Heterogeneity test showed $I^2=85\%$, so the random-effects model was used. The meta-analysis results found no statistical significance of washing your hands more than 4 times or less than 4 times a day and respiratory disease prevention (OR =0.61, 95% CI: 0.37 to 1.01). When we performed a subgroup analysis by study design, a meta-analysis of three case-control studies (14,25,26) showed that hand washing more than

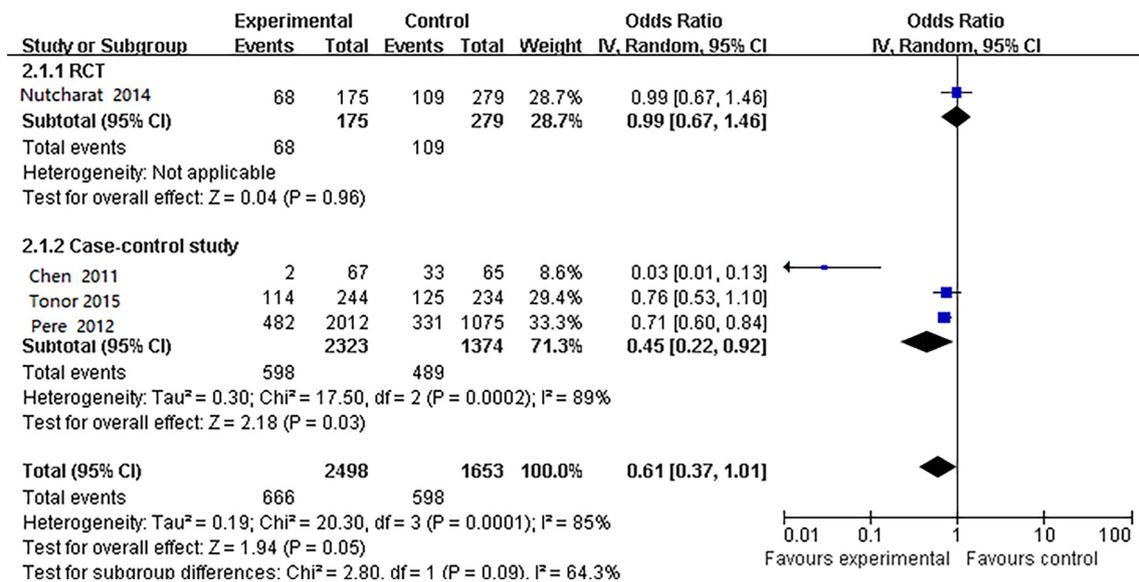


Figure 2 Comparison of the effect of hand washing over less than 4 times day *vs.* more than 4 times a day on disease prevention.

4 times per day may be a protective factor in preventing respiratory disease (OR =0.45, 95% CI: 0.22 to 0.92). The result of an RCT study (27) showed no statistical significance in the prevention effect (OR =0.99, 95% CI: 0.67 to 1.46) (Figure 2). Egger's test showed no publication bias (P=0.445), but the quality of evidence was graded low (Table S4).

Pairwise comparison of hand washing ≤4 times per day, 5–10 times per day, and >10 times per day

A case-control study showed (14) that compared to hand washing ≤4 times/day, washing hands 5–10 times per day and >10 times per day could reduce the risk at the most influenza A infection (OR =0.75, 95% CI: 0.63 to 0.91; OR =0.65, 95% CI: 0.53 to 0.80, respectively). Hand washing 10 times a day may be better at preventing infection, there was no statistical significance between hand washing 10 times per day and 5–10 times per day (OR =0.86, 95% CI: 0.70 to 1.06) (Figure 3). The quality of evidence was graded low (Table S4).

>10 times per day versus ≤10 times per day

Two case-control studies (14,15) compared the effect of hand washing more than 10 times per day to less than 10 times per day on respiratory disease prevention. The heterogeneity test showed that $I^2=86%$, so the random-

effects model was used. The results of the meta-analysis showed that compared with hand washing ≤10 times/day, hand washing >10 times/day was a protective factor against respiratory disease infection (OR =0.59, 95% CI: 0.36 to 0.97) (Figure 4). The quality of evidence was graded low (Table S4).

