Viral inactivation by quaternary ammonium compounds: A systematic review

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# Abstract

**Background**: Disinfection plays an important role in interrupting the spread of pathogen transmission. Quaternary ammonium compounds (QACs) are common disinfectants, which act by destroying lipid membranes. QACs also have demonstrated high but variable efficacy against viruses, including non-enveloped viruses despite the absence of a lipid membrane.

**Procedure**: To identify sources of variability and inform future applications of QACs for virus inactivation, we conducted a systematic review to identify impacts of environmental, viral, and experimental characteristics on reported efficacy. Efficacy was indicated by reported virus log reduction value (LRV) following exposure to the QAC at a reported concentration and duration (i.e., CT factor).

**Main findings:** The review identified 557 studies, of which 75 studies included primary data on QAC disinfection for viruses, resulting in 1012 unique data points on disinfection efficacy. In total, the identified studies assessed efficacy of 15 different QAC or QAC mixtures against 137 unique virus strains. Based on our analysis, viruses were more likely to be inactivated by QACs if they were enveloped, had larger capsids, and contained double-stranded DNA or double-stranded RNA. Type of QAC, higher CT factors, and temperatures also significantly influenced disinfection efficacy. Genome size, whether the disinfection study was conducted in a liquid matrix or on a surface, and presence of a simulated carbon load did not have a significant impact on the model.

**Conclusions**: This study and the associated dataset highlight factors influencing QAC disinfection efficacy against a huge variety of viruses, including non-enveloped viruses. Furthermore, high observed variability highlights the need for improving study standardization.

**Keywords**: Disinfection, deactivation, viruses, data analysis, Quaternary ammonium compounds, Systematic review

# Introduction

Quaternary ammonium compounds (QACs) are commonly used surface disinfectants. Since the introduction of QACs in 1915-1916 (1), the production of these surface-active agents has increased substantially (2). QACs are broadly used cationic surfactants for applications from fabric softeners and anti-static agents to disinfectants in hospitals, the food industry, schools, and other public and private areas (3–5). In 2020, QACs were classified and publicly announced as effective against SARS-CoV and SARS-CoV-21, 21, 2 (6,7). Of the U.S. Environmental Protection Agency’s (EPA) 430 listed disinfectant products to use against the spread of CoVID-19, the majority (216) contain QACs (8).

QACs are defined by their chemical structure consisting of one nitrogen atom (N) bound to four side chains (R1-R4), R being an alkyl or aryl/ hydrocarbyl group (9). The antimicrobial property of QACs derives from their structure. QACS have a hydrophilic, positively charged “head” and lipophilic tails that can interact with bacterial membranes and viral surface structures (3). The positively charged nitrogen head reacts with negatively charged phospholipid components of the bacterial membrane while the lipophilic tails interacts with the microorganism’s membrane to cause disruption and cell leakage (10,11). The hydrophobicity of QACs also enables QACs to interact with lipid-envelopes of viruses (3,12).

QACs inactivate microorganisms using a variety of distinct mechanisms. Bacterial inactivation follows a cascade of events that starts with interactions between the positively charged quaternary nitrogen of the QAC molecule and the negatively charged lipids of the cell surface. The QAC molecules penetrate and disorganize the structure of the membrane leading to leakage, degradation, and cell wall lysis(3,13,14). This interaction also provides a mechanism for QAC inactivation of enveloped viruses, as QACs interact with the lipids in viral envelopes, leading to interruption of the membrane (15,16). However, QACs can also be active against non-enveloped viruses, although this may require a much higher concentration to reach similar log reduction values than needed for enveloped viruses (3,16). One way QACs may achieve this is through the formation of micelles which encapsulate the viral particles (16). In addition, QACs are able to bind to and collapse DNA and may interact with other intracellular targets, such as proteins (3,17).

Environmental factors can likewise affect the efficiency of chemical disinfection of viruses. Negatively charged material, such as soil clay minerals, organic matter or other organic contaminations can interact with the positively charged heads of the QACs, effectively blocking their use as a disinfectant (18–21). In addition, higher temperatures typically lead to faster inactivation times (22), and the pH of a solution can also impact disinfection efficiency (23).

Knowledge about virus persistence and transmission, as well as about efficacy of disinfectants, can help to inform infection control practices. Recent viral disease outbreaks, such as from Coronaviruses (SARS-CoV-2, MERS-CoV, SARS-CoV), enteric viruses, and other viruses such as Ebola have raised questions about the transmission and persistence of viruses in the natural and built environment (24–26). Laboratory experiments to inform inactivation and persistence of these human pathogenic viruses requires access to high biosafety level (BSL) laboratories (27,28). Access to such laboratories is limited and expensive. Additionally, some human pathogenic viruses cannot be easily grown *in vitro*, for example the human norovirus (hNoV) (29). To circumvent the need for high BSL laboratories, viral surrogates are often used as a cheaper and safer alternative (21,30). A surrogate is expected to behave similarly to the target pathogen based on size, structure, physicochemical properties, or other characteristics but is chosen because is easier or safer to use. It may be a non-pathogenic virus or bacteriophage that poses little to no risk to human health compared to pathogens it represents, or may be easier to culture, detect, or quantify than the pathogens. Viral surrogates are useful tools for studying how environmental factors effect viral persistence; however, surrogates can often behave very differently than the virus they model (3,15–17). Knowing viral factors that influence inactivation by QACS can help inform surrogate selection.

In the current study, we conducted a systematic review following the “Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols “(PRISMA-P) framework to identify the key viral properties and environmental factors influencing viral disinfection efficacy of QAC disinfection (31). Within the scope of this systematic review, we compared the quantitative inactivation of viruses exposed to QACs, identified virus and study characteristics that influence efficacy of QAC inactivation, and informed the decision of which properties of surrogates are most important to consider in understanding efficacy of QACs against human virus pathogens.

