Antimicrobial resistance and bacteriophages: an overlooked intersection in water disinfection

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Abstract

This article focuses on how bacteriophages (phages), antibiotic resistance genes (ARGs) and disinfection practices intersect. Phages are considered the most abundant biological entities on Earth and they have the potential to transfer genes among their bacterial hosts, including ARGs. In the urban water cycle, phages are used as indicators of faecal pollution and surrogates for human viral pathogens but they are also known to withstand common disinfection treatments deployed to produce safe drinking/reclaimed water. Recent studies also suggest that phages have the potential to become an additional footprint to monitor water safety. A precautionary approach should therefore include phages in surveillance programs aimed at monitoring antimicrobial resistance (AMR) in the urban water cycle. This article argues that phages ought to be used to assess the efficiency of disinfection treatments (both classical and novel) on reducing the risk associated with antibiotic resistance. Finally, this article discusses contributions to the advancement of AMR stewardship in aquatic settings and is relevant for researchers and water industry practitioners.

Keywords: Antimicrobial resistance; disinfection technologies; horizontal gene transfer; urban water cycle

Highlights

- From a precautionary viewpoint, monitoring of phages and ARGs should be included when designing and developing new disinfection treatments aimed at removing possible AMR risks from treated water.
- Investments in upgrading wastewater treatment plants to decrease AMR risk in treated waters are on the horizon for the water industry.
- Deployment of disinfection to remove phages and the related AMR risk needs further assessment. The method should be cost-effective and should not trigger horizontal gene transfer side-effect. Membrane filtration methods are promising technologies to remove both phages and ARGs, but these still need to decrease in cost.

Glossary box

- Antimicrobial resistance (AMR): intrinsic or acquired ability of bacteria to withstand antimicrobial treatment.
- AMR determinants: All genes that encode for mechanisms of AMR. It should be noted that phages or other MGE are not antimicrobial resistance determinants *per se*. AMR determinants are all genes that encode for proteins involved in AMR [1].
- AMR stewardship [2]: coordinated interventions designed to promote, improve, monitor and evaluate the judicious use of antimicrobials to preserve their future effectiveness, and to promote and protect human and animal health.
- Bacteriophages: viruses that infect and replicate in bacterial cells.
- Horizontal gene transfer (HGT): is a process in which an organism (the donor) transfers genetic material to another organism (the recipient) of the same or different species.
- Mobile genetic elements (MGEs): are identified as fragments of DNA that encode a variety of virulence or resistance determinants, as well as the enzymes that mediate their own transfer and integration into new host DNA. Phages, phage-related particles, plasmids, genomic islands, integrons and integrative conjugative elements (ICEs) are MGEs [3,4].
- NDMA (*N*-Nitrosodimethylamine): a well-known DBP (disinfection-by-products) characterized by its toxic and carcinogenic effects.

1. Introduction

Antimicrobial resistance (AMR) has become a growing global public health concern due to the difficulties and increased costs in treating antibiotic-resistant infections [5,6]. In fact, AMR causes an estimated 700,000 deaths annually worldwide and that has been predicted to exponentially rise to above 10 million deaths annually by 2050 [7]. A better understanding of the mechanisms and pathways underlying AMR is therefore urgently needed to implement effective public health policies, programmes and interventions at all levels. Reclaimed water systems are not exempt from the impact of AMR. Considering that there is increasing evidence that **bacteriophages** may carry antibiotic resistance genes (ARGs) [8,9], their implications for environmental and human health should not be underestimated. Phages – viruses that infect bacterial hosts – are biological entities consisting of single or double stranded DNA or RNA surrounded by a protein coat (capsid), which is able to withstand disinfection treatments [10,11].

Disinfection is an essential step during drinking water production. Most wastewater treatment plants (WWTP) have only up to secondary treatment (focused on the removal of organic matter by activated sludge), and disinfection is mainly limited to when water is intended for reuse [9] or recreational bathing purposes. However, the quest to achieve a circular economy in the water sector [12], driven by a growing global need for reusing water, is expected to increase the application of disinfection methods and tertiary treatment technologies in WWTPs.

This review article puts the spotlight on phages and their contribution to AMR in the context of water treatment. Novel insights on the relationships between water disinfection, antimicrobial resistance, and phages and ARG are presented (**Figure 1**).