7 times per day versus 4 times per day

A randomized controlled trial study (20) showed that patients assigned to the intensive hand washing intervention group washed hands more frequently compared with the control group (7 versus 4 times a day, respectively) and developed fewer episodes of diarrheal illness (WMD =-1.68, 95% CI: -1.93 to -1.43) (Figure S1). The quality of evidence was graded low (Table S4).

Comparison of other hand washing frequency

A cohort study (37) showed that there were no significant decreases in acute respiratory tract infection (ARI) rates and influenza-like illness among adults with increased frequency of daily hand washing after adjusting for age, vaccination status, gender, educational level, occupational status, household size, and child contact. Comparing washing hands 2, 3 or 4 times per day, washing hands 5–9 times per day was associated with an adjusted ARI rates RR=1.08, 95% CI 0.87 to 1.33; 10–19 times with adjusted RR =1.22,

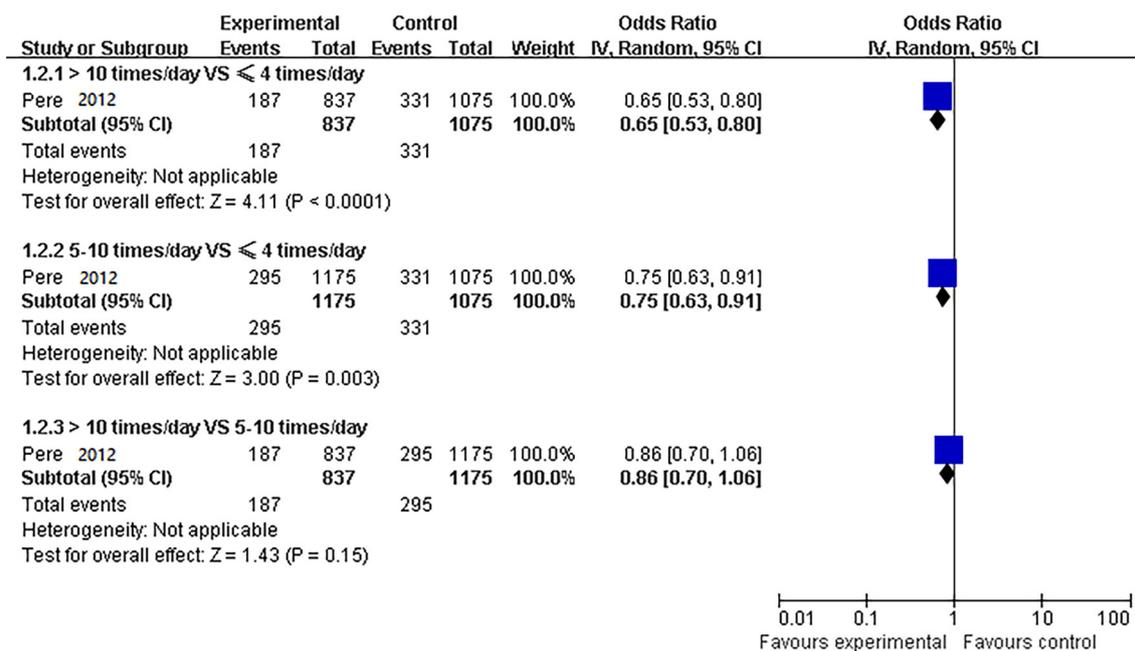


Figure 3 Comparison of different hand washing frequency for disease prevention.