# Results

## Screening process

In April 2020, we searched 3 databases (PubMed, EMBASE, and Web of Science) resulting in in 866 studies (Figure 1). After removing duplicates, 557 unique studies were identified. Titles and abstracts were screened based on inclusion and exclusion criteria, resulting in 210 references defined as likely to provide quantitative data about disinfection of viruses with QACs. These 210 references were screened during the full text review process (AV) and data of 75 references were extracted, for a total of 1012 data points describing inactivation of viruses using QACs in liquid or on surfaces.

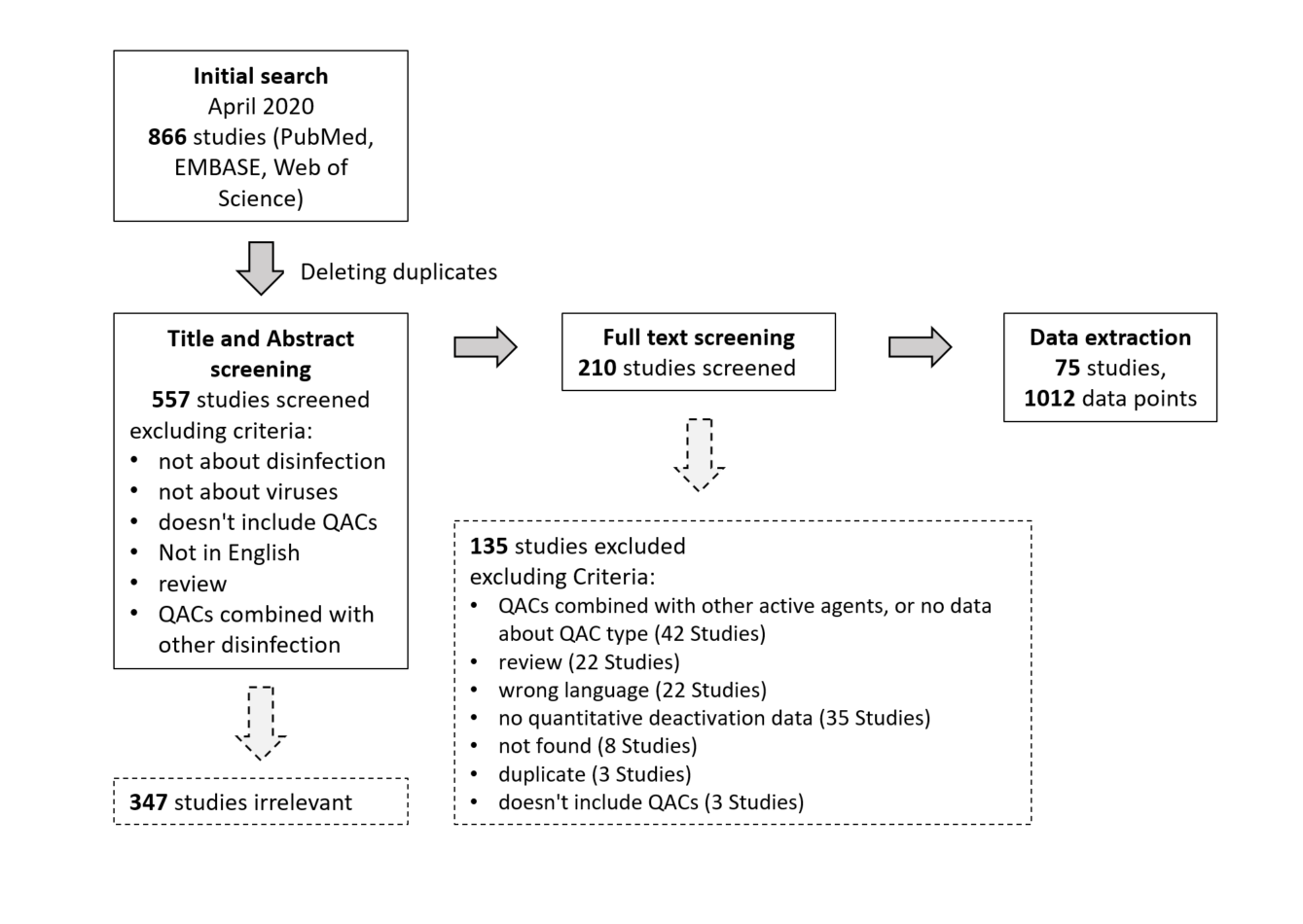


Figure 1 PRISMA-P systematic review flow chart

## Data distribution

Identified studies assessed efficacy of QACs for a wide range of both viral characteristics (viral family, viral surface, virus size, genome size, and nucleic acid type) and environmental / experimental design characteristics (carbon load, environmental surface material, and temperature). Notably, in 137 (14%) of the 1012 data points, QAC disinfection of viruses resulted in LRV above the limit of detection or below the limit of quantification.

*Viral family*

The review identified QAC disinfection data for 29 different virus families (Figure 2: Virus families identified in the systematic review and the associated number of unique data points, grouped by each unique study (grey squares). ). Of these, Picornaviridae (137 data points from 18 studies) and Adenoviridae (108 data points from 7 studies) were the most represented. Picornaviridae has the most studies contributing (Figure 2), implying broad representation of this family in the literature. The 29 virus families represent 137 different virus strains.