Figure 1.

2. Antimicrobial resistance and phages

Although substantial efforts have been made to understand the mechanisms that promote AMR [13,14], limited information is available about the extent to which phages contribute to the acquisition, maintenance and spread of this phenomenon. Among the main processes responsible for the increasing prevalence of AMR, **horizontal gene transfer** (HGT) plays an important evolutionary role that allows the movement of genetic material between both closely and distantly related organisms. This process is mediated by mobile genetic elements (MGEs), such as phages [3,15,16]. The concentration of phages in the biosphere is estimated at ~10^{31} phages, thereby increasing the likelihood of phage related HGT events occurring [17,18] (see **Box 1** for more details on HGT).

Phages are mainly involved in HGT by transduction mechanisms. In fact, many studies have provided evidence that phage particles carry genes conferring resistance to different antibiotics and, in some cases, these particles effectively transduce ARGs to recipient bacterial cells [19–21]. By doing this, phages may benefit from host survival under antibiotic selection and thus favour not only their own persistence but also the spread of transferred ARGs [22–24].

Interestingly, a recent study has shown that environmental phage fractions contain genes conferring resistance to β-lactamase and carbapenems (7.3% to 64.9%, respectively) at a greater proportion than in bacterial fractions (5 to 36.8%, respectively) [19]. Some authors, however, argued that ARGs are more abundant in bacteria than in phages [20,21]. Also, phages in the human microbiome rarely encode ARGs [25]. In clear contrast, phages from non-human sources (e.g., pig faeces, raw sewage, and freshwater and marine environments) contain a large reservoir of ARGs [26]. Despite the controversy, a recent

114 study has demonstrated that phages isolated from wastewater successfully transduced β-
115 lactamase genes into *E. coli* [27]. Further efforts are needed to elucidate the rate at which
116 phages actively contribute to the transfer of ARGs among environmental bacteria in
117 aquatic settings.

118 **Box 1. Horizontal gene transfer and phages**

119 Mobilization of genes (including ARGs) among bacterial cells occurs through three main
120 mechanisms: (i) conjugation (mediated by plasmids or conjugative transposons); (ii)
121 transformation (the uptake of free DNA from the surrounding milieu); and (iii)
122 transduction (mediated by phages). Three transduction mechanisms have been described,
123 namely generalized, specialized and lateral [10,23,28]. The latter has been recently
124 described in temperate phages of *Staphylococcus aureus* and its characteristic feature is
125 that prophages excise later in their life cycle, allowing for an exacerbated (up to 1,000
126 greater that previously observed) random packaging of host genome fragments. This
127 process will generate both true or competent phages and transducing particles containing
128 bacterial DNA, and it is considered key to bacterial evolution [28].

129 **Phage life cycles: lytic and lysogenic pathways**

130 Depending on the phage, the infection of the bacterial host may follow either a lytic or a
131 lysogenic pathway. In the lytic cycle, the infecting (or infectious) phage uses the cell
132 machinery to replicate itself, to assemble new viral particles and to lyse the host cell,
133 thereby resulting in the release of its progeny. The lysogenic (or temperate) cycle usually
134 involves the integration of the phage genome into the host chromosome and the
135 maintenance of a latent state – the prophage – that perpetuates until environmental cues
136 (nutrient imbalance, UV light, chemicals) trigger the lytic pathway (induction).

137 **Phages and transducing particles**

Errors in the packaging of phage genomes during assembly of new virions may result in the formation of viral particles containing hybrid genomes (in specialized transduction this correspond to a defective phage genome + bacterial genes) or particles containing only bacterial genome fragments (transducing particles in generalized transduction) [19–22]. Both hybrid genomes and transducing particles can infect the host, but they cannot multiply inside the host cell. Only "true" phages (those which contain the complete viral genome) are able to carry out the viral cycle, multiply inside the host and release progeny.

3. Disinfection of phages and ARGs

Phages are usually considered surrogates of human viral pathogens and thus it is important that their removal be monitored to ensure water safety. Phages have recently been suggested as more reliable indicators of the occurrence of viral pathogens than traditional indicator bacteria (*E. coli*, coliforms, etc.) [29]. New commercially available tests that utilize phage kits (BluePhage®) [30] are thus gaining market traction. Therefore, we foresee the surveillance of phages being implemented at larger scale in WWTPs and water reuse scenarios.