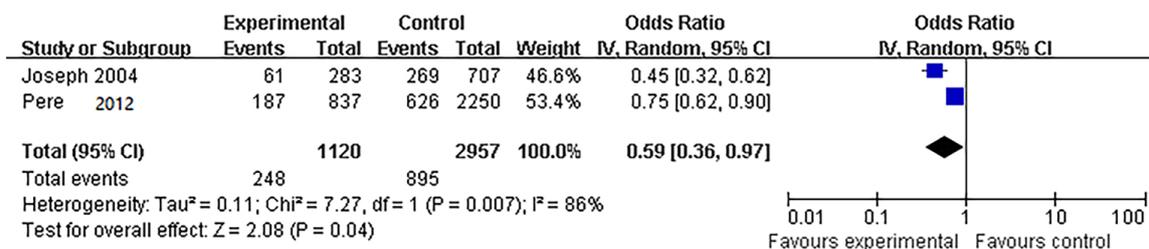


Figure 4 Comparison of the effect of hand washing more than 10 times per day on the prevention of respiratory diseases.

95% CI: 0.97 to 1.53; and ≥20 times with adjusted RR=1.03, 95% CI: 0.81 to 1.32. Washing hands 5–9 times per day was associated with an adjusted Influenza-like illness RR =0.98, 95% CI: 0.66 to 1.46; 10–19 times with adjusted RR =1.25, 95% CI: 0.82 to 1.90; and ≥20 times with adjusted RR =1.06, 95% CI: 0.68 to 1.67. The quality of evidence was graded low (Table S4).

A cross-sectional study (38) showed no significant associations were found between hand washing frequency (≤3, 3–6 and ≥6 times per day) and developing gastrointestinal or upper respiratory symptoms (runny nose adjusted OR =0.97, 95% CI: 0.64 to 1.49; itchy eyes adjusted OR =0.97, 95% CI: 0.53 to 1.79; fever adjusted OR =0.61, 95% CI: 0.25 to 1.47; Cough adjusted OR =0.98,

95% CI: 0.65 to 1.50; sore throat adjusted OR =1.42, 95% CI: 0.93 to 2.16; Earache adjusted OR =1.42, 95% CI: 0.66 to 3.02; Vomiting adjusted OR =0.85, 95% CI: 0.34 to 2.10; Diarrhea adjusted OR =1.06, 95% CI: 0.66 to 1.73). The quality of evidence was graded low (Table S4).

Discussion

A total of eight studies were included in this systematic review, when carefully analyzed showed generally no statistically significant difference in the impact of frequency of hand washing on disease occurrence or symptoms, although hand washing more than 4 times per day may be better than hand washing less than 4 times per day.

A few low-quality pieces of evidence showed that hand washing more than 10 times per day could reduce the risk of respiratory and gastrointestinal diseases better than hand washing less than 10 times per day.

According to the results of this meta-analysis, hand washing more than 10 times per day can effectively reduce the risk of respiratory disease infection compared to hand washing less than 10 times per day, as determined in another study (39). Similar results have been observed in a previous systematic review, which pooled reviews from six case-control studies on varying public health interventions. The results showed that hand washing more than 10 times per day could effectively reduce the risk of the transmission of SARS (40). As new research has been published over the past decade, this issue needs to be reassessed. Additionally, as seen in the findings of this study, hand washing more than 10 times per day was better than more than 4 times per day in the prevention of the transmission of respiratory diseases, and the difference was found to be statistically significant. In other words, to some extent, it may be because insufficient hand washing frequency every day can't ensure the reducing the risk of diseases at critical moments. These critical moments include eating, inserting or removing contact lenses, preparing food, using the toilet, changing diapers or cleaning up children who have used the toilet, blowing your nose, coughing or snoring, contacting animals and their feed or waste, disposing of garbage, handling pet food, treating wounds or caring for patients and obviously dirty hands (41,42).

Some studies have shown that hand hygiene is more effective in reducing gastrointestinal diseases than in preventing respiratory diseases (43,44). Huang *et al.*, for example, showed that hand washing 7 times per day versus 4 times per day resulted in fewer episodes of diarrheal illness (20). However, the study only included one gastrointestinal disease. Three studies showed no difference between different hand washing frequencies and the reducing the risk of respiratory diseases (27,37,38). Two possible reasons may account for this result. On one hand, hand hygiene may have different effects on the prevention of different diseases. Multiple transmission routes of respiratory infections, for example, may mean that frequent hand washing may be ineffective. On the other hand, participants were students or adults in these three studies. However, in five other studies (14,15,20,25,26), participants were patients and were matched to controls showed the opposite results. Therefore, hand washing frequency may have different effects on disease prevention for different groups.