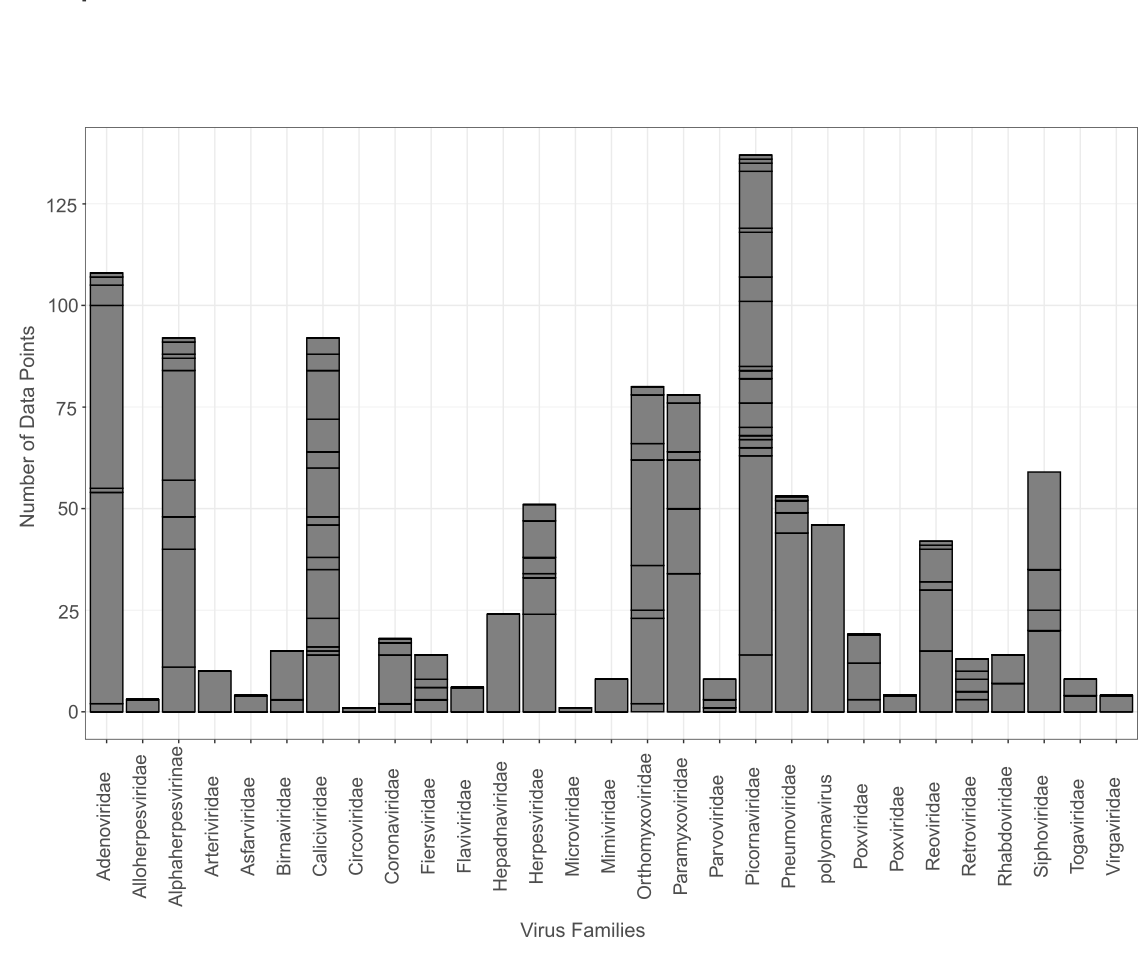


Figure 2: Virus families identified in the systematic review and the associated number of unique data points, grouped by each unique study (grey squares).

*Viral envelope*

Of the 75 studies in the systematic review, 23 (17%) included data on enveloped viruses, 40 (30%) included data on non-enveloped viruses, and 12 (9%) included data on both. The resulting 1012 data points resulted in near equal representation of enveloped (477 data points, or 47%) and non-enveloped (535 data points, or 53%) viruses.

*Nucleic acid type*

The viruses studied were dominated by three types of nucleic acids genomes (Figure   
S2): double stranded DNA (dsDNA: 418 data points, 41%), positive charged single stranded RNA (ssRNA(+): 302 data points, 30%), and negative charged single stranded RNA (ssRNA(-): 225 data points, 22%). Only 57 data points (7%) were for viruses with double stranded RNA (dsRNA), and only 10 data points (1%) for single stranded DNA viruses (ssDNA).

*Genome size*

The genome size of the viruses ranges from 1.7 kilo nucleotides (kn) to 1200 kilo base pairs (kbp). Most genomes (814, 80%) are 50kn or smaller (Figure S3)). Only 8 data points (0.8%) from one study represent viruses with a genome size bigger than 300 kbp. These datapoints represent a giant virus, Acanthamoeba polyphaga mimivirus (APMV), which has been studied as a putative pneumonia-causing agent [30]. The smallest genome size represented is the non-enveloped virus porcine circovirus (PCV2) with a genome size of 1.7 kn (32).

*Virion size*

The virion size among the 1012 data points averages 125 ± 97 nm (range of 17 nm to 600 nm). Few studies included viruses larger than 250 nm (57 data points, 6%). For a better overview of the distribution, data were classified into 50 nm size bins. Viruses were represented as follows: smaller than 50 nm (328 data points, 32%), 50 to 100 nm (264 data points, 26%), 100 nm to 150 nm (39 data points, 4%), 150 nm to 200 nm (224 data points, or 22%), 200 nm to 250 nm (100 data points, or 10%), and greater than 200 nm (57 data points, or 5.7%).

Notably the average virion size of the enveloped viruses included in the studies was larger (average 170 ± 71 nm) than the average size of the non-enveloped viruses included in the studies (85 ± 100 nm) (Figure S4).

*Temperature*

Studies primarily assessed disinfection at room temperature (RT: 20-25°C, 569 data points, 56%) or at human body temperature (37°C, 313 data points, 31%) (Figure S7). Other temperature ranges are less represented in the data set including lower temperatures between zero and 5°C (66 data points, 7%), temperatures between room and body temperature (52 data points, 5%), and temperatures above 37°C (11 data points, 1%).