Most disinfection studies to date, both in the lab and in real scale, have focused on the removal of faecal bacterial indicators (FBIs). In this context, data on phage and ARG removal are still scarce. A precautionary approach to deal with the possible AMR risk is therefore necessary. Advanced tertiary treatments (which may include certain disinfection or membrane methods) have a better potential to remove phages and **AMR determinants**. In this article, we argue that phages ought to be used to assess new disinfection treatments, so that the potential removal of phages carrying ARGs and the possible associated AMR risks are more fully comprehended.

Representative data on the responses of phages and ARGs to various disinfection methods are compiled in **Table 1**. Filtration methods have been included for comparison purposes. For the evaluation of the disinfection efficiency, it is necessary to count phage plaques or halos (lytic zones caused by infection of a sensitive bacterial host by a phage particle) on double agar overlay plaque technique [31]. In this way, the information available from the disinfection literature regarding phage disinfection originates mostly from studies targeting true phages and not transducing/defective particles. As regards disinfection of ARGs, the data shown in **Table 1** were resourced from studies targeting disinfection of, in most cases, extracellular ARGs. We have only encountered one study that targeted disinfection of ARGs in the phage fraction of wastewater samples [32]. Each disinfection method is commented on in more detail below.

Table 1.

From Table 1, we observe an overall trend: the disinfection dose to achieve a 1–Log reduction (90%) of ARG concentration is commonly greater than the dose required to achieve a similar reduction of phage counts. The specific reasons for these differences need to be analysed by taking into consideration the environmental conditions under which the disinfection assays were performed. Factors such as aqueous media composition, competing COD (chemical oxygen demand), and specific biochemical features of the ARG and phages involved may play a role in the response to a disinfectant [33,34]. Also, from the reviewed data, it is possible to conclude that disinfection of ARGs and phages is not yet cost-effective. High doses of disinfectant would be required to (i) achieve the disassembly of the viral capsid, and (ii) ensure enough contact time to

185 inactivate the ARG. If the total elimination/disinfection of ARGs or phages is still not a
186 feasible target, the alternative goal should be to monitor traditional indicators of AMR
187 such as antibiotic-resistant bacteria (ARB).

188 *3.1. Ultraviolet Radiation*

189 In wastewater treatments, generally the type of UV deployed for microbial activation is
190 the germicidal wavelength of monochromatic lamps emitting UV light at 253.7 nm (or
191 UV-C). Other wavelengths and lamps may be utilised, although UV-C is the one that is
192 most commonly used. Doses of UV are calculated as a function of the lamp or reactor
193 emission in mW per cm^{-2} versus exposure time in seconds, which in turn is equated to a
194 value in mJ. UV-C doses range between 5 and 400 mJ/cm^2, which corresponds to a
195 reduction of gene copies in the range between 0.2–6 Log [32,33,35–37]. UV-C doses to
196 achieve reduction of phage particles between 4–7 Log were relatively lower, that is
197 between 5–250 mJ/cm^2. From these values, described in detail in **Table 1**, it seems that
198 Log reductions of phages are more easily achieved by UV than Log reductions of ARG
199 copies. However, it is important to highlight that, in some cases, deployment of high UV
200 doses has been shown to increase the abundance of ARGs [38].

201 Phage genomes are enclosed by a protein shell (i.e., the capsid), which provides protection
202 against environmental challenges including UV radiation. In fact, the deactivation of
203 ARGs in phage fractions of wastewater are delayed in comparison to the deactivation of
204 ARG in bacterial fractions [32]. Other influential factors in UV disinfection are aqueous
205 media composition, such as suspended particles, which may shield ARGs and phages
206 from UV radiation, and aggregation of viruses to particles.

3.2.Chlorination

In WWTPs of USA and Canada, disinfection is often required prior to wastewater effluent discharge into the environment. The first and most widely used method of water disinfection results, unfortunately, in the generation of disinfection-by-products (DBPs). Although required in these North American countries, at global scale, disinfection of wastewater is generally not a standard practice in WWTPs [39]. In WWTPs, standard doses of chlorination are 5 to 20 mg/L versus a contact time which depends on physicochemical features of the wastewater [40]. Impairment of ARGs and phages are likely to occur by chlorine but largely depend on aqueous media composition. The dose that has been reported to reduce phages by 1–Log is 1 mg /L × 30 min. On the other hand, doses that were reported to achieve up to 6–Log units of ARG reduction ranged between 1–1000 mg/L (time and aqueous media varied) [34,36,37]. More detailed metrics on disinfection of ARGs and phages can be found in **Table 1**.

