UK Health Security Agency

Effectiveness of hand hygiene agents as alternatives to soap and water to reduce *Clostridioides difficile* in health and social care settings where care is provided

A rapid systematic review

Contents

Main messages

1. This rapid systematic review (search from 1 January 1990 to 12 December 2025) identified and summarised evidence relating to the effectiveness of hand hygiene agents, as alternatives to soap and water, to reduce transmission of *Clostridioides difficile* (*C. difficile*) in health and social care settings where care is given.

2. Two randomised cross-over studies (1, 2) and one non-randomised cross-over study (3) were included.

3. Two studies were conducted in the United States of America (USA) (1, 3) and one study was conducted in Canada (2). No studies were identified in health and social care settings, the only available evidence was from laboratories of hospitals or health centres. All studies involved healthy volunteers, without active *C. difficile* infection whose hands were experimentally contaminated with *C. difficile* spores.

4. One study evaluated the effectiveness of a hypochlorous acid containing hand hygiene agent (Vashe ®) compared to soap and water (1). The study reported that soaking hands in Vashe solution soak for one minute was significantly more effective at reducing *C. difficile* spores compared to a soap and water soak and to a soap and water handwash.

5. Two studies evaluated the effectiveness of liquid chlorhexidine soap compared to plain soap and water (2) (3). There was no clear consistent difference between liquid chlorhexidine soap and plain soap and water in reducing *C. difficile* spores in either study, although findings from one study suggested that plain soap and water was more effective (2).

6. The effectiveness of antiseptic parachlorometaxylenol wipes compared to soap and water was evaluated by one study (2). The study reported plain soap removed more *C. difficile* spores than antiseptic parachlorometaxylenol hand wipes, regardless of water temperature.

7. The evidence for outcomes that could be assessed using a modified GRADE approach was rated as very low certainty (2), and where it could not be assessed, was considered moderate to high risk of bias (1, 3). Evidence was downgraded for risk of bias as participant characteristics were not reported and there was no blinding to the intervention given. All evidence was downgraded for indirectness as the population was healthy volunteers in a laboratory of a hospital and the *C. difficile* strain used was nontoxigenic. There was also some imprecision in the results due to wide confidence intervals which crossed the option of no difference between interventions in some cases. This may be due to the small sample size.

8. In conclusion, only one study suggested that an alternative hand hygiene agent to soap and water – a hypochlorous acid-based agent – reduced *C. difficile* spores on hands more effectively. Other evidence suggested chlorhexidine soap may be as effective as soap and water, but antiseptic parachlorometaxylenol hand wipes were less effective. All evidence was from studies with small sample sizes and considered very low certainty or moderate to high risk of bias. The available evidence does not provide conclusive evidence that there is a more effective hand hygiene agent as an alternative to soap and water to reduce the transmission of *C. difficile* in settings where care is provided.

Purpose

The purpose of this rapid systematic review was to identify and summarise the available evidence on the effectiveness of hypochlorous acid or chlorine containing hand hygiene agents as alternatives to soap and water, to reduce the transmission of *C. difficile* in health and social care settings.

The review question was:

1. What is the effectiveness of hand hygiene agents, as alternatives to soap and water, to reduce transmission of *C. difficile* in health and social care settings where care is given?

This rapid systematic review was commissioned to help inform the update of UKHSA guidance on *C. difficile* infection: how to deal with the problem.

Methods

A rapid systematic review was conducted, following streamlined systematic methods to accelerate the review process and provide a timely, evidence-informed answer to the review question without compromising the rigour of the review process.

A literature search was undertaken to look for relevant high quality systematic reviews, randomised-controlled trials, quasi-experimental studies, cross-over studies, mixed methods studies, cohort studies, before-and-after studies, and letters to the editor (if they report trial results that met the review protocol's eligibility criteria and provided sufficient detail for assessment), published or available as preprint, from 1 January 1990 up to 12 December 2025. Four databases were searched. Backwards and forwards citation searching of primary studies was also performed. References that were included following full text screening were used as seed references.

The following settings, specified by subject matter experts, were agreed of interest for this review: any settings where care is given (defined as care administered by health or social

care professionals, not care of dependents). This included hospitals, care homes with or without nursing, adult social care settings and other residential settings, outpatient and community care settings, mental health facilities and healthcare facilities in prisons.

The interventions of interest for this review were hypochlorous acid or chlorine containing hand hygiene agents. These were identified by topic experts as having potential activity against *C. difficile* spores and therefore their effectiveness is of interest when considering alternatives to soap and water. The outcomes of interest for this review were effectiveness of hand hygiene agents measured by: *C. difficile* incidence rate, *C. difficile* transmission rate, secondary *C. difficile* infection, or environmental outcomes from clinical settings (spore recovery from hands).

A protocol was produced before the literature search was conducted, including the review question, the eligibility criteria, and all other methods. Full details of the methodology are provided in the protocol in Annexe A. The UKHSA health equity checklist for non-equity-focused reviews was implemented to ensure that health equity considerations were embedded throughout the review process (4).The protocol was reviewed by the UKHSA ‘How to deal with the problem' working group 2025 and was also prospectively published on PROSPERO (an international database for the prospective registration of systematic reviews) registration number: CRD420251268263. One protocol deviation was made to amend the inclusion criteria to include laboratory settings of hospitals or health centres. This was due to an absence of evidence identified from settings where care was given.

Screening on title and abstract was undertaken in duplicate by 2 reviewers for 20% of the eligible studies, with the remainder completed by one reviewer. Screening on full text was undertaken by one reviewer and checked by a second. Data extraction was performed by one reviewer and checked by a second. Data presented in figures was extracted using the linear scale of Plot digitizer.

Risk of bias assessment was conducted in duplicate by 2 reviewers using the JBI tools for randomised controlled trials and quasi-experimental studies (5).

The certainty of evidence identified in this review was assessed across studies by outcome where appropriate, using a modified version of the GRADE approach (6). This process is described in detail in Annexe A. In brief, the certainty of evidence was assessed for each outcome across 4 domains:

Limitation in study design and execution

1. Risk of bias: where results may not represent the true effect because of limitations in the design or conduct of the study.
2. Inconsistency: where studies show different effects for the same outcome.
3. Indirectness: where elements of the study differ from the review question.

4. Imprecision: a measure of how uncertain the result is.

Outcomes were given one of 4 ratings for certainty of evidence:

- very low (the true effect is probably different from the estimated effect)
- low (the true effect might be different from the estimated effect)
- moderate (the true effect is probably close to the estimated effect)
- high (the authors are confident that the true effect is similar to the estimated effect)

Outcomes from studies that were not similar enough to combine were assessed individually. In these cases, inconsistency was not assessed. GRADE was not applied where there was no measure of variability reported with the outcome for a single study (for example, confidence intervals), as imprecision could not be assessed (risk of bias and indirectness alone are insufficient to effectively use GRADE).

Evidence

In total, 3,267 studies were screened at title and abstract and 29 studies were identified to be screened at full text. However, the full text of one study was not retrieved as it was identified to be a clinical trial registry entry, without any published results, and therefore, 28 studies were screened at full text. Of these, 3 studies met the inclusion criteria.

A PRISMA diagram showing the flow of studies through the review is shown in Annexe B, and studies excluded at full text screening are available with the reasons why in Annexe C. Study characteristics are available in Annexe D, risk of bias assessments are available in Annexe E, and GRADE assessments are available in Annexe F.

Two studies were randomised cross-over studies (1, 2). One study followed a quasi-experimental design (where participants were assigned to interventions without randomisation) (3). Two studies were conducted in the USA (1, 3) and one was conducted in Canada (2).

Although the review intended to identify evidence from hospital clinical settings, care homes (with or without nursing), adult social care, outpatient and community care, mental health facilities and prisons, the only available evidence was from laboratory settings of hospitals or health centres. In these studies, the hands of healthy volunteers were experimentally contaminated by *C. difficile* spores, without causing active infection.

One study tested hand hygiene agents containing hypochlorous acid as the active ingredient (Vashe solution soak and Vashe wipes) (1). One study looked at liquid chlorhexidine gluconate in alcohol (Hibiclens ®) (3) and one study looked at antiseptic parachlorometaxylenol hand wipes (Sani-dex ® antimicrobial hand wipes) and chlorhexidine

foaming liquid skin cleanser soap (Hygienepak ®) (2). Although chlorhexidine and parachlorometaxylenol are not chlorine-based hand hygiene agents, these studies were included following discussion with the review commissioners because their chemical structures contain chlorine and they are routinely used as antiseptics.