The hand washing frequency may be affected by many factors, such as gender (38,45-47), age (45), economic conditions (48,49), education level (45,49), handwashing compliance (50,51) and religious culture (52) etc. Being a female, middle-aged and having tertiary education level can effectively improve hand hygiene behavior (45). Females may be more likely to wash hands compared to males (38,46,47). The data shows that after exposure to pollutants, handwashing frequency in high-income countries is higher (48-72%), while that in low-income countries is only 5-25% (48). The low compliance of medical staff and the general public (22-59.6%) can explain low handwashing frequency to some degree (50,51).

The compliance of hand hygiene is influenced by the expected results of preventive measures, the perception of the ability of hand hygiene, and habit (52). A clear description of the effectiveness of protective actions helps the public actively prevent pandemics (53). A systematic review showed that setting long-term hand washing reminders or regular repeated feedback can improve the enthusiasm of medical staff to wash their hands (54). The implementation of health education to promote hand hygiene can effectively support children, medical staff, and patients to improve their hand washing frequency and technique (55-60). At the same time, improvements in hand washing facilities can also increase the frequency of hand washing, such as the availability of soap or hand sanitizer, as well as a clean napkin to dry hands (41,42). Based on these differences, health workers need to develop a detailed education plan to improve people's enthusiasm for handwashing, so as to increase the handwashing frequency. These can refer to the WHO and the CDC in their hand hygiene guidelines and website materials and the need for the user (e.g., hospital) to adapt it to their culture and think of hand hygiene as a whole system (41,42).

Strengths and limitations

This study is the first systematic review to specifically explore different frequencies of hand washing on the prevention of certain diseases, which provides evidence for recommendations on the most advantageous number of times to wash hands in a day and also provides directional guidance for future research. However, this study still has its limitations. Currently, there are few high-quality studies on hand washing frequency and disease prevention. Thus, the overall number of studies included in the analysis was small. Additionally, the impact of hand washing is influenced by

many confounding factors (such as the standardization of hand washing methods, the cleaning agents used and their formulation, dose, delivery system, wash time, religious culture, and the risk of disease exposure in the population, etc.), which may reduce the reliability of the results.

It is recommended that future large-scale, high-quality hand washing frequency studies be conducted among different populations and different diseases. Besides, for the same population, the same disease prevention test should verify whether there is a certain frequency range relationship between the effect of handwashing frequency on disease prevention.

Conclusions

The results of this study indicate that the higher the frequency of hand washing, the better the effect on disease prevention. No high-quality evidence, however, indicates the best range of hand washing frequency for disease prevention. Hand hygiene is one of the basic components of infection control procedures, as washing hands frequently is a low-cost and high-efficacy health measure. Health promotion efforts should aim to expand hand hygiene education, increase public enthusiasm for washing hands, and improve health.

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Footnote

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Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at <http://dx.doi.org/10.21037/atm-20-6005>). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Appendix 1 Search strategy

PubMed (N=1294) (<https://pubmed.ncbi.nlm.nih.gov/>)

- #1. "Preventive Health Services"[Mesh]
- #2. "Preventive Medicine"[Mesh]
- #3. "prevention and control"[Subheading]
- #4. "preventive therapy"[Title/Abstract]
- #5. "preventive measures"[Title/Abstract]
- #6. Prevent*[Title/Abstract]
- #7. safeguard[Title/Abstract]
- #8. precaution[Title/Abstract]
- #9. defense[Title/Abstract]
- #10. prophylaxis[Title/Abstract]
- #11. "prevention and control"[Title/Abstract]
- #12. #1-#11/OR
- #13. "Handwashing"[Mesh]
- #14. "hand washing*" [Title/Abstract]
- #15. "hand-washing" [Title/Abstract]
- #16. "hand-cleaning" [Title/Abstract]
- #17. "hand hygiene" [Title/Abstract]
- #18. "wash* hand*" [Title/Abstract]
- #19. "clean hand*" [Title/Abstract]
- #20. #13-#19/OR
- #21. Frequency [All fields]
- #22. Times[All fields]
- #23. Seldom[All fields]
- #24. Always[All fields]
- #25. often[All fields]
- #26. #21-#25/OR
- #27. #12 AND #20 AND #26