3.3. Advanced Oxidative Processes (AOPs)

AOPs present a promising technology for microbial reduction of viruses; however, they are not yet scalable for large applications [41]. Available both as a homogeneous (only aqueous phase reagents with or without a light source) and a heterogeneous phase (solid catalyst or semiconductor involved plus a light source) [42], the main downsides to AOPs include the likelihood of microbial or ARG repair and hydroxyl (or other) radical scavenging. General comments about AOPs are listed next (with detailed appraisals in **Table 1)**. Both homogeneous and heterogeneous catalysts have been shown to be effective at removing phages, but less effective in removing ARGs. The ranges of disinfection reported of phage and ARGs, in various types of waters matrices (such as buffers or distilled water, or artificial wastewater) and in lab scale, were up to 10–Log

reductions of PFU/ml (plaque forming units per mL) for phages and to 4–Log reduction for ARGs. Also, in the case of heterogeneous photocatalysis, immobilised catalysts provide lower quantum yield because of the reduced surface area. Although more efficient, suspended catalysts have been proved to not be feasible, thus far, for deployment at large-scale because of post treatment separations. Finally, various efforts to change the characteristic of catalysts [41], such as doping, to increase absorption of visible wavelengths and result in improved quantum yield have been shown to contribute to improved disinfection [41–47]. Homogeneous photocatalysis, such as Fenton reaction, have gained traction in lab scale testing; however, ARG and phage inactivation by this method are still low or subject to recovery after post-treatment incubation (**Table 1**). More studies in the area of photo-Fenton disinfection are thus necessary [48].

3.4.Ozonation

Less frequently employed than chlorination, ozonation has a lower risk of DBPs generation during disinfection in WWTPs. However, there are significant downsides to implementing this method in large-scale applications. These include high cost, technical difficulties with dosing, and no lasting disinfectant residual concentration [48]. Ozonation doses reported to achieve inactivation of ARGs (1–6 Log) ranged between 0.20–0.9 mg O_3/mg DOC. On the other hand, inactivation of phages (4 –9 Log) required ozone doses between 0.25–0.6 mg O_3/mg DOC [37,42,49–51]. From Table 1, it seems the method is highly efficient for disinfecting both phages and ARGs. However, while considering ozonation in the context of water reuse, one must monitor DBPs such bromates and *N*-Nitrosodimethylamine (**NDMA**), as well as be aware of the need for downstream toxicity tests of treated water to avoid adverse health effects [42].

3.5. Peracetic acid and performic acid

In the search to find alternatives that are more sustainable and possess a lower risk of DBP generation than chlorine disinfection, various alternative disinfectants are currently being investigated. Peracetic acid (PAA) (CH_3CO_3H) is a new sterilizing agent, which has been gaining attention in the water treatment sector. Efficient at inactivating both bacteria and viruses, PAA possesses a lower risk of generating DBPs [48]. In fact, this method has been shown to inactivate ARB in wastewater aquatic settings [52]; however, regrowth of bacteria was observed, and might be related to the formation of the easily assimilable acetic acid [53]. Rizzo et al. [42] advised that to target ARB, PAA is not efficient enough, and needs to be used with a coadjutant disinfection method. This approach may also be necessary to disinfect phages and ARGs, which are more problematic targets for disinfection [54]. Another disadvantage of PAA is its high cost.

Alternatively, Performic Acid (PFA) (CH_2O_3) is up to 20 x faster and more efficient as a disinfectant than PAA, as evidenced by tests done on coliforms and murine norovirus in wastewater [55]. It has also been recently used for treating municipal wastewater and combined sewage overflows [54]. PFA is the strongest oxidising (oxidizing potential of 2.70 V) disinfectant currently available and it has been shown to rapidly decompose into CO_2 and water. It has been shown that this method will work more effectively at a pH of 7 and its efficiency decreases with lower temperatures [53,54]. To the best of our knowledge, PFA has not been yet explored for the disinfection of phages and ARGs and this remain to be explored; thus, the method is not covered in Table 1. Also, a major concern with PFA is the feasibility of ensuring the safety of operators during its deployment in WWTPs.