*Carbon load*

Of the 75 total studies in the systematic review, 26 (35%) included data on an added carbon source, including nutrient rich media (FBS, BSA, TPB, TBS, yeast suspension, or skimmed milk), and relevant matrices (fecal suspensions or blood). In 190 (19%) of the 1012 data points a carbon load was added.

*Experimental surface*

Most of the data collected (866 data points, 86%) were from experiments conducted in solution. A subset (146 data points, 14%) represent experiments with viruses on surfaces (Figure S1). In total, 19 out of the 75 studies investigated disinfection on surfaces with four studies comparing multiple surface materials. Evaluated surfaces include non-organic materials (stainless steel, plastic, rubber, glass, latex paint, ceramic, tiles, and aluminum) as well as organic materials (lettuce, strawberries, and hands).

*QAC type*

Benzalkonium chloride (BAC) was the most studied QAC in terms of both number of data points as well as number of Studies. 43 studies used BAC as disinfectant with 582 data points (57%). Within these 43 studies 8 used more than one QAC disinfectant, within these 4 used more than 2 different QACs. Didecyl-dimethylammonium chloride (DDAC) and mixed QACs were relatively equally represented in terms of data points with 162 (16% used in 9 studies) and 151 (15% used in 23 studies) data points. Benzethonium chloride (BEC) and didecyl dimethyl ammonium bromide (DDAB) were used by one study with 35 (3%) and 26 (4%) data points respectively. QACs that were represented by less than 10 data points (<1%) were grouped together and represented in total 42 data points (4%). Distributions of QACs are available in (Figure S8 and Table S5).

## Statistical Analysis

Backward elimination multiple linear regression was used to determine the effect of viral and environmental characteristics on the efficacy of QACs. The final multiple linear regression model with all included factors (numeric: CT-factor, temperature, virus size, and dummy variables: virus envelope, culture vs. other measurement methods, nucleic acid type, and QAC type) explained 24% of the data (Adj. R2: 0.24) with all included factors as significant (p < 0.05) (Table 1). The backward multiple linear regression models are provided in the supplemental material (Table S1-2). Genome size correlates to virus size (Spearman correlation coefficient = 0.71, p = < 2.2\*10-16), and so only virus size was included in the model to limit collinearity. Carbon load and if the experimental were conducted on a surface or in solution were excluded from the final model since they did not significantly influence the initial model (p > 0.05).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Estimate** | **Std. Error** | **t value** | **p value** |  |
| (Intercept) | -1.02 | 0.43 | -2.39 | 0.02 | \* |
| Log10 (CT factor [mg\*min/L]) | 0.57 | 0.06 | 9.88 | < 2\*10-16 | \* |
| Virus size [10 nm] | 0.03 | 0.01 | 4.93 | 9.79\*10-07 | \* |
| Temperature [°C] | -0.02 | 0.01 | -3.20 | 1.42\*10-03 | \* |
| Enveloped (Relative to non-envelope) | 1.24 | 0.15 | 8.47 | < 2\*10-16 | \* |
| Measurement in culture (Relative to Molecular) | 0.59 | 0.23 | 2.57 | 0.01 | \* |
| *Nucleic Acid (Relative to dsDNA)* |  |  |  |  |  |
| ssRNA(-) | -0.70 | 0.16 | -4.29 | 1.98\*10-05 | \* |
| ssDNA | -0.81 | 0.51 | -1.57 | 0.12 |  |
| ssRNA(+) | -0.32 | 0.15 | -2.12 | 0.03 | \* |
| dsRNA | 0.71 | 0.26 | 2.71 | 6.87\*10-03 | \* |
| *QAC Type (Relative to BAC):* |  |  |  |  |  |
| BEC | 0.76 | 0.28 | 2.75 | 6.16\*10-03 | \* |
| DDAB | -0.23 | 0.34 | -0.66 | 0.51 |  |
| DDAC | 0.38 | 0.17 | 2.27 | 0.02 | \* |
| MBAT | 0.19 | 0.43 | 0.45 | 0.65 |  |
| others | -0.49 | 0.26 | -1.89 | 0.06 |  |
| QACmix | 0.14 | 0.16 | 0.86 | 0.39 |  |
|  |  |  |  |  |  |
| **Adj. R2** | **0.24** |  |  |  |  |

Table 1: Multiple linear regression model for impact of virus characteristics for factors influencing disinfection of virus with QACs.

As expected, CT factor is significant (Table 1). Enveloped viruses are also more readily inactivated by QACs than non-enveloped viruses, with the presence of an envelope increasing LRV by, on average 1.24 ± 0.15 log. Experimental temperature was inversely associated with QAC inactivation rates of viruses, with an increase in temperature per-degree Celsius change associated with a decrease in QAC disinfection (on average -0.02 ± 0.01). Additionally, viral inactivation by QACs is significantly influenced by virus size. The model found that an increase in virus size of 10 nm corresponds to a modest increase of 0.03 ± 0.01 in LRV. Experiments that measured virus inactivation in culture (Plaque assays, Egg infective dose at 50%, Tissue Culture Infectious Dose, leaf culture) found in general a higher LRV (0.59 ± 0.23) compared to other measurement methods (qPCR, nested PCR, immune-magnetic separation, Antigen detection methods).

Nucleic acid type was also influential. With double stranded DNA as the reference category, viruses with double stranded RNA were more susceptible to QACs (0.71 ± 0.26 higher LRV). Single stranded RNA was less susceptible compared to dsDNA, with negative sense RNA inactivation reduced by 0.70 ± 0.16 LRV, and positive sense RNA reduced by 0.32 ± 0.15 LRV. ssDNA was not significantly different than dsDNA. Comparing the different QACs benzethonium chloride (BEC; 0.76 ± 0.28) and didecyl-dimethylammonium chloride (DDAC; 0.38 ± 0.17), showed higher inactivation compared to benzalkonium chloride.