Randomised cross-over studies

Nerandzic Michelle M and others conducted a randomised cross-over study in the USA to evaluate the effectiveness of a hypochlorous acid-based hand hygiene agent compared with soap and water for the reduction of *C. difficile* spores on hands (1). The study included 6 healthy adult volunteers, who were laboratory staff without any skin disease.

The study compared 2 different hand hygiene interventions with a soap and water soak or a soap and water handwash. The interventions were:

- soaking hands in Vashe solution (hypochlorous acid solution, concentration not reported)
- Vashe wipes (terry cloth wipes soaked in Vashe)

Hands of participants were experimentally contaminated with approximately 6 $\log_{10}$ colony forming units (CFU) of non-toxigenic *C. difficile* spores, alongside other bacterial spores. The liquid containing the *C. difficile* spores was poured into the study participants' cupped hands, and they rubbed it over their hands up to the wrists for 20 seconds. Participants then let their hands air-dry for 30 seconds. The contamination process was repeated 3 times.

The hand hygiene interventions were performed according to a standardised protocol, under study supervision. Hands were immersed in one gallon of Vashe solution soak for one minute and massaged vigorously. Hand hygiene with Vashe wipes involved thoroughly wiping the hands with terry cloth soaked in Vashe solution for 20 seconds.

Soap and water handwashing involved using 2mL of 0.05% triclosan liquid soap (hands rubbed with liquid soap for 20 seconds, rinsed with water and patted dry) and a soap and water soak involved hands being immersed in 1 gallon of water containing 10mL of 0.5% triclosan liquid soap and massaged vigorously for one minute. The washout period or decontamination procedure between hand hygiene interventions were not reported.

Hand contamination levels were measured at baseline and after the hand hygiene intervention. However, the study only reported results for the comparisons between soaking hands in Vashe solution and a soap and water handwash or a soap and water soak. No results were reported for the Vashe wipes compared to soap and water handwash or soap and water soak.

Soaking hands in Vashe solution significantly reduced spore count by 0.5 to 0.8 log_{10} CFU more than soap and water handwashing (p value <0.01). When compared to soap and water soak, Vashe solution soak significantly reduced spores by 0.5 to 1.2 log_{10} CFU more than soap and water (p value <0.01).

The certainty of evidence could not be assessed because the study did not report any measure of variability. Risk of bias assessment indicated that the study was at moderate to high of bias. The sample size was very small, only including 6 participants. Participant characteristics were not reported. Neither the participants nor those delivering the hand hygiene interventions were blinded to the intervention received and although this was considered less likely to influence the effectiveness of the hand hygiene agents, it could still lead to potential bias. Although not part of the risk of bias assessment, it is also important to note the population was laboratory staff, and the *C. difficile* strain used was nontoxigenic.

Another randomised cross-over study conducted in Canada between June and July 2007, compared 2 hand hygiene interventions to handwashing with soap and water for removal of *C. difficile* spores from hands (2). The study included 10 healthy adult volunteers, who were hospital laboratory workers without any skin disease.

The study compared 2 hand hygiene interventions to each other, or hand washing with plain soap (Hygenipak Instafoam) with warm (30 degrees Celsius) or cold (15 degrees Celsius) water. The interventions included:

- antiseptic parachlorometaxylenol hand wipes, containing 0.5% parachlorometaxylenol and 40% vol/vol ethanol (Sani-dex antimicrobial hand wipes)
- chlorhexidine antibacterial soap (Hygenipak foaming skin cleanser containing 2% chlorhexidine)

Hands of participants were experimentally contaminated with a suspension of non-toxigenic *C. difficile* spores at 1.4 x 10^5 CFU/mL, with 62% of the bacteria in spore form. Hand hygiene interventions were performed according to a standardised protocol, under study supervision. Handwashing with chlorhexidine antibacterial soap or plain soap included lathering hands for 10 seconds followed by rinsing with tap water and drying with paper towel. Hand hygiene with antiseptic parachlorometaxylenol hand wipes included wiping hands for 15 seconds followed by one minute of air-drying. Each participant had a gap of at least 24 hours between interventions to reduce carryover of handwashing agents or bacteria and hands were experimentally re-contaminated with *C. difficile* spores before switching to the next intervention.

The mean difference in reductions in *C. difficile* colony counts comparing hand hygiene interventions on whole hand and the palm are reported in Table 1.

Table 1. Mean difference in reductions in *C. difficile* colony counts following hand hygiene interventions

Comparison of hand hygiene interventions	Whole hand mean difference in reduction *C. difficile* colony counts (log_{10} CFU/mL)	95% CI	Palm mean difference in reduction (log_{10} CFU/plate)	95% CI
Plain soap and warm water versus antiseptic parachlorometaxylenol hand wipes	1.57	1.18 to 1.96	4.71	2.94 to 6.50
Plain soap and cold water versus antiseptic parachlorometaxylenol hand wipes	1.31	0.92 to 1.71	4.64	2.85 to 6.45
Plain soap and warm water versus chlorhexidine antibacterial soap and warm water	0.63	0.23 to 1.02	0.01	−1.77 to 1.83
Plain soap and cold water versus chlorhexidine antibacterial soap and warm water	0.37	-0.03 to 0.76	0.08	-1.74 to 1.90
Chlorhexidine antibacterial soap and warm water versus antiseptic parachlorometaxylenol hand wipes	0.94	0.55 to 1.34	4.72	2.59 to 6.59

Both plain soap with either warm or cold water removed more *C. difficile* than antiseptic parachlorometaxylenol hand wipes on both the whole hand and the palm.

Plain soap and warm water removed more *C. difficile* than chlorhexidine antibacterial soap on the whole hand but there was no difference between these on the palm. The reason for the difference in results between the palm and the whole hand are unclear. There was no clear difference between plain soap and cold water and chlorhexidine antibacterial soap and warm water for either the whole hand or palm.

Chlorhexidine antibacterial soap and warm water removed more *C. difficile* than antiseptic parachlorometaxylenol hand wipes on both the whole hand and the palm.

Evidence for all comparisons (antiseptic parachlorometaxylenol hand wipes compared to liquid plain soap and water, chlorhexidine antibacterial soap compared to plain soap and water and chlorhexidine antibacterial soap compared to antiseptic parachlorometaxylenol hand wipes) and the outcome of mean reduction *C. difficile* colony counts (log_{10} CFU/mL) was rated as very low certainty. The evidence was downgraded for risk of bias as participant characteristics were not reported. Participants were not blinded to the intervention they were given, and they administered it themselves. Evidence was downgraded for indirectness as the population was healthy volunteers in a laboratory of a hospital, and the *C. difficile* strain used was nontoxigenic. The evidence for chlorhexidine soap compared to warm or cold water and the outcome of mean reduction *C. difficile* colony counts (log_{10} CFU/mL) was also downgraded for imprecision for some comparisons due to wide confidence intervals which crossed the option of no difference between interventions. This may be due to the small sample size. As there was only one study for each of these comparisons reporting this outcome, it was not possible to assess inconsistency.

Non-randomised cross-over study

Bettin and others conducted a non-randomised cross-over study in the USA to compare the effectiveness of a chlorhexidine-based hand hygiene agent with plain liquid soap for the removal of *C. difficile* spores from hands (3).

The study involved 10 healthy adult volunteers (60% females and 40% males), who were research laboratory staff without regular patient contact.

The study compared chlorhexidine gluconate liquid soap (4% chlorhexidine gluconate in 4% alcohol; Hibiclens) with plain liquid soap (Epicare ® Skin Cleanser).

Bare hands of participants were experimentally contaminated with a bacterial suspension containing approximately 6.7 log_{10} CFU (47%) *C. difficile* spores. Spores were seeded onto the right palm and distributed by gently rubbing both palms for 10 seconds, followed by air drying before each hand hygiene intervention.

During the first week, 5 participants washed their hands with the chlorhexidine gluconate liquid soap, while 5 used plain liquid soap. In the second week, after decontamination, hands were experimentally re-contaminated and participants crossed over to the alternate intervention. Each participant did a 3-minute scrub with a surgical sponge to decontaminate the skin between interventions.

Hand hygiene interventions were performed according to a standardised protocol, under study supervision. Handwashing involved dispensing 1 mL of hand hygiene agent onto the palm, rubbing hands for 10 seconds, rinsing with tap water for 5 seconds and drying with a paper towel for 5 seconds for both the chlorhexidine gluconate liquid soap and the plain liquid soap.

Bacterial counts were assessed by measuring spores recovered from 3 sites; finger or thumb tips, fingers, and palms. Reduction in spore counts were only reported as approximate values, as shown in Table 2.

