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State of the Science Review

A systematic review of chlorine-based surface disinfection efficacy to inform recommendations for low-resource outbreak settings

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Epidemic**Background:** Infectious diseases can be transmitted via fomites (contaminated surfaces/objects); disinfection can interrupt this transmission route. However, disinfection guidelines for low-resource outbreak settings are inconsistent and not evidence-based.**Methods:** A systematic review of surface disinfection efficacy studies was conducted to inform low-resource outbreak guideline development. Due to variation in experimental procedures, outcomes were synthesized in a narrative summary focusing on chlorine-based disinfection against 7 pathogens with potential to produce outbreaks in low-resource settings (*Mycobacterium tuberculosis*, *Vibrio cholerae*, *Salmonella* spp., hepatitis A virus, rotavirus, norovirus, and Ebola virus).**Results:** Data were extracted from 89 laboratory studies and made available, including 20 studies on relevant pathogens used in combination with surrogate data to determine minimum target concentration × time (“CT”) factors. Stainless steel (68%) and chlorine-based disinfectants (56%) were most commonly tested. No consistent trend was seen in the influence of chlorine concentration and exposure time on disinfection efficacy. Disinfectant application mode; soil load; and surface type were frequently identified as influential factors in included studies.**Conclusions:** This review highlights that surface disinfection efficacy estimates are strongly influenced by each study’s experimental conditions. We therefore recommend laboratory testing to be followed by field-based testing/monitoring to ensure effectiveness is achieved in situ.

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Contaminated surfaces or objects (“fomites”) can act as vehicles for infectious disease transmission.¹ This indirect transmission pathway has been found to play an important role in outbreaks of viral respiratory and enteric diseases, such as avian influenza and norovirus.^{2–4} Likewise, contaminated surfaces and needles can contribute to the spread of Ebola in treatment units^{5–7} and indirect cholera transmission has been documented in affected communities.^{8–10}

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Cleaning, disinfection, and sterilization can interrupt infectious disease transmission via fomites. Cleaning is “the removal of visible soil from objects or surfaces” and is often used in conjunction with disinfection, defined as “a process that eliminates many or all pathogenic microorganisms, except bacterial spores, on inanimate objects”, while sterilization is “a process that destroys or eliminates all forms of microbial life.”^{11,12} Disinfection interventions in health care facilities, public spaces, or households are usually part of environmental infection prevention and control strategies deployed in response to infectious disease outbreaks.

While a wide range of disinfectants may be used in high-income settings, including chlorine, alcohol-based products, glutaraldehyde, quaternary ammonium compounds, and hydrogen peroxide,¹² disinfection in low-income and emergency settings with limited access to

basic supplies (“low-resource”) is primarily chlorine-based, as this disinfectant is inexpensive and widely available. Another advantage of chlorine is its broad microbicidal spectrum, while disadvantages include corrosive and irritant properties at high concentrations, potential surface staining, inactivation by organic matter, as well as rapid chlorine decay in solution for some chlorine compounds.^{12,13} Four chlorine compounds are commonly used in emergency response. Sodium hypochlorite (NaOCl), also referred to as household bleach, is widely available as a stabilized (pH 9–11), colorless liquid at concentrations 4,000–6,000 ppm and a shelf-life of at least a month at 25–30°C.^{13,14} Electrolyzed water – or generated NaOCl (gNaOCl) – is produced by electrolysis of a salt (NaCl) solution, has a close-to-neutral pH, and is generated on site, as it expires in days to weeks without stabilization.¹³ In addition to hypochlorous acid, gNaOCl may contain reactive oxygen species that contribute to disinfection.¹⁵ Sodium dichloroisocyanurate (NaDCC) is available as a stable powder; once in solution, however, it has a low pH (~6) and short shelf-life as it decays rapidly (within hours).¹³ It has been reported to be less sensitive to inactivation by organic matter than other chlorine compounds.¹⁶ Finally, calcium hypochlorite or high-test hypochlorite (HTH) is a salt of hypochlorite, like NaOCl, however it is more commonly found in granular form and produces solutions at pH 9–11 that are as stable as NaOCl.^{13,14}

The type and amount of chlorine-based disinfectant used will influence disinfection efficacy. Other parameters that influence disinfection efficacy include^{14,17}: (1) the target microorganism’s resistance to disinfection; (2) the nature of the fomite to disinfect, for example, porous or nonporous surfaces, and presence of organic matter; (3) characteristics of the tested disinfectant such as ingredients, mode of action and concentration; and, (4) the disinfection protocol, as pre-cleaning, disinfectant mode of application, and exposure time can influence efficacy.

Guidelines on how to clean and disinfect surfaces in infectious disease outbreaks lack consistency. For example, during the 2014 Ebola outbreak, different chlorine exposure times (not specified or up to 15 minutes) and practices (precleaning, covering spills, or direct application) were recommended.^{18–20} Recommendations for surface disinfection in cholera outbreaks also vary in terms of chlorine concentration (from 0.1% to 2.0%), exposure time (not specified or up to 10 minutes), and practices (precleaning, spraying, or direct application) – both in health care^{21–23} and household settings.^{23–26} Additionally, despite the widespread availability of chlorine types with different physicochemical characteristics, outbreak response guidelines do not generally specify which chlorine type should be used to prepare disinfection solutions.

Inconsistencies between guidelines are due, in part, to the lack of evidence to inform disinfection protocols in response to outbreaks, as highlighted during the 2014 Ebola virus disease outbreak.^{27,28} Conducting research in humanitarian settings is challenging, and a recent systematic review found no rigorous evaluation of the field effectiveness or health impacts of surface disinfection in response to outbreaks.²⁹ However, there is a large body of literature on the laboratory-based efficacy of surface disinfection – including studies from different research fields such as hospital infection control, food science, or bioterrorism prevention – that has the potential to inform disinfection interventions in response to outbreaks.

To improve consistency in guidelines and inform recommendations for infectious disease outbreak response interventions in low-resource settings, we conducted a systematic review of laboratory-based surface disinfection efficacy studies. Specifically, we sought to synthesize surface disinfection efficacy data across test organisms, surface types, and disinfectants; to provide evidence-based recommendations for disinfection of pathogens that have the potential to cause large outbreaks in low-resource settings; and to identify and

discuss cross-cutting themes and methodological considerations in surface disinfection efficacy studies as well as future research needs.

METHODS

We conducted a systematic review of scientific publications assessing chlorine-based surface disinfection efficacy following the PRISMA guidelines and reporting requirements for systematic reviews.³⁰ First, a broad search was performed to identify potentially relevant references, and titles and abstracts were successively screened. Minimal information was extracted from the abstracts and used to refine exclusion criteria prior to full text screening. Surface disinfection efficacy data from eligible full texts were then recorded and a synthesis of disinfection efficacy was prepared.

Eligibility criteria

Criteria were defined following the “Population, Interventions, Comparisons, Outcomes, and Study type” framework. The population for this systematic review consisted of microorganisms on surfaces. The studied intervention was, initially, chemical disinfection. Physical disinfection processes, such as ultraviolet light and heat, were not included. Comparisons were possible between different disinfectants or chlorine compounds; different test organisms; and different surfaces.

The outcome of interest was disinfection efficacy, defined as the proportion of pathogens or surrogate organisms that are inactivated by disinfection, estimated as:

$$\frac{[\text{organisms on surface after disinfection}]}{[\text{organisms on surface before disinfection}]}$$

No restriction was placed on study type, however only studies providing a quantitative outcome for surface disinfection efficacy were included. Articles published in English, German, French, Spanish, Portuguese, and Italian were included. Other languages were excluded. No restriction was placed on year of publication.

Information sources and search strategy

Three online databases (PubMed, Scopus, and ISI Web of Knowledge) were searched on March 17, 2017 using specific keywords and strategies, including Medical Subject Headings on PubMed and Index terms on Scopus (Table 1). The full search was repeated on May 16, 2018 to include more recent publications. On July 25, 2019, the full search was repeated again, however the screening and data extraction processes were modified to target publications relevant to chlorine disinfection against 7 selected pathogens, as described below. In addition to the online searches, references from review articles identified as potentially relevant were screened for the identification of additional eligible studies.

Screening and data collection process

Online search results were exported into EndNote X7.7.1 (Thomson Reuters, Canada) and duplicates were removed from the initial search results. References were then screened by title, abstract, and full text in Excel 2016 (Microsoft, Washington), based on the exclusion criteria described below for the initial search and first search update.