# Discussion

Based on the here used dataset, the present review suggests that QACs efficacy is influenced by viral properties, with higher efficacy against viruses that have a lipid envelope, a bigger capsid, and double stranded DNA or double-stranded RNA. QAC efficacy is also influenced by the specific compounds tested with benzethonium chloride and didecyl-dimethylammonium chloride showing higher efficacy compared to the widely used benzalkonium chloride. Experimental conditions also influence QAC efficacy, complicating comparisons between studies which may be resolved with improved efforts on standardization of experimental set-ups and reporting.

The systematic review highlighted the general efficacy of QACs against a variety of viruses, including enveloped and non-enveloped viruses. The primary driver of QAC efficacy was CT factor, which was significant and positively correlated with log reduction, as expected and widely described in the literature (3,22,33–36). Across all data, an increase in one log10 CT unit (expressed as mg\*min/L) was associated with an average increase of 0.57 LRV. QAC was effective at inactivation of both non-enveloped viruses and enveloped viruses (37), though the analysis highlights enhanced inactivation of enveloped viruses compared to non-enveloped viruses. This is in line with previous observations and the general understanding that enveloped viruses are more susceptible to QACs than non-enveloped ones (3,10,22,34,36,38,39). The mechanism of QAC inactivation may explain this finding, as QACs attach to and disrupt lipid membranes, react with the cytoplasmic membrane, and cause membrane disorganization and leakage, explaining increased activity against enveloped viruses (10,40). Reduced concentrations or contact time of QACs are necessary for enveloped viruses, like influenza or SARS-CoV-2, relative to non-enveloped viruses, like norovirus or rotavirus.

QACs can also induce micelle formation, which would contribute to inactivation of both enveloped and non-enveloped viruses (16). Micelle formation encapsulates viruses which prevents subsequent interaction with host cells (16,41). To form micelles the critical micelle concentration has to be reached. Critical micelle concentration is defined as the concentration of surfactant at or above which micelles form (36,42). Almost all surfactants added after this concentration will dissolve into micelles (43). This value depends on temperature, pressure, and the presence and concentration of additives like other surfactants or electrolytes. The Critical micelle concentration of BAC additionally depends on the composition of C12, C14, and C16-alkyl chains. Reported values range between 0.008% (80mg/L) and 0.2% (2000mg/L) (42,44,45) with varying matrixes and additives. Concentrations in the data set range from 1mg/l up to 105mg/L, with most data points at concentrations between 100 to 1000mg/L (472 datapoints, 47%). The extracted data were not sufficiently detailed to estimate individual critical micelle forming concentrations per study design.

Temperature was also identified as an influential factor, although the relationship identified in our model was an inverse relationship with inactivation, in contrast to previously reported effects of temperature on both bacterial and viral inactivation(46,47). Previous work has shown viruses are inactivated faster at higher ambient temperatures relative to lower temperatures (22),(48,49). Temperature effects might also be affected by other variables correlated with temperature, such as humidity (50,51). The efficacy of temperature on inactivation might be influenced by the mechanisms of action of QACs.Lower temperatures may reduce fluidity of the bacterial membrane and subsequently reduce interactions between cytoplasmic membrane and QAC (52,53). Viral envelopes underlie a similar reduction in fluidity at reduced temperatures (54,55). Another possible explanation might be that reduced temperatures reduce micelle formation and corresponding virus inactivation. However, the unexpected finding may instead be explainable by bias in the data set extracted from the literature review, where temperatures of experiments are not proportionally distributed amongst other factors influencing LRV. As one example, the highest reported temperature (49°C) in the dataset was applied to murine norovirus (a non-enveloped virus with higher resistance to inactivation) with only four data points (Figure S7). Nevertheless, the findings suggest additional research may be warranted to identify appropriate QAC CT factors for disinfection applications at low- or high- temperatures.

Larger viruses are also more sensitive to QACs than smaller viruses. This finding is also consistent when sub setting data by presence of a viral envelope (Figures S5-6, Tables S3-4). These observations go in line with described inactivation hierarchies in the literature, in which enveloped viruses are the most sensitive to disinfectants in general, followed by larger non-enveloped viruses, with small non-enveloped viruses as the most resistant (34,38,56,57).

The analysis highlighted that carbon load does not influence inactivation of viruses exposed to QACs. This finding is in contrast to both previous research, which has described interference of organic load with chemical disinfection (3,58–60), as well as the results of individual studies identified in the review (22,48,61–70). An explanation for the deviation of this trend in the current model might be bias in the distribution of the data underlying the analysis, combined with wide variation in inter-experimental efficacy estimates. Only 26 studies (19 %) included the addition of a carbon load; additionally, there was wide variability in the substance used to simulate a carbon load. Although we classified the addition of any carbon substance as a carbon load, QAC efficacy may be differentially impacted by various carbon sources. Due to the heterogeneity in carbon sources and their dosing (concentration) across studies, the model was underpowered to further account for the impact of concentration or type of carbon load. As discussed, studies identified within the review also identified an impact of carbon load on disinfection efficacy. Specifically, amongst the 26 studies investigating impacts of carbon loads, 16 studies directly compared disinfection efficacy of QACs with and without carbon load. In 11 of these studies, carbon load was shown to decrease QAC disinfection efficacy. Two studies were inconclusive because the log reduction value reached the limit of detection in samples both with and without carbon load and therefore have no comparable data (71,72). One study reported the need of higher concentrations of BAC to achieve the same LRV but no effect of organic load if other QACs were used (68). Another study reported a higher LRV with 5% FBS at 1 minute exposure time, and no difference in LRV at 5 minutes exposure time (73). In 11 studies, a carbon load was used in all experiments but did not include a comparison to samples without a carbon load. Although it is possible that higher QAC CT factors are needed for disinfection when carbon loads are present, the results from this analysis suggests other experimental conditions may have as much or more influence on associated LRVs.