Table 2. Reduction in *C. difficile* spore counts handwashing with chlorhexidine gluconate liquid soap and plain liquid soap

Hand hygiene intervention	Finger or thumb tips (reduction in log_{10} CFU)	Fingers (reduction in log_{10} CFU)
Chlorhexidine gluconate liquid soap	3.1	3.0
Plain liquid soap	3.2	3.1

No statistically significant difference was found between chlorhexidine gluconate liquid soap and chlorhexidine gluconate liquid soap on the finger or thumb tips, or the fingers. Reductions on the palms could not be assessed as spore counts were too high to quantify.

The certainty of evidence could not be assessed because the study did not report any measure of variability. Risk of bias assessment indicated that the study was at moderate risk of bias. The sample size was very small, only including 10 participants and participant characteristics, beyond their sex, were not reported. The study did not randomise participants to their starting intervention (chlorhexidine gluconate liquid soap or non-medicated liquid soap) and while it is possible order interventions received in could influence the results of the study, the decontamination process between interventions was likely adequate to reduce or remove this risk. Whilst not part of the risk of bias assessment, it should also be noted that the population was healthy volunteers who were laboratory staff of a hospital, and the *C. difficile* strain used was nontoxigenic.

Health inequalities

No evidence was identified on the key demographic and social factors (socio-economic status, age, race, and ethnicity) considered as of interest for this review. Only one study reported the sex of participants, however this factor is unlikely to influence the effectiveness of the hand hygiene agent (3). It is therefore not possible to draw meaningful conclusions from the available evidence about possible health inequalities in relation to this review question.

Limitations

This rapid systematic review used streamlined systematic methods to accelerate the review process. Sources of evidence searched included databases of peer-reviewed and preprint research, but an extensive search of other sources was not conducted and most article screening was completed without duplication, so it is possible relevant evidence may have been missed.

The available evidence was very limited. Only 3 experimental studies were included, all of which had a cross-over design and small sample sizes. All studies were conducted in laboratories of hospitals or health centres with healthy adult volunteers, mainly laboratory staff, so the outcomes may not reflect how hand hygiene interventions would perform in other settings such as hospital clinical settings, adult social care settings, outpatient and community care settings, mental health facilities or prisons.

The studies involved healthy volunteers, without active *C. difficile* infection rather than in the intended population of interest for this review and therefore may not reflect the effectiveness when used in practice. Two studies looked at hand hygiene interventions that contain chlorine in their chemical structure rather than as an active ingredient, which may limit the applicability of these findings to the intended interventions (2, 3).

Details of the cross-over process and washout period after application of hand hygiene intervention was not reported by one study (1). The other 2 studies reported washout periods. While residual effects of the hand hygiene intervention used first may persist and influence the response to the next hand hygiene intervention if the decontamination process is not effective, it is not deemed a major risk of bias in these studies as the outcomes reported were objective (2, 3).

One study reported that all hand hygiene interventions were performed for shorter durations than recommended by the product manufacturers, so it is unclear how well they would work if used exactly as instructed (2).

Participants in both randomised cross-over studies were not blinded to the intervention they received, and one study did not report whether the outcome assessors were blinded. However, this was considered unlikely to influence effectiveness of hand hygiene interventions as the outcomes were measured objectively (1, 2).

One study was quasi-experimental by design and therefore did not use random assignment (although differences between starting groups may not influence the effectiveness of hand hygiene interventions under the same setting) (3).

Evidence gaps

There was no directly relevant evidence for this review question. No evidence was found from hospital clinical settings, care homes, adult social care settings, outpatient or community care settings, mental health facilities, or prisons. The studies included in this review were conducted with healthy volunteers who did not have confirmed or suspected *C. difficile* infection. Only one study evaluated a hypochlorous acid-based product compared with soap and water. None of the studies reported on *C. difficile* incidence rates, transmission rates or secondary *C. difficile* infection.

Conclusion

This rapid systematic review examined the effectiveness of hand hygiene agents, as alternatives to soap and water to reduce the transmission of *C. difficile* in health and social care settings, where care is given. Three studies were included of which 2 were randomised cross-over studies and one was a non-randomised cross-over study. All evidence was from laboratory settings (of hospitals or health centres), despite efforts to locate evidence from other relevant settings, including hospital clinical settings, care homes (with or without nursing), adult social care, outpatient and community care, mental health facilities and prisons.

One randomised cross-over study found that a hypochlorous acid-based hand hygiene intervention (Vashe solution soak) was significantly more effective than soap and water in reducing non-toxigenic *C. difficile* spores on hands under laboratory conditions. The second randomised cross-over study found that chlorhexidine antibacterial soap may be as effective as plain soap and water for removal of *C. difficile* spores, but antiseptic parachlorometaxylenol hand wipes were less effective than plain soap and water. The non-randomised cross-over study found no statistically significant difference between chlorhexidine gluconate liquid soap and plain liquid soap and water for reduction of *C. difficile* spores.

The certainty of the evidence for the outcomes that could be assessed was assessed as very low, and where it could not be assessed the studies were rated as being at moderate to high risk of bias. There was no available evidence of the effect on incidence rates, transmission rates or secondary *C. difficile* infection, and no evidence in the specific population and setting of interest for the review question.

The available evidence therefore does not provide any conclusive evidence that there is a more effective hand hygiene agent as an alternative to soap and water to reduce the transmission of *C. difficile* in settings where care is provided.

Acknowledgments

We would like to thank colleagues within the All Hazards Public Health Response division who either reviewed or input into aspects of the review.

Disclaimer

UKHSA's rapid systematic reviews and evidence summaries aim to provide the best available evidence to decision makers in a timely and accessible way, based on published peer-reviewed scientific papers, and papers on preprint servers. Note that the reviews:

- use accelerated methods and may not be representative of the whole body of evidence publicly available
- have undergone an internal independent peer review but not an external peer review
- are only valid as of the date stated on the review

In the event that this review is shared externally, note additionally, to the greatest extent possible under any applicable law, that UKHSA accepts no liability for any claim, loss or damage arising out of, or connected with the use of, this review by the recipient or any third party including that arising or resulting from any reliance placed on, or any conclusions drawn from, the review.

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Annexe A. Protocol

Review question

The review question was:

1. What is the effectiveness of hand hygiene agents, as alternatives to soap and water, to reduce transmission of *C. difficile* in health and social care settings where care is given?

A search for primary evidence to answer this review question will be conducted up from 1 January 1990 to 12 December 2025.

Eligibility criteria

Table A.1. Inclusion and exclusion criteria

	Included	Excluded
Population	Adults and children (aged 2 years and above) with confirmed or suspected *C. difficile* [note 1]	Children under the age of 2
Context	To inform guidance for health and social care settings in cases of *C. difficile* outbreaks, clusters, periods of increased incidence, areas of high prevalence or single cases	
Settings	Any setting where care [note 2] is given, including: • hospitals • adult social care settings including care homes (with or without nursing) and other residential settings • outpatient and community care settings • mental health facilities • prisons Or: Laboratory settings in hospitals or health centres	Any other settings

	Included	**Excluded**
Intervention or exposure	Hand hygiene agents as alternatives to soap and water [note 3]: (all forms including liquid, gel and hand wipes) for example: • hypochlorous acid containing hand hygiene agents • chlorine containing hand hygiene agents	Hand hygiene agents that contain only alcohol as their active ingredient
Comparator	Handwashing with soap and water Hand hygiene agents compared to each other	
Outcomes	Clinical evidence of effectiveness measured by: • *C. difficile* incidence rate • *C. difficile* transmission rate • secondary *C. difficile* infection • environmental outcomes from clinical settings (spore recovery from hands)	
Language	English	Any other language
Date of publication	1 January 1990 to 12 December 2025	Studies published before 1990
Study design	Systematic reviews (high quality) Experimental studies including randomised-controlled trials, quasi-experimental studies, cross-over designs Mixed methods (quantitative element if presented separately) Observational studies including cohort studies and before and after studies The following study designs will be considered only if no systematic reviews, experimental studies, cohort or before and after studies are available: • case control studies • cross sectional studies	Non-comparative observational studies Descriptive studies including case series or case reports Modelling studies Qualitative studies

	Included	Excluded
Publication type	Peer-reviewed published research Preprints Letters to the editor (if they report trial results that meet this protocol's eligibility criteria and provide sufficient detail for assessment)	Conference abstracts Editorials News articles Grey literature

note 1: Including people who are symptomatic and positive for *C. difficile* on PCR test, but negative toxin test.
note 2: 'Care' defined as care administered by health or social care professionals (not care of dependents).
note 3: A new chemical formulation (not soap and water or agents that contain alcohol as their only active ingredient) or technology not currently standard in healthcare practice.