Titles that suggested the study would be related to chemical disinfection of a surface were included for abstract screening. Abstracts reporting a quantitative assessment of disinfection efficacy initially passed screening. During abstract screening, information was extracted, if available, on study type, test organism, disinfectant, and surface. This information was used to refine inclusion criteria for full

Table 1
Search terms used on 3 online databases

PubMed	Scopus	ISI web of knowledge
(surfac* OR fomit*) AND (resist* OR surviv* OR inactiv*) AND ((disinfect* OR clean* OR decontamina* OR sanitiz*) OR ("Equipment Contamination"[Mesh] OR "Disinfection"[Mesh] OR "Disinfectants"[Mesh] OR "Disease Outbreaks/prevention and control"[Mesh] OR "Disease Outbreaks/transmission"[Mesh]) OR ("infection control" OR "cross infection"))	ALL ((surfac* OR fomit*) AND (disinfect* OR sanitiz* OR clean* OR decontamina* OR "infection control" OR "cross infection") AND (resist* OR surviv* OR inactiv*) AND NOT water) AND INDEXTERMS ((surfac* OR fomit*) AND (disinfect* OR sanitiz* OR cleaning OR decontamina* OR "infection control" OR "cross infection"))	TS= ((surfac* OR fomit*) AND (disinfect* OR sanitiz* OR clean* OR decontamina* OR "infection control" OR "cross infection") AND (resist* OR surviv* OR inactiv*)) NOT TS=(water)

text screening. Studies evaluating the following items were excluded prior to the full text screening: (1) disinfectants including surface coatings and gaseous disinfectants, due to their limited applicability in low-resource settings; (2) living surface materials such as vegetables, meat, or skin, which do not qualify as fomite (inanimate); (3) prions and fungi, and environmental sampling where an uncontrolled amount of test organisms was on the test surface prior to disinfection. Additionally, all studies using disinfectants, surfaces, or test organisms that were recorded less than 5 times during abstract screening as well as disinfectants with multiple active ingredients were excluded prior to full text screening due to the expected limited ability to make comparisons between items used in less than 5 studies and the fact that novel or proprietary disinfectants presented in few studies are unlikely to be available in low-resource settings. In cases where it was unclear from the abstract whether a study was eligible for full text screening, it was included for determination during full text screening.

During full text screening, data were extracted from studies providing a quantitative outcome for chemical surface disinfection efficacy using a clearly defined disinfectant, test organism, and surface not previously excluded. The following parameters were recorded for included studies: test organism, preparation, washing or purification if any, and observed inactivation (eg, reported % or log reduction); surface type; disinfectant type, concentration, pH, mode of application, exposure time, neutralization method, and any available information on its safety, stability, and cost; experimental conditions, including temperature, relative humidity, carrier size, addition of soil load, drying time, mode of recovery, types of controls, positive control level, detection limit, and number of replicates; main study conclusions and comments. Please note all log inactivation reported in this manuscript refer to log₁₀.

For the second search update conducted prior to manuscript submission, title screening criteria remained the same. However, given changes to the data analysis strategy and focus on 7 selected pathogens relevant to low-resource settings (as described below), abstract and full text screening were modified to include only studies that quantitatively assessed surface disinfection efficacy against these 7 pathogens. As previously, in cases where it was unclear based on the abstract whether a study contained relevant data, it was included for full text screening.

Throughout full text data extraction, if results were only presented in graphical format without data labels, corresponding authors were contacted via email to request data labels.

A reason for exclusion was recorded for all studies that were not included after title, abstract, or full text screening. All data entry was performed independently by 2 individuals and cross-checked for accuracy. Any discrepancies were discussed and resolved through consensus.

Quality appraisal

Quality appraisal criteria relevant to microbiological laboratory testing were adapted from Yeargin et al.³ Five criteria were defined: (1) adequate controls: at least positive controls (no disinfectant), and cytotoxicity controls if the test organism is a virus quantified using a culture-based assay; (2) methods clearly described; (3) detection method that includes recovery of etiological agent from surface carrier, disinfectant neutralization, and use of culture or propagation method for quantification; (4) at least duplicate experiments; and (5) appropriate statistical analysis (eg, providing a measure of variability). A pass¹ or fail (0) score for each criterion was determined for the studies selected for full text review, giving a maximum score of 5 points. Only studies with a score of 3 or more, considered moderate quality and above, were included in the synthesis.

Data analysis

After data extraction, analysis for this systematic review was articulated in 3 phases: (1) mapping of data points across categories of test organisms, surfaces, and disinfectants; (2) synthesis of efficacy outcomes focused only on chlorine-based disinfection and pathogens relevant to low-resource settings; (3) identification of cross-cutting themes and data on factors influencing surface disinfection efficacy. Each phase is described below in more detail.

First, the numbers of data points (ie, reported efficacy outcomes for 1 test condition) recorded for each combination of test organism (bacteria, viruses, and spores), surface type, and disinfectant were mapped in color-coded tables to visualize research trends – ie, identify which items are the most studied, and which ones remain under-researched. This was also completed with the subset of the data including only chlorine compounds to compare the number of data points available.

Second, the synthesis of disinfection efficacy outcomes for this manuscript was restricted to chlorine-based disinfection, as this is the most widely used disinfectant in low-resource outbreak settings. Efficacy outcomes were stratified according to test organism, surface, and chlorine type, and reported or expressed as log reductions for a given chlorine concentration and contact time (or "CT factor," defined as the multiplication of both). Summary tables were prepared by type of test organism – gram-positive and gram-negative bacteria, viruses, and spores.

Please note that while our initial intent was to perform a meta-analysis, appropriate statistical methods could not be identified given the characteristics of the extracted data. In particular, aggregation categories could not be defined due to the heterogeneity of testing protocols. Data points were plotted using MATLAB R2017a (The

MathWorks, Inc., Natick, MA) to visualize the distribution of reported efficacies by surface type, test organism, and disinfectant.

A more qualitative approach was then selected that was in line with the objectives of the work. We identified studies using pathogens with potential to cause outbreaks in low-resource settings from the list of references processed through abstract screening. These studies were included in a summary table and narrative synthesis. The 7 selected pathogens for which data were available were: *Mycobacterium tuberculosis*, *Vibrio cholerae*, *Salmonella enterica*, hepatitis A virus, rotavirus, norovirus, and Ebola virus. *M. tuberculosis* was responsible for 1.6 million deaths in 2017, making it among the global top 10 causes of death, 95% of which in low-income countries.³¹ *V. cholerae* causes an estimated 95,000 annual cholera deaths among vulnerable populations worldwide.³² Like *V. cholerae*, *Salmonella* spp. are gram-negative bacteria responsible for diarrheal disease outbreaks. One serovar is particularly important: *S. enterica* serovar Typhi is estimated to cause 11.9 million cases of typhoid fever and 129,000 deaths per year in low- and middle-income countries,³³ and the frequency of prolonged, severe outbreaks has been reported to increase across sub-Saharan Africa.³⁴ The hepatitis A virus produces explosive outbreaks, and causes approximately 1.4 million cases per year, primarily in areas without adequate water, sanitation and hygiene.^{35,36} Rotavirus is responsible for approximately half of the global diarrheal disease burden of 215,000 annual deaths in children under 5 years, concentrated in low-income countries.^{37,38} Norovirus is also a major cause of gastroenteritis worldwide, causing approximately 200,000 deaths per year, primarily in low-income countries and among children under 5 years.^{39,40} Ebola virus is among 5 viral hemorrhagic fever agents on the list of ten priority pathogens identified on the World Health Organization (WHO) Blueprint list,⁴¹ and is responsible

for an ongoing epidemic in the Democratic Republic of the Congo that has been declared a public health emergency of international concern.⁴² Studies where a surrogate was proposed for one of the 7 selected pathogens were included in the narrative synthesis as well.

In order to guide recommendations for disinfection against the 7 selected pathogens, the US Environmental Protection Agency (US EPA) efficacy criteria for disinfectants to be used on environmental surfaces were taken as a reference⁴³: if possible based on available data, CT factors that could reliably achieve 4 log reduction in *M. tuberculosis* (or surrogates), 5 log reduction in *Salmonella* spp and other bacterial pathogens, or 3 log reduction in viral pathogens were identified.

Lastly, information recorded on study conclusions during full text screening was used to identify and inform the discussion of parameters relevant to low-resource outbreak settings and, more broadly, to the understanding of parameters influencing surface disinfection efficacy.