Genome size was not identified as an influential factor driving QAC disinfection efficacy. This finding is in contrast to disinfection studies using UV inactivation (74). A main mode of action of UV inactivation is nucleic acid dimerization (74,75), which is not relevant to currently understood mechanisms of QAC disinfection. Accordingly, the lack of correlation in our model implies that the main mode of action of QACs is not influenced by genome size of the viruses, despite the ability of QACs to bind to and compact DNA (17). Additionally, it might reflect a lack in studies investigating QAC efficacy of viruses with large genomes.

The analysis further revealed that nucleic acid type can impact the efficacy of QACs to viruses. Both positive and negative sense single stranded RNA viruses are in general more resistant to QACs compared to dsDNA viruses. Double stranded RNA viruses showed a higher sensitivity to QACs compared with dsDNA viruses. Inactivation of viruses with ssDNA was not significantly different than inactivation of dsDNA viruses. The mechanism of differential susceptibility of viruses based on nucleic acid type is unclear, and may be influenced by the ability of QACs to bind to DNA (10). However, this would also suggest increased efficacy against viruses with longer genomes, but this was not observed. Alternatively, the findings may either reflect biases in the underlying data set or might be driven by virus properties correlated to their nucleic acid type.

We observed differences in the efficacy of different quaternary ammonium compounds. Specifically, BEC and DDAC were associated with increased inactivation compared to BAC, which was the most widely used QAC in the literature (583 data points). Although the analysis revealed a higher inactivation for BEC compared to BAC, there was limited evaluation of BEC (two studies out of the 57 total). In one of these studies, in a direct comparison of ten viruses higher efficacy of BEC was observed for only two (37). This aligns with bacterial inactivation of BAC and BEC where two out of three strains where more sensitive to BEC compared to BAC (76). Our finding that DDAC is more effective than BAC aligns with previous reports (77,78). However, our finding was also based on a limited sample size of only six studies that evaluated DDAC.

We observed no significant difference in the efficacy of QAC, as a function of the CT value, for studies investigating surface disinfection compared to studies comparing disinfection in solution. This is in contrast to the general opinion that inactivation efficacy of disinfectants is lower on surfaces (79). An explanation, again, might be the distribution of experimental setups in the dataset: Only one study reported inactivation data in solution and surface (48). This study showed higher inactivation in solution at room temperature. However, at 2 °C, the results show similar inactivation levels for the surface applied tests as well as in solution, but inactivation was generally lower compared to room temperature.

# Conclusion

The identification of factors that influence QAC inactivation of viruses informs both future experimental designs as well as potential QAC concentrations and exposure times for novel viruses. For example, there was controversy about the efficacy of QACs, specifically benzalkonium chloride (BAC), on surface disinfection of SARS-CoV-2 during its emergence (12). As SARS-CoV-2 is a small, ssRNA, enveloped virus, QACs are likely effective based on our current analysis, in line with subsequent experimental reports (6). This analysis can also inform future experimental design, in particular the selection of surrogates for future inactivation studies of high BSL level human viral pathogens. For example, appropriate surrogates for pathogens should have the same surface type as the pathogen (presence or absence of viral envelope), a similar capsid size, and a similar nucleic acid type, whereas genome size is a less important factor.

Although we identified a number of significant virus characteristics and environmental conditions that influence QAC efficacy against viruses, there is substantial inter-study variability that remains unexplained. Specifically, we found low explanatory performance of the risk factors on LRVs of QACs (R2 = 24%). These findings suggest unidentified or hidden variables influencing the relationship between CT of QACs and corresponding LRVs of viruses. Inter-experimental variation in study design or experimental set-up may be a driving factor. Future studies should seek to identify the hidden drivers of this variability. For example, inter-experimental sources of variation include: 1) the inconsistency of study design and sampling; 2) variance in measurement methods such as PCR and culture measurements; and 3) insufficient reporting on experimental set-up, consistency, and reproducibility. Additionally, analysis of the data was complicated by incomplete or missing information in a number of studies. Most notably: 1) information on disinfectants in proprietary formulations; 2) limit of detection of quantitative assays; 3) variation, such as standard deviation, in replicates; 4) information about concentrations of QACs or carbon loads; 5) pH of the solution; and 6) cytotoxicity of QACs to host cells when using culturing methods for quantification. Additionally, information about virus properties such as size were often not reported, particularly for some specific strains. Although one can predict from a species or genus level an average size, there might be an unaccounted difference within different strains.

As disinfection studies are continually published, it would be beneficial to define a standard set of experimental parameters to increase comparability across future studies and help inform future meta-analyses. This could include parameters on standard viral concentrations, contact times, temperatures, disinfectant concentrations, application method, and quantification method. Another possibility could be to establish a validation test organism for experiments, such as the use of bacteriophage MS2 in UV inactivation studies (80). Using a standard bacteriophage or other indicator for disinfection studies may help to increase comparability between experiments, or at least highlight the impact of different study protocols on virus disinfection estimates. Given the influence of an envelope on QAC efficacy, we suggest that studies include both enveloped and non-enveloped viruses, for example Phi6 and MS2. Benchmarking observed inactivation of standardized viral targets would provide insights into the relative efficacy of study protocols.

The analysis was based on a systematic review identifying 75 studies on disinfection of 29 families of viruses, yielding a pool analysis of 1012 unique data points. Substantial inter-study variability in methods, results, and reporting highlights the challenges of meta-analysis studies, limits the predictability of disinfection efficacy of untested viruses, and suggests the need for more rigorous standardization and reporting of disinfection studies in peer-reviewed literature.

# Methods

In this systematic review, data about disinfection of viruses with QACs were collected following the PRISMA-P guidelines (31).