Background

This protocol is part of a series of reviews commissioned to support the updating the Infection Prevention and Control (IPC) section of the following guideline: Clostridioides difficile infection: how to deal with the problem.

Identification of studies

The following databases will be searched for studies published up to 1 September 2025: Ovid Medline, Ovid Embase, Ovid Emcare and Web of Science Preprint Citation Index. The search strategy is presented below.

Backwards and forwards citation searching will be carried out using references that are included at full text screening as seed papers. Citation searching will use Lens.org via CitationChaser. References that are included following full text screening will be used as seed references.

Screening

Title and abstract screening will be undertaken in duplicate by 2 reviewers for at least 20% of the eligible studies, with the remainder completed by one reviewer. Disagreement will be resolved by discussion or with involvement of a third reviewer where necessary.

Screening on full text will be undertaken by one reviewer and checked by a second. Case control and cross-sectional studies will be considered at the full text screening stage if no systematic reviews, experimental studies, cohort studies or before-and-after studies are

identified to answer the review question. Letters to the editor will be included only if they report trial results that meets this protocol's eligibility criteria and provide sufficient detail for assessment.

Data extraction

Summary information for each study will be extracted and reported in tabular form. Information to be extracted will include country, study period, study design, intervention and comparator, participants, results, and any relevant contextual data. This will be undertaken by one reviewer and checked by a second.

Risk of bias assessment

We will perform risk of bias assessment at the primary study level using the relevant JBI checklist (5). Risk of bias will be assessed by 2 reviewers independently with disagreements resolved through discussion or with a third reviewer.

Certainty of evidence

If appropriate, the certainty of evidence identified within this review will be assessed using a modified version of the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) framework (6).

Certainty of evidence will be assessed at the outcome level, and be rated as one of 4 levels:

- very low (the true effect is probably different from the estimated effect)
- low (the true effect might be different from the estimated effect)
- moderate (the true effect is probably close to the estimated effect)
- high (the authors are confident that the true effect is similar to the estimated effect)

The certainty of evidence will be assessed by one reviewer (and checked by a second) for each outcome across 4 domains:

1. Limitation in study design and execution (risk of bias: where results may not represent the true effect because of limitations in the design or conduct of the study.
2. Inconsistency: where studies show different effects for the same outcome of interest (only assessed where there are 2 or more studies measuring the same outcome). Inconsistency will be rated down if, for example, the point estimates are not similar, or the confidence intervals do not overlap, heterogeneity will be considered.
3. Indirectness: where elements of the study differ from the intended elements in the review question (for example, the outcome of interest has not been directly measured).

This will be rated down if the population, intervention, comparator, or outcome of interest have not been directly measured.

4. Imprecision: a measure of how uncertain the estimate is. Imprecision will be rated down if the confidence intervals cross the line of no effect, or if the reviewer judges that the confidence intervals are overly wide and so the true effect is likely to be different at the upper versus the lower end of the confidence interval.

Publication bias will not be used to assess the quality of the evidence in this review

Evidence may be downgraded one or 2 levels following the assessment of quality or upgraded if there is a large magnitude of effect or clear dose-response gradient.

Synthesis

Where studies are similar enough to combine and present data in a consistent format, a narrative synthesis will be produced to interpret the findings. The narrative synthesis will include: the number of studies, the number of participants in each study, effect size and variance and a summary of the risk of bias across studies reporting each outcome will be summarised and presented. Alternatively, if studies present methodological differences that would make synthesis inappropriate, a narrative summary of each study will be provided.

Health inequalities

Variations among individuals experiencing health inequalities will be reported as part of the evidence synthesis, as these factors may influence outcomes for *Clostridioides difficile* infections: socio-economic status, sex, race, and ethnicity. Differences in outcomes by age and any variation or disparities between settings (for example, health care or social care) will also be assessed if the data are available.

Search strategy

Ovid MEDLINE(R) ALL (1 January 1990 to 11 December 2025)

1. exp Clostridioides difficile/ (12,411)
2. exp Clostridium Infections/ (34,167)
3. clostridium difficile.tw,kf. (15,617)
4. clostridioides difficile.tw,kf. (5,455)
5. clostrid* infection*.tw,kf. (558)
6. C difficile.tw,kf. (9,994)
7. "C.difficile".tw,kf. (106)
8. C diff.tw,kf. (227)
9. "C.diff".tw,kf. (105)
10. CDAD.tw,kf. (777)
11. CDI.tw,kf. (10,590)
12. or/1-11 (49,711)
13. exp Hand Hygiene/ (8,660)
14. (hand* adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (10,643)
15. (hand* adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or chlorine or gel* or foam* or spray* or wipe*)).tw,kf. (4,070)
16. (skin adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (3,545)
17. (skin adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*)).tw,kf. (4,969)
18. (dermal adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (138)
19. (dermal adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*)).tw,kf. (600)
20. primel.tw,kf. (1)
21. spectricept.tw,kf. (0)
22. sterilox.tw,kf. (25)
23. Hypochlorous Acid/ (3,363)
24. hypochlorous acid*.tw,kf. (3,291)
25. HClO.tw,kf. (902)
26. HOCl.tw,kf. (2,902)
27. hypochlorite.tw,kf. (10,566)
28. chloranol.tw,kf. (0)
29. exp Anti-Infective Agents, Local/ (278,515)
30. (anti-infect* adj4 (local* or topical* or agent*)).tw,kf. (2,720)

31. (antiinfect* adj4 (local* or topical* or agent*)).tw,kf. (177)
32. (anti-septic* adj4 (local* or topical* or agent* or persistent)).tw,kf. (18)
33. (antiseptic* adj4 (local* or topical* or agent* or persistent)).tw,kf. (1,881)
34. (anti-sepsis* adj4 (local* or topical* or agent* or persistent)).tw,kf. (16)
35. (antisepsis* adj4 (local* or topical* or agent* or persistent)).tw,kf. (121)
36. (anti-microbial* adj4 (local* or topical* or agent*)).tw,kf. (614)
37. (antimicrobial* adj4 (local* or topical* or agent*)).tw,kf. (40,853)
38. (anti-bact* adj4 (local* or topical* or agent*)).tw,kf. (1,567)
39. (antibact* adj4 (local* or topical* or agent*)).tw,kf. (18,061)
40. (anti-viral* adj4 (local* or topical* or agent*)).tw,kf. (803)
41. (antiviral* adj4 (local* or topical* or agent*)).tw,kf. (16,282)
42. (bactericid* adj5 (local or topical or agent*)).tw,kf. (1,535)
43. microbicid*.tw,kf. (7,225)
44. microbiocid*.tw,kf. (196)
45. yeasticid*.tw,kf. (21)
46. virucid*.tw,kf. (2,533)
47. sporicid*.tw,kf. (607)
48. (hand* adj2 shield*).tw,kf. (38)
49. or/13-48 (400,104)
50. 12 and 49 (1,317)
51. limit 50 to (comment or editorial or letter or news or newspaper article) (53)
52. 50 not 51 (1,264)
53. limit 52 to yr="1990 -Current" (1,086)

Embase (1 January 1990 to 11 December 2025)

1. exp Clostridioides difficile/ (23,115)
2. exp Clostridium infection/ (49,298)
3. clostridium difficile.tw,kf. (23,941)
4. clostridioides difficile.tw,kf. (7,364)
5. clostrid* infection*.tw,kf. (648)
6. C difficile.tw,kf. (15,170)
7. "C.difficile".tw,kf. (543)
8. C diff.tw,kf. (1,159)
9. "C.diff".tw,kf. (387)
10. CDAD.tw,kf. (1,367)
11. CDI.tw,kf. (16,936)
12. or/1-11 (78,254)
13. exp hand washing/ (23,745)
14. (hand* adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (14,655)