RESULTS

Screening process

A total of 12,730 references were retrieved in the initial (2017) and second (2018) online searches on 3 databases. After removal of 2,118 duplicates, 10,612 titles and 1,985 abstracts were screened (Fig 1). During abstract screening, 800 studies were identified as likely to provide a quantitative efficacy assessment of surface disinfection efficacy. Twenty-nine additional studies were found by screening references from relevant reviews. In the second search update (in 2019), which focused on 7 pathogens of interest, 1,096 new publications were identified and 3 studies were included for full text screening. During full text screening, data were extracted from

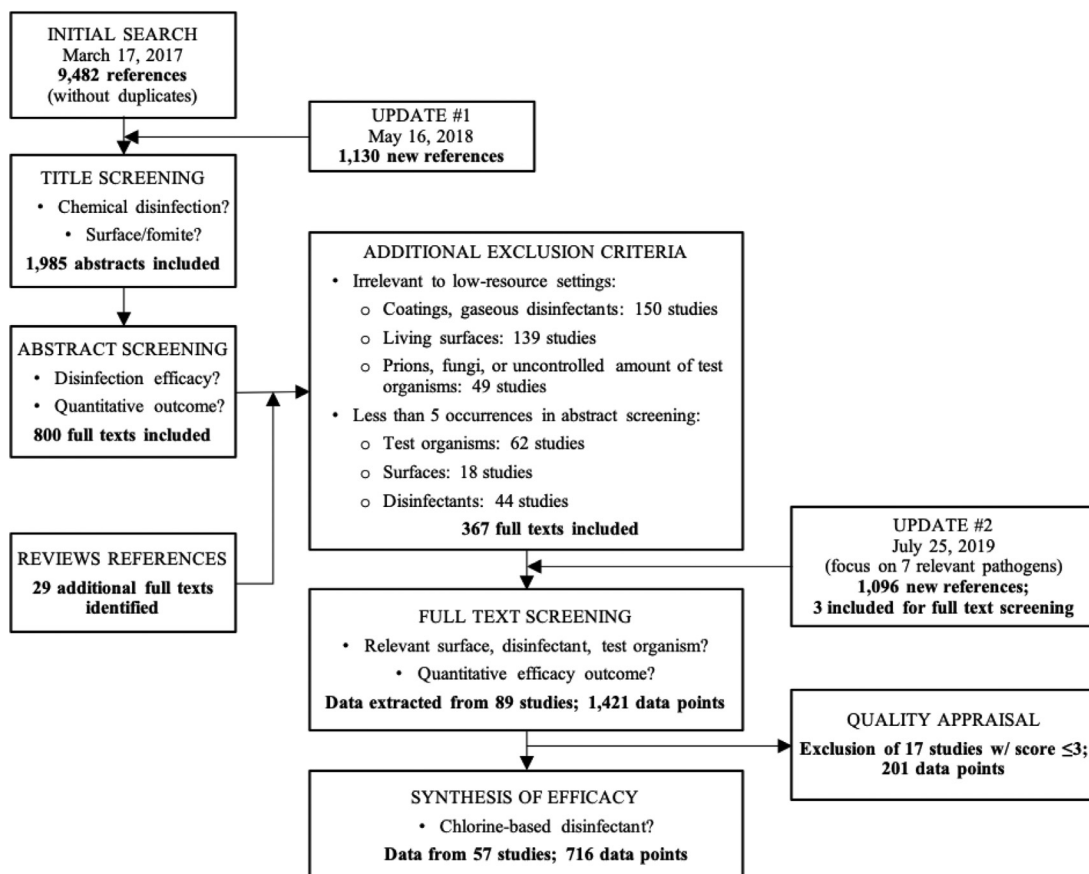


Fig 1. PRISMA systematic review flow chart.

Table 2

Distribution of data points from studies that passed full text. Blue shades intensity increases with increasing numbers

# Data points	Chlorine	Alcohols	GAH	H ₂ O ₂	PAA	CHX	ClO ₂	Others	TOTAL
Ceramic	Bacteria	5	0	0	0	0	0	3	14 (1.0%)
	Virus	6	0	0	0	0	0	0	
	Bacterial spores	0	0	0	0	0	0	0	
Rubber	Bacteria	5	0	2	1	1	0	2	26 (1.8%)
	Virus	0	0	0	0	0	0	0	
	Bacterial spores	13	0	2	0	0	0	0	
Fabric	Bacteria	4	40	0	0	0	0	0	54 (3.8%)
	Virus	5	4	0	0	0	0	0	
	Bacterial spores	0	1	0	0	0	0	0	
Glass	Bacteria	10	10	1	2	0	0	4	82 (5.8%)
	Virus	3	12	1	2	0	0	0	
	Bacterial spores	12	2	4	2	13	0	4	
Wood	Bacteria	1	0	0	0	0	0	0	71 (5.0%)
	Virus	2	0	0	0	0	0	2	
	Bacterial spores	53	0	4	0	5	0	4	
Plastics	Bacteria	37	16	0	0	0	4	0	203 (14%)
	Virus	53	19	24	0	3	4	4	
	Bacterial spores	32	0	0	0	1	0	6	
Stainless steel	Bacteria	313	130	7	31	3	28	2	971 (68%)
	Virus	177 *	79	25	36	26	5	1	
	Bacterial spores	67	1	4	0	6	0	15	
TOTAL	798	314	74	74	58	41	32	30	1421
	(56%)	(22%)	(5.2%)	(5.2%)	(4.1%)	(2.9%)	(2.3%)	(2.1%)	

CHX, chlorhexidine; ClO₂, chlorine dioxide; GAA, glutaraldehyde; H₂O₂, hydrogen peroxide; PAA, peracetic acid.

*Including 2 data points from 1 study retrieved during the second search update in 2019.

89 studies, for a total of 1,421 data points. Of these, 716 data points were from 57 studies using chlorine-based disinfectants and that had received a quality score >3. Ultimately, 20 studies were included in the narrative synthesis focusing on 7 selected pathogens relevant to low-resource outbreak settings.

Distribution of data points

In studies that passed full text screening (1,421 data points), test organisms included bacteria (675 data points, 48%), viruses (495 data points, 35%), and spores (251 data points, 18%) (Table 2). The distribution between types of test organisms was similar in studies using chlorine-based disinfectants (Table 3): from a total of 716 data points for chlorine-based disinfection, bacteria accounted for 398 data points (56%), viruses for 201 data points (28%), and spores for 117 data points (16%).

The most commonly studied surface was stainless steel, with 971 data points (68%) out of 1,421, followed by plastics, wood, glass, fabric, rubber, and ceramic (Table 2). Nonporous surfaces were used in 90% of the tested conditions (1,282 data points). Chlorine-based disinfectants were also studied mostly on

nonporous surfaces, with only 12% of the tested conditions (88 data points) on porous surfaces.

Chlorine-based disinfectants were the most studied disinfectants (798 data points, 56%) followed by alcohols (314 data points, 22%). Other disinfectants included glutaraldehyde, hydrogen peroxide, peracetic acid, chlorhexidine, and chlorine dioxide, each of them accounting for less than 5% of all data points. Among chlorine compounds, NaOCl accounted for almost half of the data (339 data points, 47%), followed by NaDCC (218 data points, 30%), and gNaOCl (150 data points, 21%; Table 3). HTH was used in only 2 studies, for a total of 7 data points (1%).

Quality scores

Of the 89 studies that were selected for data extraction after full text screening, almost half (47%, n = 42) had a quality score of 5; a third had a quality score of 4 (34%, n = 30). Seventeen studies (19%) were excluded following quality appraisal, including fifteen that had a score of 3 and 2 with a score of 2 points. Please note studies with a quality score of 3 were included in the narrative summary.