3.6. Monochloramine (NH_2Cl)

Monochloramine (NH_2Cl) is a less efficient disinfectant than chlorine but also less prone to generate DBPs such as trihalomethanes. Although NH_2Cl has an overall low reactivity towards carbohydrates, proteins, and nucleic acids [34] disinfection was still feasible. In fact, this method of disinfection has been applied to avoid microbial regrowth in membrane bioreactors that treat secondary wastewater effluent prior to reverse osmosis (see discussion on membrane methods below) [56]. Results were more promising in buffers than in wastewater, with doses ranging from 1228 mg × min/L for 1–Log removal of phages [57] to 1.5–3.0 $\times 10^5$ mg × min/L for 4 to 6–Log removal of ARGs [33]. However, it should be noted that this method is not yet scalable for disinfection of phages and ARGs and further investigations are warranted.

3.7. Filtration methods

Our rationale for including filtration methods in the current discussion is that they have competitive removal rates when compared to chemical, UV and AOPs-mediated disinfection. The aim of filtration treatments is not inactivation of ARGs, phages or bacteria, but rather their physical removal from drinking and wastewater. Membrane-based processes present a wide array of removal efficiencies, membrane setups, applications and materials, and costs. They are generally applied to complement other disinfection methods in the water treatment process chain.

Filtration methods are typically classified according to their size-exclusion cutoffs, as follows: membrane filtration (MF) allows separation of particles greater than ~100 nm; ultrafiltration (UF) is the separation of macromolecules with molecular weight between ~1 kDa to 1000 kDa; nanofiltration (NF) can remove both macromolecules and ions (~1 kDa or less), while reverse osmosis (RO) can remove ions (~100 Da or less) [58]. As a

301 matter of comparison, most phages range in size from ~20 to 200 nm in length [59],
302 which is a relatively low variability and might be unlikely to cause major effects on the
303 exclusion response of phages to disinfection (although experimental data are lacking). On
304 the other hand, phage genomes can vary from ~3.0 kb to over 500 kb [60], whereas ARGs
305 range from ~200 bp to over 2000 bp [61]. As can be seen from **Table 1**, UF, NF and RO
306 can achieve the highest removals for both phages and ARGs (4.4–7 Log for phages, and
307 5.9–9.5 Log for ARGs) [62–64] when compared to all other methods. To be effective,
308 these membranes however require pre-treatment of water to prevent clogging. Also, NF
309 and RO treatments require post-treatment of membrane concentrate and high energy
310 input, which means that careful feasibility assessments are necessary to remove phages
311 and ARGs prior to implementing these solutions at a larger scale [42].
312
313 **4. Knowledge gaps and outstanding questions**
314 From a precautionary point-of-view, stakeholders acting on global **AMR stewardship**
315 should be informed about where to devote their efforts [65]. To date, the risk that phages
316 pose to ARG spread in aquatic settings has not been established. Questions about the
317 relationship between phages and ARGs in the context of AMR and disinfection are
318 discussed in the Outstanding questions box. A few clues to address these questions are
319 also presented as follows:

320 I. In a disinfection system, it is not currently possible to specifically target phages
321 containing ARGs. Methods of disinfection applied to reduce phage particles, if cost-
322 effective, could meet the criteria of the precautionary approach to mitigating AMR
323 risks relating to phage particles in aquatic settings.

II. It is not yet possible to distinguish between true phages and transducing particles. Advanced microscopy techniques such as Transmission Electron Microscope (TEM) could help in assessing alterations in the morphology of phage particles caused by disinfection treatments. Investigations on developing more accessible methodologies to assess the different ways in which disinfection methods affect various phages particles are needed.

III. A clearer correlation between the decrease of AMR risk in aquatic settings and the disinfection of both phages and ARGs needs to be established so that AMR efforts can be best applied.

IV. As faecal indicator bacteria (FIB) play a role in assessing the microbiological risks of water sources, future studies should examine the relationships between indicator phages, ARGs, and AMR risk. Our group is currently working to assess the efficacy of novel disinfection methods on the reduction of phages, ARGs and the overall HGT risk. We encourage other research groups to also pursue this effort, and to focus on removal or reduction of other MGEs from aquatic settings.