Cochrane library (N=133) (<https://www.cochranelibrary.com/>)

- #1. MeSH descriptor: [Preventive Health Services] explode all trees
- #2. MeSH descriptor: [Preventive Medicine] explode all trees
- #3. Prevent*:ti,ab,kw
- #4. safeguard:ti,ab,kw
- #5. precaution:ti,ab,kw
- #6. defense:ti,ab,kw
- #7. prophylaxis:ti,ab,kw
- #8. "prevention and control":ti,ab,kw
- #9. "preventive therapy":ti,ab,kw
- #10. "preventive measures":ti,ab,kw
- #11. #1-#10/OR
- #12. MeSH descriptor: [hand hygiene] explode all trees

- #13. "hand washing*":ti,ab,kw
- #14. "hand-washing":ti,ab,kw
- #15. "hand-cleaning":ti,ab,kw
- #16. "wash* hand*":ti,ab,kw
- #17. "clean hand*":ti,ab,kw
- #18. #12-#17/OR
- #19. Frequency
- #20. Times
- #21. seldom
- #22. always
- #23. often
- #24. #19-#23/OR
- #25. #11 AND #18 AND #24

Web of Science (N=962) (<https://apps.webofknowledge.com/>)

- #1. TOPIC: "Prevent*"
- #2. TOPIC: "safeguard"
- #3. TOPIC: "precaution"
- #4. TOPIC: "defense"
- #5. TOPIC: "prophylaxis"
- #6. TOPIC: "prevention and control"
- #7. TOPIC: "preventive therapy"
- #8. TOPIC: "preventive measures"
- #9. #1-#8/OR
- #10. TOPIC: "Handwashing*"
- #11. TOPIC: "hand washing*"
- #12. TOPIC: "hand-washing"
- #13. TOPIC: "hand-cleaning"
- #14. TOPIC: "hand hygiene"
- #15. TOPIC: "wash* hand*"
- #16. TOPIC: "clean hand*"
- #17. #10-#16/OR
- #18. All fields: "Frequency"
- #19. All fields: "times"
- #20. All fields: "seldom"
- #21. All fields: "always"
- #22. All fields: "often"
- #23. #18-#22/OR
- #24. #9 AND #17 AND #23

Embase (N=1349) (<https://www.embase.com/>)

- #1. 'prevention and control'/exp
- #2. 'defense'/exp
- #3. 'prophylaxis'/exp
- #4. 'Prevent*':ab,ti

- #5. 'safeguard ':ab,ti
- #6. 'precaution ':ab,ti
- #7. 'preventive therapy':ab,ti
- #8. 'preventive measures':ab,ti
- #9. #1-#8/OR
- #10. 'hand washing'/exp
- #11. 'handwashing*':ab,ti
- #12. 'hand washing*':ab,ti
- #13. 'hand-washing':ab,ti
- #14. 'hand-cleaning':ab,ti
- #15. 'hand hygiene':ab,ti
- #16. 'wash* hand*':ab,ti
- #17. 'clean hand*':ab,ti
- #18. #10-#17/OR
- #19. frequency
- #20. times
- #21. seldom
- #22. always
- #23. often
- #24. #19-#23/OR
- #25. #9 AND #18 AND #24

CBM (N=1376) (<http://www.sinomed.ac.cn/>)