Table 3

Distribution of data points included in the synthesis of chlorine-based disinfection efficacy. Green shades intensity increases with increasing numbers

# Data points		NaOCl	gNaOCl	NaDCC	HTH	Cl2	TOTAL
Ceramic	Bacteria	1	4	0	0	0	11 (1.5%)
	Viruses	6	0	0	0	0	
	Bacterial spores	0	0	0	0	0	
Fabric	Bacteria	4	40	0	0	0	53 (7.4%)
	Viruses	5	4	0	0	0	
	Bacterial spores	0	0	0	0	0	
Glass	Bacteria	8	2	0	0	0	25 (3.5%)
	Viruses	3	0	0	0	0	
	Bacterial spores	12	0	0	0	0	
Rubber	Bacteria	3	2	0	0	0	18 (2.5%)
	Viruses	0	0	0	0	0	
	Bacterial spores	13	0	0	0	0	
Wood	Bacteria	1	0	0	0	0	24 (3.4%)
	Viruses	1	0	0	1	0	
	Bacterial spores	21	0	0	0	0	
Plastic	Bacteria	19	6	2	2	0	83 (12%)
	Viruses	33	16	0	1	0	
	Bacterial spores	2	0	2	0	0	
Stainless steel	Bacteria	63	48	189	2	2	502 (70%)
	Viruses	79 *	28	23	1	0	
	Bacterial spores	65	0	2	0	0	
TOTAL		339	150	218	7	2	716
		(47%)	(21%)	(31%)	(1.0%)	(0.3%)	

Cl, dilution of a saturated chlorine solution; gNaOCl, electrolyzed water or on-site generated NaOCl; HTH, high-test calcium hypochlorite; NaDCC, sodium dichloroisocyanurate; NaOCl, sodium hypochlorite.

*Including 2 data points from 1 study retrieved during the second search update in 2019.

Eighty-five studies (96%) appeared to use appropriate detection methods that included recovery of the etiological agent from surface carrier, disinfectant neutralization, and culture or propagation method for quantitation of the agent; 84 (94%) had adequate controls; and, 82 (92%) performed experiments in duplicate at least. In 16 studies (18%), methods lacked clarity and/or insufficient experimental details were provided, particularly regarding disinfection procedures (eg, disinfectant concentration or recovery). Lastly, 54 (61%) did not obtain the point for “appropriate statistical analysis,” the most common reason being that no measure of variability was provided for the disinfection efficacy outcome.

Synthesis of disinfection efficacy

The editable Excel spreadsheet containing all data extracted from 89 studies (1,421 data points) – including chlorine-based and other

disinfectants – is made available in supplementary materials (File S1). Additionally, summary tables for studies that provided a quantitative assessment of chlorine-based disinfection efficacy and had a quality score >3 are presented in supplementary materials for gram-positive bacteria (Table S1), gram-negative bacteria (Table S2), viruses (Table S3), and spores (Table S4).

As an example of the observed variability, which precluded meta-analysis, reported log reductions against bacteria and viruses achieved by sodium hypochlorite on stainless steel spread across up to 8 log for a given concentration-exposure time (“CT”) factor (Fig 2).

Reported chlorine disinfection efficacy outcomes against the 7 selected pathogens with potential to cause outbreaks in low-resource settings are summarized in Table 4, and narratively in text below. Please note we found no data in this review to inform surface disinfection efficacy against *Shigella* spp. or pathogens listed on the WHO Blueprint list of priority pathogens⁴¹ other than the Ebola virus.

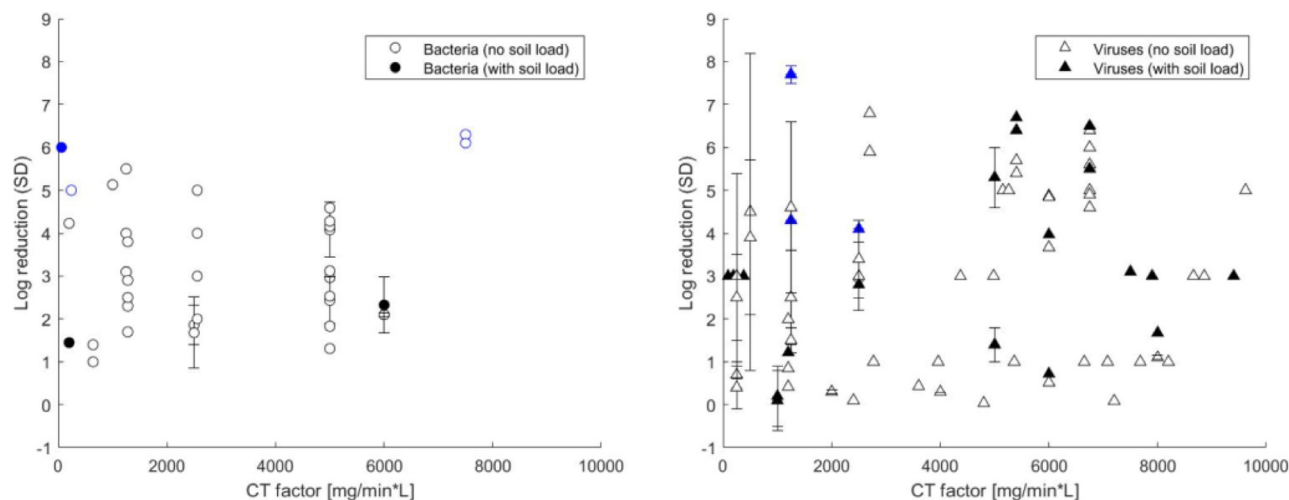


Fig 2. Reported log reductions for sodium hypochlorite (NaOCl) concentration-exposure time (“CT”) factors up to 10,000 mg min/L against bacteria and viruses. Error bars correspond to standard deviations when reported in the publications. Blue symbols correspond to studies where the limit of detection for the upper bound of the log reduction (y-axis) was reached.

Mycobacterium tuberculosis

M. tuberculosis were used in only 1 study where exposure to 6,000–10,000 ppm NaOCl and NaDCC for 1 minute (CT = 6,000–10,000 mg × min/L) on stainless steel produced 2.08–3.20 log reduction in the absence of soil load and 2.28–3.22 log reductions when sputum was added.⁴⁴ Best et al⁴⁴ noted that *M. tuberculosis* is not typically used in disinfection studies due to safety concerns and slow growth, and tested *M. smegmatis* as a potential nonpathogenic and faster-growing surrogate for *M. tuberculosis*. Varying levels of resistance were observed depending on the tested disinfectant. When exposed to sodium hypochlorite, *M. smegmatis* appeared less resistant than *M. tuberculosis*, highlighting the need to interpret surrogate testing results with caution.⁴⁴ Other bacteria from the same genus, *M. terrae*, underwent 8.19 log reduction following exposure to 2,500 ppm sodium hypochlorite for 5 minutes (CT = 12,500 mg × min/L).⁵⁴ The limited log reduction observed for treatment with CT up to 10,000 mg × min/L for *M. tuberculosis* and the high variation in log reductions for related surrogates highlights the need for further evaluation of effective disinfection strategies. Specifically, there is a data gap on exposure times and concentrations producing CT factors higher than 10,000 mg × min/L against *M. tuberculosis*. Treatments producing CT factors below 10,000 mg × min/L are likely insufficient for *M. tuberculosis* disinfection.

Vibrio cholerae and *Salmonella* spp

Vibrio cholerae and *Salmonella* spp. are gram-negative bacterial pathogens similar to the more commonly used bacteria *Escherichia coli* and *Pseudomonas* spp. The review identified 6 studies investigating disinfection of *V. cholerae* (n = 1) and *Salmonella* spp. (n = 5).

The resistance of *V. cholerae* to chlorine was evaluated in a study focused on improving US preparedness to bioterrorism threats.⁴⁵ Chlorine (pH-amended bleach, 6,200 ppm, pH 6.8) was dosed by a hand-held sprayer until coupons of glass, aluminum, wood, carpet, and concrete were visibly wetted. Reported reductions were >6 log on glass and aluminum after 15 minutes exposure time (CT = 93,000 mg × min/L) and approximately 5 log on carpet after 2 disinfectant applications and 30 minutes exposure time (CT = 186,000 mg × min/L). Efficacy could not be evaluated on wood and concrete due to poor recoveries.

The studies investigating surface disinfection efficacy of *Salmonella* spp. included 4 studies on *Salmonella enterica* serovar Enteritidis

and 1 on *S. Typhimurium*. Exposure of *S. Enteritidis* to NaOCl (800 ppm) resulted in 3 log reduction after 30 minutes on viscose without soil load (CT = 24,000 mg × min/L).⁴⁶ On stainless steel, Riazi et al⁴⁸ reported increasing log reductions (up to 5 log) in *S. Enteritidis* with increasing NaOCl concentrations (up to 512 ppm) applied for 5 minutes in absence of soil load (CT = 2,560 mg × min/L), whereas Tuladhar et al,⁴⁹ who assessed the effect of wiping a surface contaminated with *S. Enteritidis* with a cloth soaked in NaDCC (1,000 ppm), observed a maximum inactivation of 2.9 log after 20 minutes (CT = 20,000 mg × min/L). In contrast, Ni et al^{47,50} observed >6 log reduction in *S. Typhimurium* after applying electrolyzed water (gNaOCl, 40–80 ppm, pH 5.7–6.5) on fabric without soil load for 5 minutes (CT = 200–400 mg × min/L) and >5.6 log reduction when gNaOCl was sprayed on stainless steel with a longer exposure time (30 minutes, CT = 1,200–2,400 mg × min/L) in presence of 20% pig slurry.

