

**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

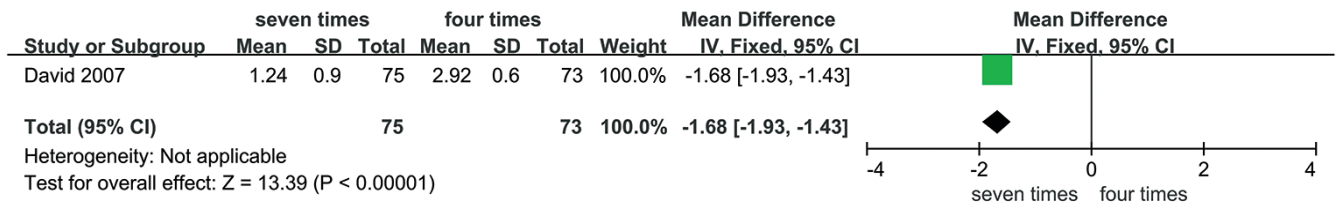
Study	Bias domains include in Newcastle-Ottawa Quality Assessment Scale										Total points
	Selection			Comparability				Exposure			
	Is the case definition adequate?	Representativeness of the cases	Selection of controls	Definition of controls	Study controls for--select the most important factor	Study 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 <sup>1</sup>	Serious <sup>2</sup>	Not serious	Not serious	None	454	175	279	OR 0.99; (0.67 to 1.46)	⊕⊕○○ LOW
CCS (3)	Not serious	Serious <sup>2</sup>	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 <sup>1</sup>	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 <sup>1</sup>	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 <sup>1</sup>	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 <sup>1</sup>	Serious <sup>2</sup>	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 <sup>1</sup>	Not serious	Serious <sup>3</sup>	Not serious	None	148	75	73	WMD -1.68; (-1.93 to -1.43)	⊕⊕○○ LOW

<sup>1</sup>, downgrade one level: the risk of bias is high due to the limitations of study design; <sup>2</sup>, downgrade one level: heterogeneity of data synthesis results,  $I^2 > 50\%$ ; <sup>3</sup>, 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.