15. (hand* adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or chlorine or gel* or foam* or spray* or wipe*)).tw,kf. (5,271)
16. (skin adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (5,681)
17. (skin adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*)).tw,kf. (7,183)
18. (dermal adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (194)
19. (dermal adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*)).tw,kf. (830)
20. primel.tw,kf. (1)
21. spectricept.tw,kf. (0)
22. sterilox.tw,kf. (32)
23. exp hypochlorous acid/ (4,947)
24. hypochlorous acid*.tw,kf. (3,897)
25. HClO.tw,kf. (611)
26. HOCl.tw,kf. (3,419)
27. hypochlorite.tw,kf. (11,546)
28. chloranol.tw,kf. (0)
29. exp topical antiinfective agent/ (893,126)
30. (anti-infect* adj4 (local* or topical* or agent*)).tw,kf. (3,154)
31. (antiinfect* adj4 (local* or topical* or agent*)).tw,kf. (439)
32. (anti-septic* adj4 (local* or topical* or agent* or persistent)).tw,kf. (28)
33. (antiseptic* adj4 (local* or topical* or agent* or persistent)).tw,kf. (2,662)
34. (anti-sepsis* adj4 (local* or topical* or agent* or persistent)).tw,kf. (19)
35. (antisepsis* adj4 (local* or topical* or agent* or persistent)).tw,kf. (160)
36. (anti-microbial* adj4 (local* or topical* or agent*)).tw,kf. (955)
37. (antimicrobial* adj4 (local* or topical* or agent*)).tw,kf. (51,580)
38. (anti-bact* adj4 (local* or topical* or agent*)).tw,kf. (2,386)
39. (antibact* adj4 (local* or topical* or agent*)).tw,kf. (22,393)
40. (anti-viral* adj4 (local* or topical* or agent*)).tw,kf. (1,267)
41. (antiviral* adj4 (local* or topical* or agent*)).tw,kf. (21,937)
42. (bactericid* adj5 (local or topical or agent*)).tw,kf. (1,772)
43. microbicid*.tw,kf. (8,866)
44. microbiocid*.tw,kf. (238)
45. yeasticid*.tw,kf. (25)
46. virucid*.tw,kf. (2,743)
47. sporicid*.tw,kf. (693)
48. (hand* adj2 shield*).tw,kf. (58)
49. or/13-48 (1,038,929)

50. 12 and 49 (3,988)
51. limit 50 to (conference abstract or conference paper or "conference review" or editorial or letter) (969)
52. 50 not 51 (3,019)
53. limit 52 to yr="1990 -Current" (2,881)

Ovid Emcare <1 January 1990 to 11 December 2025>

1. exp Clostridioides difficile/ (1,795)
2. exp Clostridium infection/ (12,314)
3. clostridium difficile.tw,kf. (4,562)
4. clostridioides difficile.tw,kf. (1,631)
5. clostrid* infection*.tw,kf. (94)
6. C difficile.tw,kf. (2,519)
7. "C.difficile".tw,kf. (49)
8. C diff.tw,kf. (110)
9. "C.diff".tw,kf. (16)
10. CDAD.tw,kf. (272)
11. CDI.tw,kf. (3,398)
12. or/1-11 (17,027)
13. exp hand washing/ (10,722)
14. (hand* adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (5,342)
15. (hand* adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*)).tw,kf. (1,545)
16. (skin adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (1,155)
17. (skin adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*)).tw,kf. (1,353)
18. (dermal adj5 (disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*)).tw,kf. (27)
19. (dermal adj5 (sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*)).tw,kf. (126)
20. primel.tw,kf. (0)
21. spectricept.tw,kf. (0)
22. sterilox.tw,kf. (20)
23. hypochlorous acid/ (691)
24. hypochlorous acid*.tw,kf. (394)
25. HClO.tw,kf. (32)
26. HOCl.tw,kf. (251)

27. hypochlorite.tw,kf. (3,800)
28. chloranol.tw,kf. (0)
29. exp topical antiinfective agent/ (175,857)
30. (anti-infect* adj4 (local* or topical* or agent*)).tw,kf. (708)
31. (antiinfect* adj4 (local* or topical* or agent*)).tw,kf. (155)
32. (anti-septic* adj4 (local* or topical* or agent* or persistent)).tw,kf. (2)
33. (antiseptic* adj4 (local* or topical* or agent* or persistent)).tw,kf. (627)
34. (anti-sepsis* adj4 (local* or topical* or agent* or persistent)).tw,kf. (2)
35. (antisepsis* adj4 (local* or topical* or agent* or persistent)).tw,kf. (36)
36. (anti-microbial* adj4 (local* or topical* or agent*)).tw,kf. (114)
37. (antimicrobial* adj4 (local* or topical* or agent*)).tw,kf. (7,904)
38. (anti-bact* adj4 (local* or topical* or agent*)).tw,kf. (720)
39. (antibact* adj4 (local* or topical* or agent*)).tw,kf. (2,847)
40. (anti-viral* adj4 (local* or topical* or agent*)).tw,kf. (95)
41. (antiviral* adj4 (local* or topical* or agent*)).tw,kf. (2,406)
42. (bactericid* adj5 (local or topical or agent*)).tw,kf. (209)
43. microbicid*.tw,kf. (1,593)
44. microbiocid*.tw,kf. (46)
45. yeasticid*.tw,kf. (10)
46. virucid*.tw,kf. (490)
47. sporicid*.tw,kf. (173)
48. (hand* adj2 shield*).tw,kf. (10)
49. or/13-48 (203,291)
50. 12 and 49 (1,122)
51. limit 50 to yr="1990 -Current" (1,120)

Web of Science Preprint Citation Index

Date of search: 11 December 2025

TS=("Clostridioides difficile") OR TS=("Clostridium infection*") OR TS=("clostridium difficile") OR TS=("C diff*") OR TS=("C.diff*") OR TS=(CDAD)

AND

TS=("hand hygiene") OR TS=(hand* NEAR/5 disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*) OR TS=(hand* NEAR/5 sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or chlorine or gel* or foam* or spray* or wipe*) OR TS=(skin NEAR/5 disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*) OR TS=(skin NEAR/5 sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact* or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*) OR TS=(dermal NEAR/5 disinfect* or wash* or scrub* or clean* or decontaminat* or sterilis* or steriliz*) OR TS=(dermal NEAR/5 sanitis* or sanitiz* or antisep* or anti-sep* or anti-infect* or antiinfect* or anti-bact* or antibact*

or anti-viral* or antiviral* or gel* or foam* or spray* or wipe*) OR TS=(primel) OR TS=(spectricept) OR TS=(sterilox) OR TS=(hypochlorous acid*) OR TS=(HClO or HOCl or hypochlorite or chloranol) OR TS=(anti-infect* NEAR/4 local* or topical* or agent*) OR TS=(antiinfect* NEAR/4 local* or topical* or agent*) OR TS=(anti-septic* NEAR/4 local* or topical* or agent* or persistent) OR TS=(antiseptic* NEAR/4 local* or topical* or agent* or persistent) OR TS=(anti-sepsis* NEAR/4 local* or topical* or agent* or persistent) OR TS=(antisepsis* NEAR/4 local* or topical* or agent* or persistent) OR TS=(anti-microbial* NEAR/4 local* or topical* or agent*) OR TS=(antimicrobial* NEAR/4 local* or topical* or agent*) OR TS=(anti-bact* NEAR/4 local* or topical* or agent*) OR TS=(antibact* NEAR/4 local* or topical* or agent*) OR TS=(anti-viral* NEAR/4 local* or topical* or agent*) OR TS=(antiviral* NEAR/4 local* or topical* or agent*) OR TS=(bactericid* NEAR/4 local* or topical* or agent*) OR TS=(microbicide*) OR TS=(microbiocide*) OR TS=(yeasticid*) OR TS=(virucid*) OR TS=(sporicid*) OR TS=(hand* NEAR/2 shield*)