E. coli and *P. aeruginosa* are 2 gram-negative bacteria widely used in surface disinfection efficacy testing and results from studies using these bacteria may provide insight into disinfection of other gram-negative bacteria including *V. cholerae* and *Salmonella* spp. (Table S2). Relatively high log reductions (4.7 to >7.3) were reported for *E. coli* across a range of surfaces and CT factors (200–50,000 mg × min/L). The CT factors reported to achieve 5 to >6 log reductions in the only study with *V. cholerae* identified in this review were much higher (93,000–186,000 mg × min/L) than those observed to achieve similar log reduction values (LRVs) in *E. coli*.⁴⁵ However, in experiments using NaDCC on 6 different surfaces (CT = 200–2,000 mg × min/L), we observed consistently higher reductions in culturable *V. cholerae* (5.4–7.0 log) compared to *E. coli* (1.4 to >6.9).⁶³ *E. coli* and *Salmonella* spp. exhibit similar sensitivities to gNaOCl.^{47,50} While less than 3 log reductions were observed in *Salmonella* spp. in 2 studies, with CT factors between 20,000 and 24,000 mg × min/L for NaOCl and NaDCC,^{46,49} Riazi et al⁴⁸ reported 2, 4, and 5 log reductions in *P. aeruginosa*, *E. coli*, and *S. Enteritidis*, respectively, exposed to NaOCl (CT = 2,560 mg × min/L).

Notably, high variation in LRVs are observed between studies investigating gram-negative bacteria, with no clear relationships to CT factors, in line with findings for all bacteria (Fig 2). Nevertheless, conservative approaches to disinfection (use of high chlorine concentrations with long exposure times) could be selected. For example, CT factors on the order of 200,000 mg × min/L could be targeted for disinfection in cholera outbreaks; however, as this may be challenging to implement in practice and data suggest that lower CT factors may

Table 4
Selected chlorine disinfection efficacy outcomes against pathogens relevant to low-resource settings

Test organism	Surface	Study	Disinfectant	Concentration (mg/L)	Exposure time (min)	CT factor (mg × min/L)	Soil load	LRV	Comments	
<i>Mycobacterium tuberculosis</i>	Stainless steel	Best et al ⁴⁴	NaOCl, NaDCC	6,000–10,000	1	6,000–10,000	None	2.08–3.20		
		Best et al ⁴⁴	NaOCl, NaDCC	6,000–10,000	1	6,000–10,000	Sputum	2.28–3.22		
<i>Vibrio cholerae</i>	Aluminum	Calfee et al ⁴⁵	NaOCl	6,200	15	93,000	None	>6.0	Spray, pH 6.8	
	Glass	Calfee et al ⁴⁵	NaOCl	6,200	15	93,000	None	>6.0	Spray, pH 6.8	
	Carpet	Calfee et al ⁴⁵	NaOCl	6,200	30	186,000	None	>6.0	Spray (2x), pH 6.8	
<i>Salmonella</i> spp.	Cotton, fabric	Kusumaningrum et al ⁴⁶	NaOCl	800	10–30	8,000–24,000	None	2.0–3.0	Viscose	
		Ni et al ⁴⁷	gNaOCl	40–80	5–10	200–800	None	>6.0	pH 5.70	
	Stainless steel	Riazi et al ⁴⁸	NaOCl	128/512	5	640/2,560	None	<1.0/5.0	pH 7.2	
		Tuladhar et al ⁴⁹	NaDCC	250/1,000	20	5,000–20,000	0.03% BSA	1.5/2.9	Wiping	
		Tuladhar et al ⁴⁹	NaDCC	250/1,000	20	5,000–20,000	1% stool	1.8/2.9	Wiping	
	Hepatitis A virus	Aluminum	Ni et al ⁵⁰	gNaOCl	50/70	30	1,500/2,100	20% pig fecal slurry	>5.64	Spray, pH 6.51–6.56
			Ni et al ⁵⁰	gNaOCl	18	30	540	20% pig fecal slurry	4.26	Spray, pH 10.08
Rotavirus	Plastic	Jean et al ⁵¹	NaOCl	3,000	5	15,000	None	~5	Polystyrene	
		Abad et al ⁵²	NaOCl	1,250	10	12,500	None	2.58	Polystyrene	
		Abad et al ⁵²	NaOCl	1,250	10	12,500	20% stool	1.12	Polystyrene	
	Stainless steel	Jean et al ⁵¹	NaOCl	3,000	5	15,000	None	~5	LDPE, PVC	
		Martin et al ⁵³	NaOCl	10,000	30	300,000	1% BSA, 1% yeast extract	4.0		
		Martin et al ⁵³	NaOCl	10,000	30	300,000	1% BSA, 1% yeast extract	4.0		
		Jean et al ⁵¹	NaOCl	3,000	5	15,000	None	~5		
Ebola virus	Stainless steel	Sabbah et al ⁵⁴	NaOCl	2,500	5	12,500	ASTM	4.41		
		Abad et al ⁵²	NaOCl	1,250	10	12,500	None	2.76	Polystyrene	
		Abad et al ⁵²	NaOCl	1,250	10	12,500	20% stool	1.62	Polystyrene	
Human norovirus	Aluminum	Sattar et al ⁵⁵	NaDCC	800	10	800	10	8,000	10% stool	
	Seat belt fabric	Smither et al ⁵⁶	NaOCl	8,000	10	80,000	None	>5	Yambuku variant	
		Smither et al ⁵⁶	NaOCl	8,000	10	80,000	None	>3	Yambuku variant	
	Stainless steel	Cook et al ⁵⁷	NaOCl	1,000–5,000	5	5,000–25,000	ASTM	>6	Makona variant	
		Cook et al ⁵⁸	NaOCl	1,000–5,000	5	5,000–25,000	ASTM	>6	Mayinga, Kikwit, Makona variant	
Human norovirus	Ceramic	Smither et al ⁵⁹	NaOCl	5,000	15	75,000	Human blood	0.9*	Makona variant	
		Park et al ⁶⁰	gNaOCl	18.8/188	10/1	188	1% stool	3.0	pH 5.5–6.2	
	Stainless steel	Park et al ⁶⁰	gNaOCl	18.8/188	5/1	94/188	1% stool	3.0	pH 5.5–6.2	
		Tuladhar et al ⁴⁹	NaDCC	1,000	20	20,000	1% stool/0.03% BSA	1.5/1.7	Wiping	
		Moorman et al ⁶¹	gNaOCl	250 ppm	10–30 min	2,500–7,500	None	1.6–5.0	pH 7.0	
		Moorman et al ⁶¹	gNaOCl	250 ppm	10–30 min	2,500–7,500	ASTM	<0.3	pH 7.0	
		Cromeans et al ⁶²	NaOCl	200–1,000 ppm	5 min	1,000–5,000	10% FBS	<0.5, <1	GI.13SP and GI.5SP	

*Unclear if results are for aluminum and/or stainless steel.

be efficacious, future disinfection efficacy assessments should test lower CT factors against *V. cholerae*. For *Salmonella* spp., using CT factors >24,000 mg × min/L is likely necessary to ensure 5 log reduction and further studies are needed to confirm the efficacy and reliability of gNaOCl at low CT factors (<3,000 mg × min/L) against gram-negative bacteria.

An additional complication with gram-negative bacteria is the ability to enter a viable but nonculturable (VBNC) stage where they lose the ability to grow on culture media but remain infectious and can withstand greater oxidative stress.⁶⁴ Recent results from our laboratory and the broader literature suggest that disinfection efficacy may be overestimated for bacteria that can enter VBNC, which includes *V. cholerae*, *M. tuberculosis*, and other pathogenic bacteria.^{63–66} This was not investigated in studies identified through this review. We recommend further research be conducted to better understand the resistance of VBNC pathogenic bacteria to surface disinfection and their relevance to disease transmission.

Hepatitis A virus and rotavirus

The hepatitis A virus and rotavirus are 2 viruses causing frequent outbreaks of enteric disease.^{35,38} The hepatitis A virus was used in 4 and rotavirus in 2 surface disinfection studies identified in this review.