## Eligibility criteria

Criteria were defined using the Population, Intervention, Comparison, Outcome and Study type (PICOS) framework (31). The population chosen was all viruses (regardless of host type) suspended in liquid or inoculated onto surfaces. The intervention was disinfection with QACs. Comparisons were made between viral characteristics (virus size, virus genome size, presence of viral envelope) and environmental factors (carbon load and temperature). Carbon load was defined as additives, with an expected reaction with QACs due to their load or chemical structure, such as: blood, soil, fecal suspensions, FBS/FCS (fetal bovine/calw serum), milk, yeast extract, wheat, and others. The outcome that was considered was log reduction value (LRV), defined as the log10-transformed number of viruses surviving disinfection minus the log-transformed total number of viruses present before the disinfectant was applied. All studies with a quantitative measure of CT value, defined as the concentration of disinfectant (in mg/L) multiplied by the application time (in min), and reported or calculable LRV of quaternary ammonium compound disinfection were included. Only studies in English were included and no restrictions were made in terms of year of publication.

## Information sources and search strategy

The three following databases were searched in April 2020: PubMed, EMBASE, and Web of Science. We used the following generic search string adapted for the databases listed above:

(disinfection OR disinfectant OR inactivation) AND (virus OR bacteriophage OR viral) AND (“quaternary ammonium compounds” OR “benzalkonium chloride” OR QUAT)

## Selection process and data collection process

Search results of the three databases were collected and uploaded into the open-source software Zotero (Zotero Software 5.0). Duplicates were deleted and the resulting library was imported to Covidence (www.covidence.org), which was used to facilitate the following selection process. Initially, two independent researchers (Anne Vescovi: AV and Elyse Stachler: ES) screened titles and abstracts to exclude irrelevant studies, based on exclusion criteria listed below. Conflicts were resolved through discussions between ES and AV. Next, full text screening (by AV with ES verifying 10% of studies for quality control) was completed to decide eligibility of the study to be included in the current review. Studies that did not meet the following criteria were excluded from the current review: 1) original study with unique data (no review articles); 2) quantitative data on viral inactivation; 3) included QACs with known applied concentrations; 4) QACs are not mixed with non-QAC active ingredients or bound on surfaces; 5) all relevant study information was included; 6) written in English

One reviewer (AV), with a second independent reviewer (ES) screening 10% of studies to verify data accuracy and consistency, extracted data. The information listed in Table 2 was extracted from the studies and recorded in a centralized spreadsheet. If information about the analyzed virus characteristics were missing, they were gathered from previously published peer-reviewed literature to complete the dataset.

|  |  |  |
| --- | --- | --- |
| Study information | Experimental information | Virus characteristics |
| Title | **Used disinfectants** | Virus name and strain |
| First Author | **Concentration** | Genus |
| Year | **Log10 reduction value** | Family |
| Link (DOI) | Number of samples | **Envelope (Yes/No)** |
|  | Standard deviation | Bacteriophage (Yes/No) |
|  | **Temperature** | Disease (if mammalian) |
|  | **Time** | Host (if bacteria) |
|  | **Solution or Surface** | **Genome size** |
|  | Application(sprayed/pipetted) | **Nucleic acid type** |
|  | Dry time | Cycle (lytic, temperate, Lysogenic) |
|  | **Measurement method (molecular-/ culture-/antibody-based methods)** | Capsid (Icosahedral/Prolate/Helical) |
|  | pH | **Virion size** |
|  | **Carbon load** | Isoelectric point |

## 

Table 2 Overview of the collected information. Variables included in the analysis are emboldened.

## Data analysis

Data were analysed with the open-source software R (version: R x64 3.6.3 and R i386 3.6.3) and the integrated development environment RStudio (RStudio, version: R-3.6.3). Packages were used for structuring the data and plotting (used packages: “hexbin”, “readvl”, “tidyverse”, “ggplot2”, “reshape2”, “dplyr”). When studies reported incubation at room temperature, the temperature of the experiment was set to 22 °C for further analysis.

## Statistical analysis

Backward elimination multiple linear regression was used to determine the effect of viral and environmental characteristics on the efficacy of QACs. The log reduction value (LRV; defined as the log-transformed number of viruses surviving disinfection minus the log-transformed total number of viruses present before the disinfectant was applied) was modelled as a function of the reported CT factor (defined as the concentration of the QAC the virus is exposed to multiplied by the duration of exposure), viral characteristics (virus size, virus genome size, nucleic acid type, and presence of a viral envelope), environmental characteristics (carbon load, QAC type, surface applied or in solution, and temperature), and experimental characteristics (method of measurement). Virus characteristics included in the initial model were chosen due to data availability and the potential influence on QAC ability to disinfect virus through the disruption of the viral envelope (presence of a viral envelope), formation of micelles (virus size), and binding of DNA (virus genome size and nucleic acid type). The environmental characteristics, which were widely reported in the studies and could affect the outcome, were selected to include in the model: 1. Temperature, which may increase QAC activity. 2. Carbon load, which may decrease QAC activity by acting as a quencher. 3. QAC type, several QACs have been reported to have higher inactivation abilities. 4. Application on surfaces, suspensions dried on surfaces can protect viruses by forming multiple layers.

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# Conflict of interest:

None to report.

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7 Figure legend

Figure 1: PRISMA-P systematic review flow chart

Figure 2: Virus families identified in the systematic review and the associated number of unique data points, grouped by each unique study (grey squares)

Figure S1: Distribution of surfaced. y axis: number of unique data points used on different surfaces sorted by the surface material. Squares represent studies; square length represents number of data points.

Figure S2: Distribution of nucleic acid types in the data set

Figure S3: distribution of genome size in the complete dataset

Figure S4: Distribution of data points by virus sizes in nm, colored by non-enveloped (red) and enveloped (blue) viruses

Figure S5: log reduction over CT factor of 4 size groups of non-enveloped viruses.

Figure S6: log reduction over CT factor of 4 size groups of enveloped viruses.