- #1. " 预防 "[常用字段：智能]
- #2. " 防控 "[常用字段：智能]
- #3. " 防护 "[常用字段：智能]
- #4. " 防治 "[常用字段：智能]
- #5. " 防患 "[常用字段：智能]
- #6. " 防御 "[常用字段：智能]
- #7. " 防卫 "[常用字段：智能]
- #8. " 防范 "[常用字段：智能]
- #9. " 公共卫生 "[不加权：扩展]
- #10. " 预防卫生服务 "[不加权：扩展]
- #11. #1-#10/OR
- #12. " 手卫生 "[不加权：扩展]
- #13. " 洗手 "[常用字段：智能]
- #14. " 手部清洁 "[常用字段：智能]
- #15. #12-#14/OR
- #16. " 次 "[全部字段：智能]
- #17. " 频率 "[全部字段：智能]
- #18. #16 OR #17

- #19. #11 AND #15 AND #18

Wan Fang (N=1421) (<http://www.wanfangdata.com.cn/>)

- #1. " 预防 "[主题]
- #2. " 防控 "[主题]
- #3. " 防护 "[主题]
- #4. " 防治 "[主题]
- #5. " 防患 "[主题]
- #6. " 防御 "[主题]
- #7. " 防卫 "[主题]
- #8. " 防范 "[主题]
- #9. #1-#8/OR
- #10. " 洗手 "[主题]
- #11. " 手卫生 "[主题]
- #12. " 手部清洁 "[主题]
- #13. #10-#12/OR
- #14. " 次 "[全部]
- #15. " 频率 "[全部]
- #16. #14 OR #15
- #17. #9 AND #13 AND #16

CNKI (N=2029) (<https://www.cnki.net/>)

- #1. 预防 [主题]
- #2. 防控 [主题]
- #3. 防护 [主题]
- #4. 防治 [主题]
- #5. 防患 [主题]
- #6. 防御 [主题]
- #7. 防卫 [主题]
- #8. 防范 [主题]
- #9. #1-#8/OR
- #10. 洗手 [主题]
- #11. 手卫生 [主题]
- #12. 手部清洁 [主题]
- #13. #10-#12/OR
- #14. 次 [全文]
- #15. 频率 [全文]
- #16. #14 OR #15
- #17. #9 AND #13 AND #16

Table S1 The risk of bias of randomized controlled trial

Study	Bias domains included in the Cochrane risk-of-bias					
	Random sequence generation	Allocation concealment	Blinding of participants and personnel; Blinding of outcome assessment	Incomplete outcome data. Assessments should be made for each main outcome (or class of outcomes)	Selective reporting	Other sources of bias
Huang 2007, (20)	Low	Unclear	Unclear	Low	Unclear	Unclear
Mangklakeree 2014, (27)	Low	Unclear	Unclear	Low	Unclear	Low

Table S2 The risk of bias of cross-sectional studies

Study	Bias domains include in Agency for Healthcare Research and Quality (AHRQ)										
	Define the source of information (survey, record review)	List inclusion and exclusion criteria for exposed and unexposed subjects (case and controls) or refer to previous publications	Indicate time period used for identifying patients	Indicate whether or not subjects were consecutive if not population-based	Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants	Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements)	Explain any patient exclusions from analysis	Describe how confounding was assessed and/or controlled.	If applicable, explain how missing data were handled in the analysis	Summarize patient response rates and completeness data collection	Clarify what follow-up, if any, was expected and the percentage of patients for which in complete data or follow-up was obtained
Thumma 2009, (38)	Yes	Yes	No	Yes	Unclear	Unclear	Yes	Yes	Yes	No	No

Table S3 The risk of bias of cohort and case-control studies

Study	Bias domains include in Newcastle-Ottawa Quality Assessment Scale										Total points
	Selection			Comparability				Exposure			
	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Study controls for--select the most important factor	Study controls for any additional factor (the criteria could be modified to indicate specific control for a second important factor)	Ascertainment of expose	Same method of ascertainment for case and controls	Non-Response rate		
Godoy 2012, (14)	★	★	★	★	★	★	★	★	★	..	8★
Lau 2004, (15)	★	★	★	★	★	..	★	★	7★
Torner 2015, (26)	★	★	★	★	★	★	..	★	★	★	8★
Chen 2011, (25)	★	★	..	★	★	★	..	★	★	★	7★
Merk 2014, (37)	★	..	★	..	★	★	★	★	★	★	7★

Newcastle-Ottawa Scale is a 'star system' and a star (★) means that the content of the study matches this item.