In the first study using the hepatitis A virus, 1.12 and 2.58 log reductions were observed after 10 minutes exposure to 1,250 ppm NaOCl (CT factor = 12,500 min × mg/L) on polystyrene with and without 20% stool, respectively.⁵² On stainless steel, using the American Society for Testing and Materials (ASTM) standard soil load, the hepatitis A virus was exposed to 2,500 ppm for 5 minutes (CT factor = 12,500 min × mg/L), resulting in a 4.4 log reduction.⁵⁴ In another study using polystyrene and stainless steel carriers, a concentration of 1% NaOCl (10,000 ppm) was required to achieve 4 log reduction in 30 minutes (CT = 300,000 min × mg/L) in the presence of 1% bovine albumin and 1% yeast extract, and it was noted that the hepatitis A virus was particularly resistant to drying.⁵³ Lastly, sodium hypochlorite was identified as the most efficacious disinfectant in 1 study using the hepatitis A virus on stainless steel, aluminum, copper, high-density polyethylene and polyvinyl chloride, with approximately 5 log reduction after 5 minutes exposure to 3,000 ppm NaOCl (CT = 15,000 min × mg/L) across all materials.⁵¹ Please note the last 2 studies were initially excluded from the review due to a lack of details in the methods regarding procedures and data analysis (quality scores of 3), but were included in this narrative summary.

Human rotavirus was used as test organism in 2 studies included in this review. With 10% stool, 1.7 log reduction was observed after 10 minutes exposure to 800 ppm NaDCC (CT = 8,000 mg × min/L) on stainless steel.⁵⁵ On plastic carriers, after 10 minutes exposure to 1,250 ppm NaDCC (CT = 12,500 mg × min/L), 2.8 and 1.6 log reduction in human rotavirus occurred without soil load and with 20% stool, respectively.⁵²

In a study comparing hepatitis A virus, human rotavirus, and bacteriophage B40-8 exposed to NaOCl on polystyrene (CT = 12,500 mg × min/L), log reductions were lower for B40-8 (0.7–1.3 log) compared to hepatitis A virus (1.1–2.6 log) and human rotavirus (1.6–2.8) but the differences were not statistically significant.⁵² Wiping a stainless steel surface with 1,000 ppm NaDCC reduced simian rotavirus by 4.2–5.7 log after 20 minutes (CT = 20,000 mg × min/L) under “clean” (0.03% BSA) and “dirty” (1% stool) conditions.⁴⁹

These results overall suggest that CT factors higher than 15,000 mg × min/L (and possibly as high as 300,000 mg × min/L on soiled surfaces) should be targeted for disinfection of the hepatitis A virus. The 2 human rotavirus studies included in this review reported a maximum of 2.8 log reduction after exposure to a CT factor of 12,500 mg × min/L; targeting at least 20,000 mg × min/L may be sufficient

to achieve 3-log reduction based on surrogate testing, however further confirmation is required given the limited evidence available.

Norovirus

Human norovirus was used in 4 studies, all of which used RNA-based assays to assess disinfection efficacy because no culture-based infectivity assay is available.

Park et al⁶⁰ noted that 1 minute exposure to gNaOCl (pH 5.6–6.2) at 188 ppm (CT = 188 mg × min/L) and would be sufficient to achieve 3 log reduction on both ceramic and stainless steel with 1% stool as soil load, while Moorman et al⁶¹ reported only 1.3 and <0.3 log reduction with and without ASTM standard soil load, respectively, after exposure to 250 ppm gNaOCl at pH 7.0 for 10 minutes on stainless steel (CT = 2,500 mg × min/L). Increasing exposure time only had an effect in absence of soil load, with 5.0 log inactivation in human norovirus reported after 30 minutes (CT = 7,500 mg × min/L).⁶¹ Cromeans et al⁶² noted limited reductions in human norovirus dried on stainless steel after exposure to 1,000 ppm NaOCl for 5 minutes (CT = 5,000 ppm), with <0.5 log reduction in GI.13 norovirus and <1.0 log reduction GI.5 norovirus in presence of 10% fetal bovine serum as soil load. Tuladhar et al⁴⁹ observed 1.5 (with 1% stool) and 1.7 (without soil load) log reductions 20 minutes after wiping stainless steel with a cloth soaked in 1,000 ppm NaDCC (CT = 20,000 mg × min/L).

Several surrogates have been proposed for human norovirus to overcome the absence of infectivity assay, including murine norovirus and MS2,^{3,15,49,60,62,67–70} feline calicivirus,^{3,62,67–72} and Tulane virus.^{61,62} Comparisons between human norovirus and the proposed surrogates were made in 4 studies. Park and Sobsey⁶⁹ determined that similar times (CT = 94–380 mg × min/L) were required for a 3-log inactivation in human norovirus, murine norovirus, and MS2 exposed to gNaOCl on stainless steel and noted that MS2 resembles human norovirus in terms of persistence and elution from surfaces. Moorman et al⁶¹ also tested gNaOCl (CT = 250–7,500 mg × min/L) on stainless steel and observed similar inactivation in Tulane virus (3.0–4.1 log) and human norovirus (1.6–5.0 log) with increasing exposure times in absence of soil load. Cromeans et al⁶² observed that murine norovirus and Tulane virus were similarly resistant to human norovirus GII (<0.5 log reduction in RNA levels) and more resistant than feline calicivirus (>2.5 log reduction) to NaOCl on stainless steel (CT = 1,000–5,000 mg × min/L). However, Tuladhar et al⁴⁹ observed inconsistencies in the correlation between murine norovirus and human norovirus inactivation in experiments where NaDCC was applied on by wiping stainless steel (CT = 5,000–20,000 mg × min/L).

Overall, no single virus stands out as a particularly good surrogate for human norovirus in this review and evidence to support chlorine-based disinfection efficacy against human norovirus remains weak; gNaOCl appears as an interesting option but with inconsistent results reported so far. Further testing of gNaOCl to verify reproducibility and alternative disinfectants should be considered. In the absence of additional data to refine estimates, disinfection for norovirus should be conservative and rely on combinations of chlorine concentrations and exposure times that yield CT factors substantially greater than 20,000 mg × min/L in order to achieve at least 3 log reduction.

Ebola virus

The Ebola virus was evaluated in 4 surface disinfection studies, all published following the 2014 West African Ebola outbreak. In the first study, the Ebola virus Makona variant was exposed to 1,000–5,000 ppm NaOCl on stainless steel for 1 and 5 minutes in presence of the ASTM standard soil load and complete inactivation (>6 log reduction) was observed after 5 minutes at both concentrations (CT = 5,000–25,000 mg × min/L).⁵⁷ In the second study, similar

experimental procedures were used to compare 3 Ebola virus variants, including the Makona variant, isolated during the 2014 West African outbreak, the Mayinga variant, from the first reported Ebola outbreak, in 1976 in Zaire (now DRC), and the Kikwit variant, from an outbreak in 1995. Concentrations of 1,000–5,000 ppm NaOCl achieved complete inactivation of all 3 virus variants within 5 minutes, as observed in the previous study, however the Makona variant was more resistant to lower chlorine concentrations (0.05%–0.1%) than previous variants of the Ebola virus.⁵⁸ In a third study, where aircraft-relevant surfaces were used, sodium hypochlorite (8,000 ppm, with <1% potassium permanganate) achieved complete inactivation – ie, >5 log reduction on painted aluminum, >3 log reduction on seat-belt strapping material – of the Ebola Yambuku variant (from the 1976 outbreak in the DRC) after 10 minutes (CT = 80,000 mg × L/min).⁵⁶ In contrast to these 3 studies, incomplete inactivation of the Ebola virus (Makona variant) in a human blood matrix after exposure to 5,000 ppm NaOCl for 15 minutes on aluminum and stainless steel was reported in a recent publication, with a reduction of only 0.9 log compared to complete inactivation when the virus was mixed with cell growth medium.⁵⁹

In a comparison of 4 bacteriophages, the enveloped Phi6 was found to undergo slightly lower inactivation (4.1 log) than that reported for the Ebola virus when exposed to NaOCl (5,000 ppm) on stainless steel for 5 minutes (CT = 25,000 mg × min/L) and was therefore proposed as an appropriate surrogate for surface disinfection efficacy testing for Ebola settings.⁷³ Further testing using Phi6 showed complete inactivation on 3 surface types following a 15-minute exposure to 5,000 ppm (CT = 75,000 mg × min/L) NaOCl, gNaOCl, HTH, and NaDCC, independently of chlorine type, soil load, and mode of application.⁶³

As complete inactivation of the Ebola virus and surrogate was reported in all studies where testing was conducted without blood (CT = 5,000–80,000 mg × min/L), a conservative recommendation would be to target at least 80,000 mg × min/L for disinfection in Ebola settings when there is no visible blood on surfaces. Alternative disinfectants should be investigated for the disinfection of blood spills from Ebola patients.