Date limit 1990 to the present

Results: 53

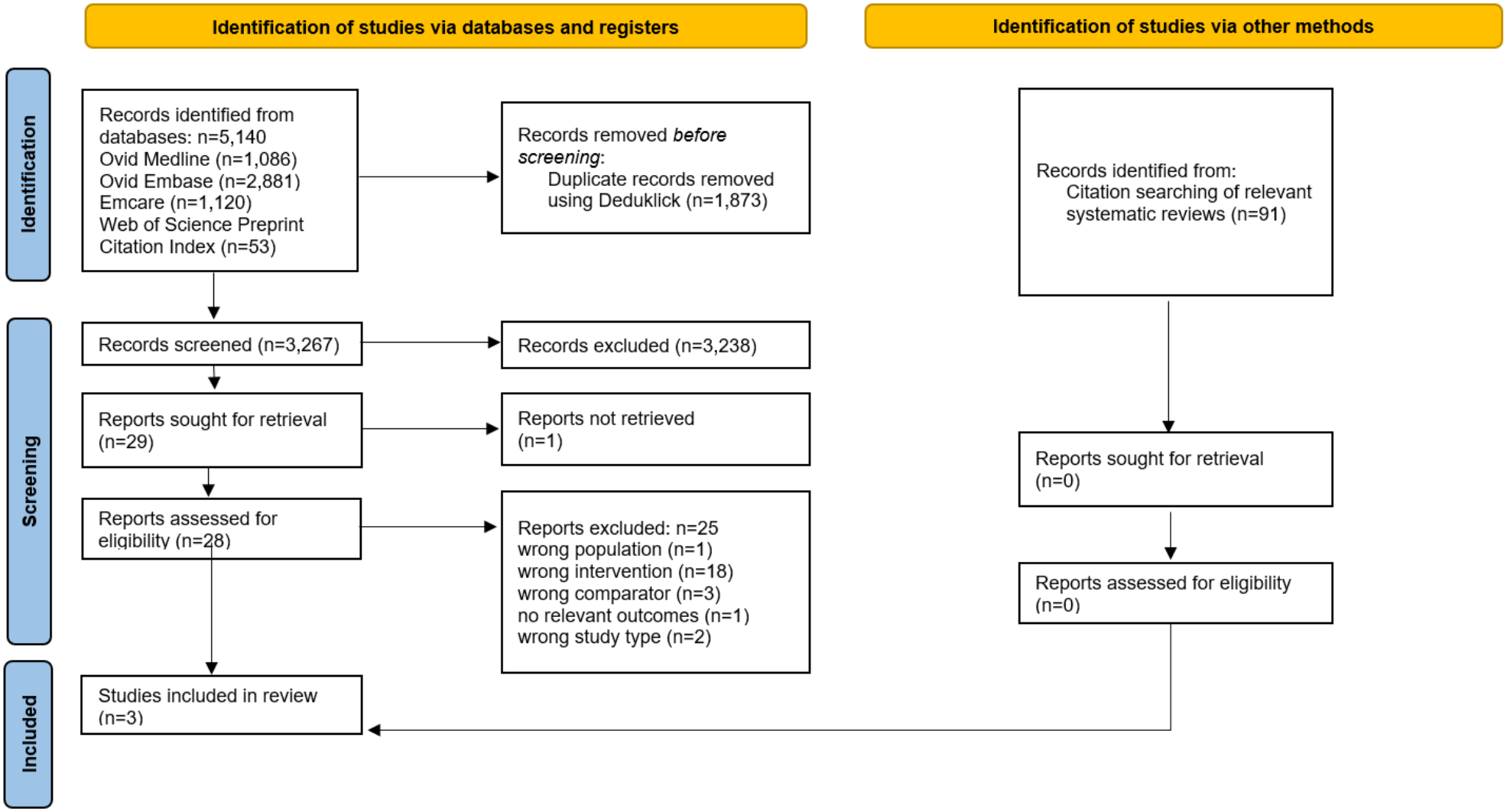
Deviations

There was one protocol deviation:

The inclusion criteria for settings were amended to include laboratory settings in hospitals or health centres. This was due to an absence of evidence identified from settings where care is given.

Annexe B. Study selection flowchart

Figure B.1. PRISMA diagram



Text version of Figure B.1. PRISMA diagram

A PRISMA diagram showing the flow of studies through this review, ultimately including 3 studies.

From identification of studies via databases and registers, n=5,140 records identified from databases:

- Ovid Medline (n=1,086)
- Ovid Embase (n=2,881)
- Emcare (n=1,120)
- Web of Science Preprint Citation Index (n=53)

From these, records removed before screening:

- duplicate records removed using Deduklick (n=1,873)

n=3,267 records screened, of which n=3,238 were excluded, leaving n=29 papers sought for retrieval, of which n=1 was not retrieved.

No studies were included from identification of studies via citation searching.

Of the n=28 papers assessed for eligibility, n=25 reports were excluded:

- wrong population (n=1)
- wrong intervention (n=18)
- wrong comparator (n=3)
- no relevant outcomes (n=1)
- wrong study type (n=2)

n=3 papers included in the review.

Annexe C. Excluded full texts

Wrong population (One study)

Nerandzic MM and others. 'Sensitizing Clostridium difficile Spores with Germinants on Skin and Environmental Surfaces Represents a New Strategy for Reducing Spores via Ambient Mechanisms' Pathogens and Immunity 2017: volume 2, issue 3, pages 404 to 421

Wrong intervention (18 studies)

Aldeyab MA and others. 'Strategy for improving and maintaining compliance with adequate hospital hand hygiene practices' Journal of Hospital Infection 2011: volume 77, issue 1, pages 87 to 88

Barker AK and others. 'On the hands of patients with Clostridium difficile: A study of spore prevalence and the effect of hand hygiene on C difficile removal' American Journal of Infection Control 2017: volume 45, issue 10, pages 1,154 to 1,156

Boyce JM and others. 'Lack of association between the increased incidence of Clostridium difficile-associated disease and the increasing use of alcohol-based hand rubs' Infection Control and Hospital Epidemiology 2006: volume 27, issue 5, pages 479 to 483

Bui LN and others. 'Chlorhexidine bathing and Clostridium difficile infection in a surgical intensive care unit' The Journal of Surgical Research 2018: volume 228, pages 107 to 111

Cadnum JL and others. 'Transfer of clostridium difficile spores by nonsporicidal wipes and improperly used hypochlorite wipes: Practice + product = perfection' Infection Control and Hospital Epidemiology 2013: volume 34, issue 4, pages 441 to 442

Cheng VCC and others. 'Containment of Clostridium difficile infection without reduction in antimicrobial use in Hong Kong' European Journal of Clinical Microbiology & Infectious Diseases: official publication of the European Society of Clinical Microbiology 2015: volume 34, issue 7, pages 1,381 to 1,386

Horn K and others. 'Hydrogen peroxide vapor room disinfection and hand hygiene improvements reduce Clostridium difficile infection, methicillin-resistant Staphylococcus aureus, vancomycin-resistant enterococci, and extended-spectrum beta-lactamase' American Journal of Infection Control 2015: volume 43, issue 12, pages 1,354 to 1,356

Hsu J and others. 'Prevention of endemic healthcare-associated Clostridium difficile infection: reviewing the evidence' The American Journal of Gastroenterology 2010: volume 105, issue 11, pages 2,327 to 2,340

Isaacson D and others. 'Novel handwashes are superior to soap and water in removal of Clostridium difficile spores from the hands' American Journal of Infection Control 2015: volume 43, issue 5, pages 530 to 532

Kassakian SZ and others. 'Impact of chlorhexidine bathing on hospital-acquired infections among general medical patients' Infection Control and Hospital Epidemiology 2011: volume 32, issue 3, pages 238 to 243

Kengen R and others. 'Chlorhexidine washing in intensive care does not reduce bloodstream infections, blood culture contamination and drug-resistant microorganism acquisition: an interrupted time series analysis' Critical Care and Resuscitation: Journal of the Australasian Academy of Critical Care Medicine 2018: volume 20, issue 3, pages 231 to 240

Knight N and others. 'Clostridium difficile colitis: a retrospective study of incidence and severity before and after institution of an alcohol-based hand rub policy' American Journal of Infection Control 2010: volume 38, issue 7, pages 523 to 528

Kundrapu S and others. 'A randomized trial of soap and water hand wash versus alcohol hand rub for removal of Clostridium difficile spores from hands of patients' Infection Control and Hospital Epidemiology 2014: volume 35, issue 2, pages 204 to 206

Nerandzic MM and others. 'Unlocking the Sporicidal Potential of Ethanol: Induced Sporicidal Activity of Ethanol against Clostridium difficile and Bacillus Spores under Altered Physical and Chemical Conditions' PLOS One 2015: volume 10, issue 7, e0132805

Nerandzic MM and others. 'A Cumulative Spore Killing Approach: Synergistic Sporicidal Activity of Dilute Peracetic Acid and Ethanol at Low pH Against *Clostridium difficile* and *Bacillus subtilis* Spores' Open Forum Infectious Diseases 2016: volume 3, issue 1, page 206

Tomas ME and others. 'A Novel, Sporicidal Formulation of Ethanol for Glove Decontamination to Prevent Clostridium difficile Hand Contamination During Glove Removal' Infection Control and Hospital Epidemiology 2016: volume 37, issue 3, pages 337 to 339

Whitaker J and others. 'Designing a protocol that eliminates Clostridium difficile: a collaborative venture' American Journal of Infection Control 2007: volume 35, issue 5, pages 310 to 314

Zafar AB and others. 'Effectiveness of infection control program in controlling nosocomial Clostridium difficile' American Journal of Infection Control 1998: volume 26, issue 6, pages 588 to 593

Wrong comparator (3 studies)

Edmonds SL and others. 'Effectiveness of hand hygiene for removal of Clostridium difficile spores from hands' Infection control and Hospital Epidemiology 2013: volume 34, issue 3, pages 302 to 305

Jabbar U and others. 'Effectiveness of alcohol-based hand rubs for removal of Clostridium difficile spores from hands' Infection Control and Hospital Epidemiology 2010: volume 31, issue 6, pages 565 to 570