V. The cost-effectiveness and feasibility of disinfection technologies to remove phages and ARGs should be carefully considered. Two case-studies in large-scale are briefly presented next in the treatment of hospital wastewaters [66] and toilet-to-tap reuse scenarios (https://www.ocsd.com/). While these studies resulted in a measurable reduction in ARB and ARGs, the deployment of such treatments requires high financial investment. The Grundfos BioBooster system [66] claimed reduction of pharmaceuticals and ARB using a combined point-of-use tertiary treatment to treat hospital wastewater (Herlev hospital, Denmark). Treatment included a membrane bioreactor/filters, ozone above 4 mg O_3/ mg DOC^{-1}, followed

by granular activated carbon and UV, thus resulting in complete removal of ARB. In the BioBooster system, phages were not monitored; however, a 4–5 Log reduction in waterborne virus was achieved. Investment necessary for the BioBooster system ranged between 3.3–4.7 million euros. Another example comes from California Orange County Sanitation District (https://www.ocsd.com/), which used an advanced water treatment facility to treat wastewater for both aquifer refill and potable reuse. In their case, treatment methods included chlorination, micro-filtrations, reverse-osmosis, ultraviolet disinfection and advanced oxidation systems. Although ARGs were reduced to levels under the detection limit (<50 copies per L) after treatment, they did increase back in the aquifer and in the distribution systems [67].

VI. It should be noted that the water sector does not assess the potential risk associated to phages carrying ARGs. Nanofiltration and reverse osmosis methods have been shown to reduce the amount of phages + transducing particles + ARGs and other MGEs. Subject to further feasibility studies, they might be the only current solution to target these various types of AMR contaminants.

5. Concluding remarks

The role of phages in the acquisition and spread of ARGs in aquatic settings is now undisputable. Our opinion is that, from a precautionary viewpoint, the monitoring of phages and ARGs should be included when designing and developing new disinfection treatments aimed at removing possible AMR risks. Currently, such studies have proved more feasible with infectious phages, although transducing phage particles and other MGEs should also be considered. Our conclusion from the review is that in water disinfection and antimicrobial resistance research, bacteriophages really matter.

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581

582 **Table and Figure captions**

583

584 **Table 1. Responses of phages and ARGs to various disinfection treatments.**

585

586 **Figure 1. A potential intersection between phages, antimicrobial resistance and**
587 **disinfection practices.** Aquatic settings (circle 1): these include urban water cycle
588 wastewater treatment and drinking water systems. Phage–mediated HGT risks (circle 2):
589 there are several unassessed AMR risks in aquatic settings. These include ARB, MGEs,
590 ARGs (in the form of free DNA), true phages and transducing particles. Disinfection
591 treatments (circle 3): the need and the feasibility of disinfection methods to remove
592 phage–mediated HGT risks needs to be assessed further. Arrows indicate that, from a
593 precautionary viewpoint, monitoring phages and ARGs should be included when
594 designing and developing new disinfection treatments aimed at removing possible AMR
595 risks from aquatic settings. All icons were obtained from The Noun Project
596 (https://thenounproject.com).

597

598 **Table 1.**

Process	Target	Dose/Treatment	Log Reduction[a]	Aquatic Environmental Settings[c]	Ref.
UV-C (253.7 nm) germicidal)	Phages	5.94–178.2 mJ/cm^2	7	wastewater	[32]
		5–250 mJ/cm^2	4.5–5.5	buffer/wastewater	[68]
	ARGs	5–178 mJ/cm^2 (ARG in phage genomes)	0.2[b]–1	mesocosm	[32]
		10–400 mJ/cm^2	<1–4	wastewater	[37]
		50–250 mJ/cm^2	3–6	buffers	[33]
		10–150 mJ/cm^2	<1	wastewater/ drinking water	[34]
Advanced Oxidative Processes (AOPs)	Phages	UV > 295 nm plus 0–25 mg/L H_2O_2 (15 min)	1–2.5	buffers, surface water	[43]
		UVA/B/C/or sunlight plus TiO_2 photocatalysis in solution or immobilised (2–2280min)	1–10 (not scalable)	lab matrices, distilled water, wastewater	[69]