Parameters influencing disinfection efficacy

We identified 4 parameters influencing disinfection efficacy: disinfectant application mode, chlorine compound used, surface type, and soil load. Data relevant to each of them are summarized hereafter, followed by a discussion of experimental parameters that likely influenced efficacy results reported in the laboratory studies included in this review.

Disinfectant application mode

Disinfectant application mode, including immersion, spraying, and wiping, is one of the parameters influencing efficacy and a recurrent theme in studies included in this review. Of the 89 studies investigated, the most common disinfectant application modes were pipetting (n = 54, 61%), immersion (n = 20, 22%), spraying (n = 8, 9%), or wiping (n = 5, 6%). Because disinfection is often combined with cleaning procedures, wiping was investigated and was found to have an effect on viruses and spores even in absence of disinfectant, suggesting that the mechanical action of wiping contributes to reducing contamination levels on surfaces.^{49,74} With spraying, ensuring contact between disinfectant and test organisms can be challenging.^{75,76} Additionally, chlorine loss during the process – from spray nozzle to the targeted surface – is a concern.⁷⁷ Ni et al (2016) found consistent increases in efficacy with increasing disinfectant spraying time from 0.5 to 2 minutes and keeping similar exposure times after spraying. A proposed explanation for variable efficacies observed between

studies is the use of different spraying equipment, such as gas-powered pressurized sprayers producing high spray velocities and hand-held spray bottles.⁷⁸

Although there are likely differences in the impact of disinfectant application mode on efficacy, only 3 studies directly compared application modes.^{79–81} Immersion into pH-amended bleach (6,000–6,700 ppm, pH 6.8) was observed to result in higher log reductions compared to spraying, particularly on porous surfaces such as carpet, paper, and wood.⁸¹ In contrast, a comparison of wiping and spraying showed similar efficacies against *C difficile* spores, though spraying was considered less appropriate for health care settings as it required extended drying times and would not remove dirt and debris.⁷⁴

Lastly, Park et al⁸⁰ noted an increase in efficacy when surface carriers were subject to shaking after immersion into electrolyzed water, with >3.12 log reduction in *S aureus* achieved within 5 minutes exposure to electrolyzed water (52.8 ppm, pH 2.55), compared to 1.50–1.67 log reduction without shaking.

Chlorine compounds

Five studies directly compared chlorine compounds, and showed that available chlorine compounds generally achieve similar disinfection efficacies on surfaces. Bloomfield et al⁸² reported lower LRVs following a 5-minute exposure to 250 ppm NaDCC compared to NaOCl at the same concentration against *S aureus* (2.4 vs 4.9 to >6.2 log reduction), *P aeruginosa* (3.7 vs 3.7–4.3 log reduction), and *E faecium* (2.2 vs 3.1 log reduction) on stainless steel. At 2,500 ppm, both NaDCC and NaOCl achieved at least 6 log reduction in each test organism. Gallandat et al⁸³ observed similar efficacies of NaOCl, gNaOCl, NaDCC, and HTH (5,000 ppm) against both *E coli* and Phi6 after 10–15 minutes on 3 nonporous surfaces, with minimum 5.9 and 3.1 log reductions, respectively. At higher concentrations, Aarnisalo et al⁸⁴ observed 3.1 and 2.2 log reductions (without/with 2% pork meat) in *L monocytogenes* after 30 seconds exposure to 22,400 ppm NaDCC and >3.6 and 2.5 log reductions (without/with 2% pork meat) after 30 seconds exposure to 27,000 ppm NaOCl. Lombardi et al⁸⁵ reported slightly lower efficacies of NaOCl compared to HTH against low pathogenic avian influenza virus on stainless steel (3.1 vs 3.7 log reductions) and wood (0.0 vs 1.9 log reductions) after 10 minutes exposure to 750 ppm. Julian et al¹⁵ found that electrolyzed water and household bleach (NaOCl) were also similar against murine norovirus and MS2 using 500–2,500 ppm for 30 seconds (Table S3).

Surface type

As previously described, identified studies tested a variety of surface materials. Overwhelmingly, the most common material was stainless steel. Among the 89 studies identified, 27 (30%) tested more than one surface, including 13 (15%) that used porous surfaces (ceramic, fabric, rubber or wood). Porous surfaces were identified as more challenging to disinfect in 8 of these 13 studies. For chlorine-based disinfection in particular, Baek et al⁸⁶ observed lower inactivation in *E coli* on wood (3.34 log) and rubber (4.69 log) compared to nonporous surfaces such as stainless steel, glass, and polyethylene (5.05–5.18 log) after exposure to NaOCl. While Park et al⁸⁰ found no difference in gNaOCl efficacy against *S aureus* on 5 nonporous surfaces (1.7–1.9 log), Kim et al⁸⁷ reported consistently lower log reductions in *S aureus* on scratched polyethylene, polypropylene, glass, and stainless steel compared to the same surfaces without scratches after disinfection with NaOCl. The influence of surface structure on disinfection is further affirmed with observations in another study that *E coli* was more challenging to disinfect on heavy duty tarp compared to smoother surfaces like nitrile and stainless steel.⁸³ Lombardi et al⁸⁵ observed lower log reductions in low pathogenic avian influenza virus on wood (0.0–1.9) compared to metal (3.1–3.7) and plastic

(3.9–4.3) surfaces after exposure to 750 ppm NaOCl or HTH for 10 minutes. Julian et al¹⁵ compared gNaOCl and NaOCl on polyvinyl chloride and stainless steel; surface type was statistically significant for murine norovirus inactivation (overall higher on polyvinyl chloride) but not for MS2. Yeargin et al⁸⁸ also found that glass and polyester were easier to disinfect than cotton using 5,000 ppm NaOCl with a 5-minute exposure time, with 4.5–5.5 log reduction in murine norovirus and feline calicivirus on glass, 4.3–5.1 log reduction on polyester, and 3.1 log reduction on cotton. Additionally, Yeargin et al⁸⁸ noted that surface porosity and hydrophobicity impacted both disinfection efficacy and viral recovery for testing, with the latter potentially biasing disinfection efficacy estimates.

Soil load

Chlorine is inactivated by organic matter¹⁴ and, as a consequence, the selection of a soil load for surface disinfection efficacy testing can affect observed results. A wide range of matrices representing soiled surfaces were used in included studies, from the standard ASTM formulation with tryptone, bovine serum albumin, and bovine mucin to human stool, serum, or shrimp and pork meat (Tables S1–S4). The impact of specific matrices used to represent soil loading on observed efficacies are unclear from this review, with the notable exception of the results reported above for the Ebola virus, which was more resistant to disinfection in human blood compared to other testing media.⁵⁹ Given the known impacts of chlorine demand on chlorine disinfection efficacy, the large variation in impacts of soil loading on efficacy is surprising. However, 1 potential explanation is the variation in chlorine demand of chosen specific matrices. For example, the formulation recommended in the ASTM standard has a low chlorine demand, and as such may not be appropriate to assess chlorine-based disinfection against pathogens that are shed in bodily fluids such as vomit and stool.⁸³ The soil load selection is a good example of the trade-off needed between testing conditions that are relevant to real-world applications (eg, working with surfaces in contact with seafood) and generating efficacy data that can be compared to the existing literature and support the development of guidelines.

Experimental parameters

One finding from this systematic review is that, while there is a large body of literature on surface disinfection efficacy with a majority of high-quality studies, experimental protocols, and observed surface disinfection efficacies are highly variable between studies, including the use of different preparation methods for the test organisms (eg, washing bacteria); inoculation methods (eg, liquid vs dry aerosols for spores); surface carrier size (from <1 to 100 cm²); drying times (from no drying to 24 hours) and conditions (temperature, relative humidity); disinfectant application mode (eg, immersion, spraying, or wiping), concentration and exposures time; soil loads; neutralization procedures and media; and recovery methods postdisinfection (eg, vortexing, sonicating, swabbing, and scraping were described).