Figure S7: distribution of temperature, colours representing temperature ranges

Figure S8: Distribution of different QACs, squares represent studies. Abbreviations: BAC: benzalkonium chloride, BEC: benzethonium chloride, DDAB: didecyl dimethyl ammonium bromide, DDAC: didecyl-dimethylammonium chloride, MBAT: mono; bis (tri-methyl ammonium methylene chloride)-alkyl (C9-15) toluene, others: CTAB: Cetyltrimethylammonium bromide, dimethylalkonium bromide, CPC: cetylpyridinium chloride, QAC1: 2-Hexadecanoyloxyethyl)triethylammonium Bromide, QAC2: 1-(2-Dodecanoyloxyethyl)pyridinium Bromide, QAC3: Eicosanoyloxyethyl pyridinium Bromide, QAC5: 1-(2-Hexadecanoylaminoethyl)pyridinium Chloride, QAC6: Tributyl-(2-dodecanoyloxyethyl)ammonium Bromide, QACmix: a mix of different quaternary ammonium compounds.

8 Table legend

Table 1: Multiple linear regression model for impact of virus characteristics for factors influencing disinfection of virus with QACs.

Table 2: Overview of the collected information. Variables included in the analysis are emboldened.

Table S1: Backwards elimination multiple Regression model 1

Table S2: Backwards elimination multiple Regression model 2, excluded “In Solution or on Surface”

Table S3: Linear regression model of the subset of enveloped viruses. NA no data for these factors in the subset.

Table S4: Linear regression model of the subset of non-enveloped viruses. NA no data for these factors in the subset.

Table S5: Overview abbreviation and abundance of QACs in this study

SI Dataset file legend:

Data points

|  |  |
| --- | --- |
| **Colum name** | **Description** |
| Abbreviation | Abbreviation of the virus Name |
| Full Virus Name | The original full virus name used in the study of the origin of this data point |
| Surface [e=enveloped, n=non-enveloped] | Structure of the outer most layer of the virus of this data point, “e” describes lipid enveloped viruses, “n” describes viruses without lipid envelope |
| Temp © | The Temperature in degrees Celsius, “RT” stands for room temperature and was reported either as room temperature or between 20 and 23 degrees Celsius. |
| Percentage of inactivation | If deactivation was reported in percentage instead of Log reduction value, percentage of inactivation was used to calculate the log reduction value. Value in percent. |
| log removal | Log transformed deactivation of the virus |
| LOD/LOQ | To highlight if the limit of detection or quantification was reached, the log reduction value is added into this column |
| time (min) | Disinfectant exposure time in minutes |
| CT (mg\*min/L) | CT value; concentration times exposure time in mg times minutes divided by liter |
| Disinfectant - active ingredient | The name of the quaternary ammonium compounds reported in the study of origin, if multiple quaternary ammonium compounds were used they are listed and separated with a comma. |
| Abbreviation QAC | For easier handling, quaternary ammonium compounds were abbreviated and grouped, see list of abbreviations in supplementary material. |
| C (mg/L) | Concentration of quaternary ammonium compound used for this data point in milligram per liter. In case of multiple quaternary ammonium compounds the sum is reported. |
| concentration reported in literature | The concentration and unit reported in the study of origin. |
| drytime (min) | If viruses were dried before deactivation, the dry time is reported here in minutes. |
| material | If viruses were applied on a surface before exposure, the surface material is reported here. |
| method of application | If viruses were applied on a surface before exposure, the method of application of the quarterly ammonium compound(s) is described here |
| carbon load | If a carbon load was added to the experiment the reported carbon load and reported concentrations are listed here |
| Replicates technical | Number of technical replicates |
| Replicates biological | Number of biological replicates |
| standard deviation | Standard deviation as reported in the study |
| measurement type | The name of the measurement method of virus deactivation as reported as in the study |
| measurement (culture or PCR) | Abbreviation of the measurement type (see above): culture based methods are defined as “c”, PCR based methods are defined as “p”, and antigen based methods are defined as “a”. |
| pH | The reported pH of the experiment, if reported. |
| Title | Title of the Study of the origin of this data point |
| First Author | Name of the first author of the study of origin of the data point |
| Studies by number | Abbreviation of the studies in this dataset. One number represents one study |

Virus properties

|  |  |
| --- | --- |
| Abbreviation | Abbreviation of the virus Name |
| Full Virus Name | The original full virus name used in the study of the origin of this data point |
| Genus | Genus of this virus |
| Family | Family of this virus |
| Host | Virus Host |
| genome\_size [kilo bases] | Genome size of the virus in kilo bases |
| nucleic acid type | Nucleic acid type of the virus abbreviations as followed: ssRNA(-): negative sense single stranded RNA, ssRNA(+): positive sense single stranded RNA, dsRNA: double stranded RNA, ssDNA: single stranded RNA  dsDNA: double stranded DNA |
| Surface(e=enveloped,n=non-envel.) | Structure of the outer most layer of the virus of this data point, “e” describes lipid enveloped viruses, “n” describes viruses without lipid envelope |
| size orig. With range | Virus size, as described in the literature with reported ranges in nanometers |
| Size[nm] | Average virion size in nanometer |
| size extracted from ( str= strain, sp=species, type=type, gen= genus, fam=family) | Viruses sizes were extracted from the literature based on: str= strain, sp=species, type=type, gen= genus, fam=family |
| Title | Title of the Study of the origin of this data point |
| First Author | First author of the Study of the origin of this data point |
| Year | Year of publication |
| DOI | DOI of the Study of the origin of this data point |
| Temperatures © | The Temperature in degrees Celsius, “RT” stands for room temperature and was reported either as room temperature or between 20 and 23 degrees Celsius. |
| Reference for virus size | References of the virus size |
| second reference for virus size | In case of multiple references for virus size, others are listed here |