Otter JA and others. 'Evaluation of a Novel Hypochlorous Acid Based Hand Hygiene Product with Sporicidal Activity in an Inpatient Ward Setting' Preprints 2024

No relevant outcomes (1 study)

Gay TW. 'Hand hygiene and Clostridium difficile' Annals of Internal Medicine 2007: volume 147, issue 1, pages 69 to 70

Wrong study type (2 studies)

Macleod-Glover N and others. 'Efficacy of cleaning products for C. difficile: environmental strategies to reduce the spread of Clostridium difficile-associated diarrhea in geriatric rehabilitation' Canadian Family Physician Medecin de famille Canadien 2010: volume 56, issue 5, pages 417 to 423

Vonberg RP and others. 'Infection control measures to limit the spread of Clostridium difficile' Clinical Microbiology and Infection: the official publication of the European Society of Clinical Microbiology and Infectious Diseases 2008: volume 14 Supplement 5, pages 2 to 20

Annexe D. Data extraction table

Abbreviations: *B. atrophaeus*: *Bacillus atrophaeus*, *B. subtilis*: *Bacillus subtilis*, *B. thuringiensis*: *Bacillus thuringiensis*, C: Celsius, *C. difficile*: *Clostridioides difficile*, CFU: Colony forming units, CHX: Chlorhexidine, CI: Confidence Interval, mL: millilitre, NA: not applicable, NS: Not significant, PCMX: Parachlorometaxylenol, SD: Standard Deviation, USA: United States of America

Study, study design	Country, time period	Population	Experimental hand contamination	Allocation of study participants	Intervention (liquid, gel or handwipes)	Comparator	Outcome (bacterial counts)	Outcome (reduction in spore count)
Bettin K and others, 1994 (3) Non-randomised cross-over study	USA, time period not reported	10 healthy volunteers (research laboratory staff without regular patient contact) Sex: females: 6 out of 10 (60%) males: 4 out of 10 (40%) Health inequalities: Not reported	Bare hands experimentally contaminated with 100µL bacterial suspension 6.7 log_{10} CFU (47% *C. difficile* spores), spores seeded into right palm, participants gently rubbed palmar side of both hands for 10 seconds, followed by air drying for 5 minutes	1st week: 5 volunteers washed with soap and 5 with chlorhexidine 2nd week: same routine was followed for each volunteer, using the alternate cleansing agent	4% chlorhexidine gluconate in 4% alcohol (liquid) - Hibiclens 1 mL dispensed onto right palm, hands rubbed vigorously for 10 seconds, rinsed with tap water for 5 seconds, dried with paper towel for 5 seconds. Washout and recontamination process: After completion of the hand hygiene experiment, each participant did a 3-minute scrub with a Betadine surgical sponge (to decontaminate skin)	Non-medicated liquid soap (Epicare Skin Cleanser) 1 mL dispensed onto right palm, hands rubbed vigorously for 10 seconds, rinsed with tap water for 5 seconds, dried with paper towel for 5 seconds After completion of hand-washing experiment: 3-minute scrub with Betadine surgical sponge (to decontaminate skin)	**Finger/thumb tips:** Mean count estimate before bare hands wash: 5.2 log_{10} CFU Mean count estimate after wash (bare hands): CHX: 2.1 log_{10} CFU, SD 0.2 soap: 2.0 log_{10} CFU, SD 0.2 p value (soap vs CHX after wash): NS **Fingers:** Mean count estimate before wash: 5.5 log_{10} CFU Mean count estimate after wash (bare hands): CHX: 2.5 log_{10} CFU, SD 0.3 soap: 2.4 log_{10} CFU, SD 0.2 p value (soap vs CHX after wash): NS **Palms:** Mean count estimate before wash (bare hands): 5.7 log_{10} CFU Mean count estimate after wash: CHX and soap: not assessable (spore count too high to quantify)	Spore reduction- Washing bare hands with liquid soap and CHX (estimated): **Finger/thumb tips:** soap: 3.2 log_{10} CFU CHX: 3.1 log_{10} CFU p value: NS **Fingers:** soap: 3.1 log_{10} CFU CHX: 3 log_{10} CFU p value: NS **Palms:** soap and CHX: not assessable
Nerandzic MM and others, 2013 (1)	USA, time period not reported	6 healthy volunteers (laboratory staff without dermatological	Hands experimentally contaminated with non-toxigenic *C.*	Order of hand disinfection procedures for each volunteer was assigned	Vashe (electrochemically generated hypochlorous acid solution 0.025 to	Soap and water handwash: 2mL of 0.05% triclosan liquid soap (hands rubbed with	Not reported	Mean log_{10} reduction in recovery of nontoxigenic *C. difficile* spores from baseline (data estimated from figure using PlotDigitizer):

Study, study design	Country, time period	Population	Experimental hand contamination	Allocation of study participants	Intervention (liquid, gel or handwipes)	Comparator	Outcome (bacterial counts)	Outcome (reduction in spore count)
Randomised cross-over study		disease) Sex: not reported Health inequalities: Not reported	*difficile* spores (4.5 mL of a liquid inoculum containing 6 log_{10} CFU of *C. difficile*, *B. atrophaeus*, *B. thuringiensis* or *B. subtilis* spores)	by a computer-generated random numbers list	0.05%) Vashe solution soak: hands immersed in 1 gallon of solution soak for 1 minute Vashe wipes (terry cloth soaked in Vashe): cloths used to thoroughly wipe the hands for 20 seconds Combined intervention: soap and water wash followed by Vashe solution soak	liquid soap for 20 seconds, rinsed with water and patted dry) Soap and water soak: hands immersed in 1 gallon of water containing 10mL of 0.5% triclosan liquid soap and massaged vigorously for 1 minute		Soap and water handwashing: 2.0 to 2.1 log_{10} CFU Soap and water soak: 1.6 to 2.1 log_{10} CFU Vashe solution soak: 2.6 to 2.8 log_{10} CFU Vashe wipe: 0.8 to 1.4 log_{10} CFU Vashe solution soak vs soap and water soak: 0.5 to 1.2 log_{10} CFU reduction (p value <0.01) Vashe solution soak vs soap and water handwash 0.5 to 0.8 log_{10} CFU reduction (p value < 0.01)
Oughton MT and others, 2009 (2) Randomised cross-over study	Canada, June to July 2007	10 healthy volunteers (hospital laboratory workers without skin disease) Sex: not reported Health inequalities: Not reported	Hands experimentally contaminated with non-toxigenic *C. difficile* spores 1.4 x 10^5 CFU/mL (62% spores)	Order of interventions for each volunteer was assigned by computer-generated random number lists	Antiseptic hand wipes containing 0.5% PCMX and 40% vol/vol ethanol (Sani-dex antimicrobial hand wipes, PDI) Hands wiped for 15 seconds followed by air-drying for 1 minute Warm water (30 degree C) with liquid antibacterial soap 2% Chlorhexidine Hygenipak foaming skin cleanser (10	Warm water (30 degree C) with liquid plain soap Hygenipak Instafoam (10 seconds lathering, rinsed with tap water, dried with paper towel) Cold water (15 degree C) with liquid plain soap Hygenipak Instafoam (10 seconds lathering, rinsed with tap water, dried with paper towel)	**Mean *C. difficile* colony counts after hand hygiene interventions (Whole hand):** PCMX antiseptic hand wipe: 3.25 log_{10} CFU/mL (95% CI = 3.04 to 3.45) Warm water and antibacterial CHX soap: 2.31 log_{10} CFU/mL (95% CI = 2.04 to 2.58) Warm water and plain soap: 1.99 log_{10} CFU/mL (95% CI = 1.80 to 2.09) Cold water and plain soap: 1.90 log_{10} CFU/mL (95% CI = 1.58 to 2.22)	**Mean reduction in *C. difficile* colony counts after hand hygiene interventions (estimated Whole hand):** Warm water and plain soap vs PCMX antiseptic hand wipes: 1.57 log_{10} CFU/mL (95% CI= 1.18 to 1.96) Cold water and plain soap vs PCMX antiseptic hand wipes: 1.31 log_{10} CFU/mL (95% CI= 0.92 to 1.71) Warm water and antibacterial CHX soap vs PCMX antiseptic hand wipes: 0.94 log_{10} CFU/mL (95% CI= 0.55 to 1.34) Warm water and plain soap vs warm water and antibacterial CHX soap: 0.63 log_{10} CFU/mL (95% CI= 0.23 to