DISCUSSION

In this systematic review, we extracted 1,421 data points from 89 potentially relevant studies and found that: (1) there is high variability and no clear trends in reported disinfection efficacy of outbreak-relevant pathogens and surrogates; (2) this high variability likely reflects inconsistent and highly variable testing procedures; (3) the majority of included studies evaluated chlorine-based disinfection of stainless steel surfaces, which have limited relevance to low-resource outbreak settings, and pathogens with potential to produce outbreaks in low-income contexts remain underresearched; (4)

improvements in consistency, reproducibility, and reporting are necessary in surface disinfection efficacy studies; and (5) the selected qualitative approach to synthesize surface disinfection efficacy data from pathogen and surrogate testing nevertheless informs recommendations for disinfection in outbreak settings.

We identified 20 studies using pathogens particularly relevant to low-resource settings in surface disinfection efficacy testing: *M tuberculosis*,¹ *V cholerae*,¹ *Salmonella* spp.,⁵ hepatitis A virus,⁴ rotavirus,² human norovirus,³ and the Ebola virus.⁴ Given the variation in study findings, we advocate conservative approaches to field disinfection relying on high CT factors for chlorine, driven largely by the assumption that chlorine-based disinfectants are readily available. We recommend following pathogen-specific target CT factors rather than aggregating pathogens by type (ie, bacteria, virus, and protozoa), as we believe that the latter would misrepresent the complexity of surface disinfection efficacy data. Specifically, the data extracted in this review support use of the following conservative target CT factors: >15,000 mg × min/L for the hepatitis A virus, >20,000 mg × min/L for rotavirus and norovirus, and >80,000 mg × min/L for the Ebola virus, as relatively consistent evidence – although from small numbers of studies – was available for these pathogens. Data for gram-negative bacteria exhibited particularly high variability, leading to a cautious (and possibly unrealistic) recommendation of 200,000 mg × min/L for *V cholerae* and >24,000 mg × min/L for *Salmonella* spp. Insufficient data were available to formulate a recommendation for disinfection of *M tuberculosis*. The appropriate combination of chlorine concentration and exposure time to achieve a given CT factor should be selected depending on safety concerns (as high chlorine concentrations may be irritant and corrosive) and practical considerations with regards to applicable exposure times, in compliance with relevant environmental and safety guidelines. Additional rigorous research is needed to better resolve (lower) recommendations for specific pathogens to ensure adequate protection of human health. Furthermore, studies to inform disinfection against the WHO Blueprint priority viruses⁴¹ other than the Ebola virus are needed.

Submission of this manuscript coincided with the COVID-19 pandemic⁸⁹; surface disinfection efficacy data for coronaviruses and potential surrogates was synthesized in the recent systematic review by Kampf et al,⁹⁰ where it is noted that exposure of human coronavirus (E229) to 1,000 ppm NaOCl for 1 minute (CT = 1,000 mg × min/L) achieved >3 LRV. In line with the findings in our review, all relevant studies identified by Kampf et al⁹⁰ were carried out using stainless steel surface carriers, thus highlighting the need for further research to be conducted on other surface types.”

In this review, we also identified themes relevant to outbreak response in surface disinfection efficacy evaluations, including the influence of disinfectant application mode, selected chlorine compound, surface type, and soil load. Each of these is briefly summarized hereafter. Increases in observed inactivation with mechanical action (shaking during immersion, wiping) were reported, as well as specificities of spraying (equipment, spray characteristics, and spraying time matter). Comparisons between the 4 chlorine compounds typically available in emergency response suggest no clear difference in terms of efficacy. Several studies mentioned that porous surfaces are more challenging to disinfect – and to use in laboratory testing – than nonporous surfaces, which is a concern in outbreaks in health care facilities, where mattresses, linens, cloths may need to be disinfected, and in households, where an even wider range of porous materials (wood, ceramic, and carpets) may be found. Soil load is expected to impact chlorine disinfection efficacy, as chlorine is inactivated by organic matter, though this review did not observe systematic reductions in efficacy due to soil load. This may be due to the wide range of substances used as soil loads. Notably, no matrix was identified that is known to accurately mimic bodily fluids excreted by

cholera or Ebola patients and the development of appropriate soil load mixtures for laboratory testing is still needed.

Surface disinfection efficacy testing standards such as the ASTM Quantitative Disk Carrier test method⁹¹ recommend the use of 1-cm stainless steel disks as surface carriers and chlorine is a widely available disinfectant that has been used for over a century.⁹² It is therefore not surprising that most studies focus on stainless steel and chlorine. What is striking, however, is the variability in reported efficacies for similar test organisms, surface types, and disinfectants (Fig 2), which made it infeasible to define appropriate aggregation categories to perform a meta-analysis. We hypothesize that this variability reflects the variability in testing procedures, which was also identified as a concern in large-scale, interlaboratory studies for the development of standard methods, where researchers advocated for the use of large numbers of replicates.^{79,82,93} For the registration of bactericidal disinfectants, the US EPA requires tests to be carried out following the Association of Official Analytical Collaboration International standard methods using 60 surface carriers on at least 3 different days, with a qualitative outcome after disinfection (positive/negative),⁴³ while the quantitative ASTM standard method requires triplicate controls and a minimum of 5 replicates.⁹¹ In this review, the average number of replicates for data points that passed full text screening was 3.9. A quantitative decision-making framework has recently been proposed by Parker et al⁹⁴ to determine whether the achieved reproducibility for surface disinfection efficacy experiments is acceptable, and we recommend this be more widely adopted in reporting surface disinfection laboratory efficacy results. Lastly, the data extraction spreadsheet (available in Supplementary Materials) includes 1,421 data points that can be filtered by any user depending on the test organism, surface, or disinfectant of interest. We believe this database is a useful tool for those who are designing surface disinfection experiments or searching for specific disinfection efficacy data. This review focused on chlorine-based disinfectants because of their widespread availability and use in low-resource settings, however data were also extracted from studies using other disinfectants (Table 2) and our dataset could be further analyzed to synthesize surface disinfection efficacy outcomes for alternative disinfectants or include additional test organisms.

We recommend further research that integrates components from existing standards (eg, stainless steel) for methods validation, while expanding testing to conditions relevant to low-resource settings (eg, porous surfaces, spraying). In view of the variability in efficacy outcomes observed in this review, replicating experiments over different days or periods of time to account for within-laboratory differences and changes in the resistance of test organisms, as suggested by Bloomfield et al,⁹³ seems sensible. The following experimental information should be reported at a minimum: test organism preparation (eg, washing, growth phase); surface inoculation method, including soil load and test organism concentration, drying time and conditions (eg, temperature, relative humidity); surface carrier size; disinfectant ingredients, preparation, storage, concentration, application mode and exposure time; recovery methods; number of replicates (for both test and control carriers) and time of testing.

Besides the inability to conduct a meta-analysis, the work presented herein had several limitations. While LRVs are the most widespread measure of disinfection efficacy, they are sensitive to the inoculation level and recovery of test organisms in positive controls,^{81,93} which can affect comparisons between studies. A second limitation is the reliance on study descriptors (test organisms, surfaces, and disinfectants) during abstract screening to refine full text inclusion criteria, which may have resulted in the exclusion of some studies relevant to low-resource settings. Similarly, relevant studies published in languages other than those understood by our review team may have been missed. Furthermore, the requirement to only include disinfectants that have 5 or more relevant publications may

have limited our ability to identify novel disinfectants potentially usable in outbreak response. Additionally, this systematic review primarily focused on the scientific literature. Alternative approaches such as screening US EPA-registered disinfectants for ease of use, cost, and international availability may allow identification of other relevant information for the development of recommendations for disinfection in low-resource settings.

CONCLUSIONS

The variability in reported disinfection efficacies between the studies included in this review highlights how challenging it may be for policy makers to use laboratory results to develop recommendations for surface disinfection in outbreaks, particularly in low-resource contexts. We therefore recommend that laboratory experiments increase reproducibility, for example through replicating experiments over different days or periods of time. More importantly, the high variation in the well-controlled laboratory experiments highlights the need for demonstrating sufficient efficacy under field conditions. We therefore strongly recommend field-based testing and monitoring to ensure effectiveness is achieved in situ. An efficient strategy for laboratory testing could be to conduct studies comparing priority pathogens to test organisms that have been widely studied, such as *S aureus* and *P aeruginosa*, feline calicivirus, murine norovirus and MS2, or *Bacillus* spp., so that existing data (available in Supplementary Materials) could be extrapolated to inform surface disinfection guideline development in low-resource settings with minimal additional laboratory testing.

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SUPPLEMENTARY MATERIALS

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