Table S4 GRADE evidence profile

No. of studies	Certainty assessment					No. of patients			Effect value (95% CI)	Certainty
	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	Sample	Intervention	Control		
Prevent infections from diseases										
≤4 times/day vs. >4 times/day										
RCT (1)	Serious ¹	Serious ²	Not serious	Not serious	None	454	175	279	OR 0.99; (0.67 to 1.46)	⊕⊕○○ LOW
CCS (3)	Not serious	Serious ²	Not serious	Not serious	None	3,697	1,087	2,610	OR 0.45; (0.22 to 0.92)	⊕⊕○○ LOW
≤4 times/day vs. 5-10 times/day										
CCS (1)	Serious ¹	Not serious	Not serious	Not serious	None	2,250	626	1,624	OR 0.75; (0.63 to 0.91)	⊕⊕○○ LOW
≤4 times/day vs. >10 times/day										
CCS (1)	Serious ¹	Not serious	Not serious	Not serious	None	1,912	518	1,394	OR 0.65; (0.53 to 0.80)	⊕⊕○○ LOW
5-10 times/day vs. >10 times/day										
CCS (1)	Serious ¹	Not serious	Not serious	Not serious	None	2,012	482	15,30	OR 0.86; (0.70 to 1.06)	⊕⊕○○ LOW
≤10 times/day vs. >10 times/day										
CCS (2)	Serious ¹	Serious ²	Not serious	Not serious	None	4,077	1,143	2,934	OR 0.59; (0.36 to 0.97)	⊕⊕○○ LOW
2-4 times/day vs. 5-9 times/day										
CS (1)	Not serious	Not serious	Not serious	Not serious	None	1,532	351	1,181	RR 1.08; (0.87 to 1.33)	⊕⊕○○ LOW
2-4 times/day vs. 10-19 times/day										
CS (1)	Not serious	Not serious	Not serious	Not serious	None	1,119	351	768	RR 1.22; (0.97 to 1.53)	⊕⊕○○ LOW
2-4 times/day vs. ≥20 times										
CS (1)	Not serious	Not serious	Not serious	Not serious	None	915	351	564	RR 1.03; (0.81 to 1.32)	⊕⊕○○ LOW
Episodes of diarrheal illness										
4 times/day vs. 7 times/day										
RCT (1)	Serious ¹	Not serious	Serious ³	Not serious	None	148	75	73	WMD -1.68; (-1.93 to -1.43)	⊕⊕○○ LOW

¹, downgrade one level: the risk of bias is high due to the limitations of study design; ², downgrade one level: heterogeneity of data synthesis results, $I^2 > 50\%$; ³, downgrade one level: population (AIDS patients) is indirect evidence. CI, confidence interval; OR, odd ratio; RR, risk ratio; WMD, weighted mean difference; RCT, randomized controlled trial; CCS, case-control study; CS, cohort study.

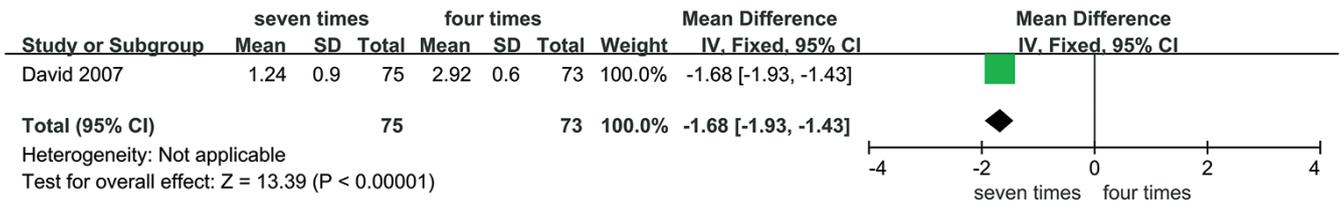


Figure S1 Comparison of the effect of hand washing 7 vs. 4 times per day on the prevention of diarrhea.