Study, study design	Country, time period	Population	Experimental hand contamination	Allocation of study participants	Intervention (liquid, gel or handwipes)	Comparator	Outcome (bacterial counts)	Outcome (reduction in spore count)
					seconds lathering, rinsed with tap water, dried with paper towel) Washout and recontamination process: Each participant had a gap of at least 24 hours between interventions to reduce carryover of handwashing agents or bacteria and hands were experimentally recontaminated with *C. difficile* spores before switching to the next intervention.			1.02) Cold water and plain soap vs warm water and antibacterial CHX soap: 0.37 log_{10} CFU/mL (95% CI= -0.03 to 0.76) **Mean reduction in *C. difficile* colony counts after hand hygiene interventions (estimated palmar surface):** Warm water and plain soap vs PCMX antiseptic hand wipes: 4.71 log_{10} CFU/plate (95% CI= 2.94 to 6.50) Cold water and plain soap vs PCMX antiseptic hand wipes: 4.64 log_{10} CFU/plate (95% CI= 2.85 to 6.45) Warm water and antibacterial CHX soap vs PCMX antiseptic hand wipes: 4.72 log_{10} CFU/plate (95% CI= 2.59 to 6.59) Warm water and antibacterial CHX soap vs warm water and plain soap: 0.01 log_{10} CFU/plate (95% CI= -1.77 to 1.83) Warm water and antibacterial CHX soap vs cold water and plain soap: 0.08 log_{10} CFU/plate (95% CI= -1.74 to 1.90)

Annexe E. Risk of bias assessment

Table E.1. Risk of bias assessment for randomised controlled trials

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Q12	Q13	Comments (including reason for no)
Nerandzic MM and others, 2013 (1)	Yes	Unclear	Unclear	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Q2: Study does not describe whether allocation was concealed from participants prior to assignment Q3: Participant characteristics not clearly reported Q4: Participants were not blinded Q5: Study personnel delivering interventions were not blinded
Oughton MT and others, 2009 (2)	Yes	Unclear	Unclear	No	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Q2: Study does not describe whether allocation was concealed from participants prior to assignment Q3: Participant characteristics not clearly reported Q4: Participants were not blinded Q5: No information provided Q6: No information provided

Critical appraisal was done using the JBI checklist for randomised controlled trials (5)

List of questions:
Q1: Was true randomisation used for assignment of participants to treatment groups?
Q2: Was allocation to treatment groups concealed?
Q3: Were treatment groups similar at the baseline?
Q4: Were participants blind to treatment assignment?
Q5: Were those delivering treatment blind to treatment assignment?
Q6: Were outcome assessors blind to treatment assignment?
Q7: Were treatment groups treated identically other than the intervention of interest?
Q8: Was follow-up complete and, if not, were differences between groups in terms of their follow-up adequately described and analysed?
Q9: Were participants analysed in the groups to which they were randomised?
Q10: Were outcomes measured in the same way for treatment groups?
Q11: Were outcomes measured in a reliable way?
Q12: Was appropriate statistical analysis used?
Q13: Was the trial design appropriate, and any deviations from the standard RCT design (individual randomisation, parallel groups) accounted for in the conduct and analysis of the trial?

Table E.2. Risk of bias assessment for Quasi-experimental studies

Study	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Comments (including reason for no)
Bettin K and others, 1994 (3)	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Q4: No control group, cross-over study design (participants acted as their own control)

Critical appraisal was done using the JBI checklist for Quasi-experimental studies (5)

List of questions:

Q1: Is it clear in the study what is the 'cause' and what is the 'effect' (i.e. there is no confusion about which variable comes first)?
Q2: Were the participants included in any comparisons similar?
Q3: Were the participants included in any comparisons receiving similar treatment/care, other than the exposure or intervention of interest?
Q4: Was there a control group?
Q5: Were there multiple measurements of the outcome both pre and post the intervention/exposure?
Q6: Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analysed?
Q7: Were the outcomes of participants included in any comparisons measured in the same way?
Q8: Were outcomes measured in a reliable way?
Q9: Was appropriate statistical analysis used?

Annexe F. GRADE assessment of certainty of evidence

Abbreviations: CFU: colony forming units, CI: confidence interval, *C. difficile*: *Clostridioides difficile*, mL: millilitre

Table F.1. Antiseptic parachlorometaxylenol hand wipes compared to liquid plain soap and water on mean log_{10} reduction *C. difficile* colony-forming units

Certainty assessment							Finding	Certainty
Number of studies and endnote reference	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**		
Spore recovery from hands (mean log_{10} reduction *C. difficile* colony counts (log_{10} CFU/mL)								
1 Oughton MT and others, 2009 (2)	Randomised trials	Serious [note 1]	Not assessed [note 2]	Very serious [note 3]	None	None	Washing with warm or cold water and liquid plain soap achieved a greater mean reduction in *C. difficile* colony counts than antiseptic parachlorometaxylenol hand wipes. Warm water and plain soap: 1.57 log_{10} CFU/mL (95% CI: 1.18 to 1.96) Cold water and plain soap: 1.31 log_{10} CFU/mL (95% CI: 0.92 to 1.71).	⊕◯◯◯ Very low [note 1, 2, 3]

Notes

Note 1. Participant characteristics and allocation concealment not reported. No blinding of participants. Intervention self-administered.
Note 2. Only one study, not possible to assess inconsistency.
Note 3. Population is healthy volunteers. Study was conducted in laboratory settings within the settings of interest. *C. difficile* strain used was nontoxigenic.

Table F.2. Liquid chlorhexidine soap compared to plain soap and water on mean log_{10} reduction *C. difficile* colony-forming units

Certainty assessment							Finding	Certainty
Number of studies and endnote reference	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**		
Spore recovery from hands (mean log_{10} reduction *C. difficile* colony counts (log_{10} CFU/mL)								
1 Oughton MT and others, 2009 (2)	Randomised trials	Serious [note 1]	Not assessed [note 2]	Very serious [note 3]	Serious [note 4]	None	Washing with warm or cold water and plain soap achieved a small mean reduction in *C. difficile* colony counts compared to be liquid chlorhexidine soap although the confidence interval does include 0 for the cold water comparison. Warm water: 0.63 log_{10} CFU/mL (95% CI: 0.23 to 1.02); cold water: 0.37 log_{10} CFU/mL (95% CI: −0.03 to 0.76).	⊕◯◯◯ Very low [note 1, 2, 3, 4]

Notes

Note 1. Participant characteristics and allocation concealment not reported. No blinding of participants. Intervention self-administered.
Note 2. Only one study, not possible to assess inconsistency.
Note 3. Population is healthy volunteers. Study was conducted in laboratory settings within the settings of interest. *C. difficile* strain used was nontoxigenic.

Note 4. CI (cold water) includes no difference.

Table F.3. Liquid chlorhexidine soap compared to antiseptic parachlorometaxylenol hand wipes on mean log_{10} reduction *C. difficile* colony-forming units

Certainty assessment							Finding	Certainty
Number of studies and endnote reference	**Study design**	**Risk of bias**	**Inconsistency**	**Indirectness**	**Imprecision**	**Other considerations**		
Spore recovery from hands (mean log_{10} reduction *C. difficile* colony counts (log_{10} CFU/mL)								
1 Oughton MT and others, 2009 (2)	randomised trials	Serious [note 1]	Not assessed [note 2]	very serious [note 3]	None	None	Washing with warm water and liquid chlorhexidine soap achieved a greater mean reduction in *C. difficile* colony counts than parachlorometaxylenol hand wipes: 0.94 log_{10} CFU/mL (95% CI: 0.55 to 1.34)	⊕◯◯◯ Very low [note 1, 2, 3]

Notes

Note 1. Participant characteristics and allocation concealment not reported. No blinding of participants. Intervention self-administered.

Note 2. Only one study, not possible to assess inconsistency.

Note 3. Population is healthy volunteers. Study was conducted in laboratory settings within the settings of interest. *C. difficile* strain used was nontoxigenic.

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UK Health Security Agency (UKHSA) prevents, prepares for and responds to infectious diseases and environmental, radiological and chemical hazards, to keep all our communities safe, save lives and protect livelihoods. We provide scientific and operational leadership, working with local, national and international partners to protect the public's health.

UKHSA is an executive agency, sponsored by the Department of Health and Social Care.

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Completed: 10 March 2026

Published: July 2026
Publication reference: GOV-20837 (PHR034c)

Suggested citation: Bhatia A, Brown S, Prakash S, Walker J, Carville S. 'Effectiveness of hand hygiene agents as alternatives to soap and water to reduce *Clostridioides difficile* (*C. difficile*) in health and social care settings where care is provided: a rapid systematic review' UKHSA 2026