	ARGs	UVA-B/H_2O_2 UV @ 320–450nm plus 20 mg/L to 340 mg/L H_2O_2 (up to 240 min)	0[b]–4 (not scalable)	wastewater	[70]
		33–72 mg × min /L chlorine and 50–130 mJ/cm^2 and 10 mg/L for UV/H_2O_2.	4	buffers, wastewater	[44]
		Fe^{2+}/H_2O_2 molar ratio 0.1 and a H_2O_2 [0.01mol/L] pH=3.0 120 min Fenton > UV/H_2O_2	2.5–3.8	wastewater	[45]
		UV/Fe/H_2O_2 $[Fe^{2+}]_0$= 5 mg/L plus$[H_2O_2]_0$= 50 mg/150 min ARGs persisted	97% total DNA	wastewater	[46]
		TiO_2-graphene based composite, Xenon lap=63 W/m^2	Some removal	wastewater	[71]

Chlorination	Phages	30 mg x min/L	1	mesocosms	[32]
	ARGs	15–450 mg x min/L	<1–2	drinking water, wastewater	[37]
		1–20 mg Cl_2/L, 2 mg x 30 min (initial [ ] 10^5 copies/µl), DNA fragmentation and reduction, genomic DNA more sensitive than plasmid borne DNA/ARG	70% reduction DNA signal	ultrapure water	[72]
		1–20 mg x min/L ARG in phages	0.1[b]–0.6[b]	mesocosms	[32]
		50 –150 mg x min/L	4–6	buffers	[33]
		180–1000 mg x min/L – extracellular fragmented plasmid and 16S	NR (various) Likely to occur	buffers	[34]

		rDNA depended on aqueous media composition. 10–100 mg x min/L intracellular DNA			
Ozonation	Phages	0.25–0.6 mg O_3 x mg DOC, MS_2 tipically inactivated in WWTP doses (0.25– 1 mg O_3 x mg DOC)	4–9	buffers	[49]
	ARGs	0.1–200 mg x min/L	1–3	wastewater	[37]
		0.8–0.12 mg x min/L	4–6	buffers	[33]
		0.1– 1 mg x min/L 15 mg x L (15 min) plasmid DNA	NR (DNA fragmented)	buffers	[34]
		(27–178 mg/L) 177.6 mg /L O_3 (corona discharge, time not mentioned	1.7–2.5	wastewater	[73]

		0.25–0.75 mg O_3/g DOC x 10 –40 min, various ARGs	2–6	wastewater	[51]
		0.2–0.9 mg O_3 /g DOC various HRT, depends on wastewater features, reduces ARGs, selects for bacterial resistance, recovery upon few days storage	various	wastewater	[42]
Peracetic acid	Phages	0–10 mg/L x 30–120 min plus UV-C @ 20 mJ/cm^2 (low reduction alone or in wastewater)	1–5	buffer, wastewater	[68]
		1254 mg x min/L greater removal in buffers	1	buffers, wastewater	[57]
	ARGs	25 mg/L x 15 min (plasmids reduced transforming activity)	0.3[b]	buffer	[74]

Monochloramine	Phages	1228 mg x min/L greater removal in buffers	1	buffers, wastewater	[57]
	ARGs	1.5–3.0 x10^5 mg x min/L (not scalable)	4–6	buffers	[33]
Ultrafiltration (~ 1kDa to 1000 kDa)	Phages	Polyamide polysulfone membrane 10–40 psi	0.3[b]–1.8	tryptic soy broth	[75]
		Membrane of polyvinylidene-fluoride 0.05 µm (0.2 to 0.6 Bar)	~0.1[b]–1	wastewater	[76]
		Various types of membranes and membrane sizes	2–7	wastewater, drinking water	[37,58]
		Various sizes (review chapter) 0.01–0.5 µm membranes	6	drinking water	[77]
	ARGs	Various sizes (reviews) (increase reported in treated water)	1–6	drinking water	[37,50]

		1.2 µm–1kDa PVDF, and cellulose membranes Millipore	0.9[b]–5.9	wastewater	[78]
		polysulfone polyamide membrane 0.15MPa, 80-100 KDa	iARG[d] removed	swine wastewater	[62]
		2.5–300KDa, 2–24bar polyether sulfone and polyamide thin	0.1[b]–3.1	filtered secondary wastewater, distilled water	[63]
Nanofiltration (~1kDa or less)	Phages	polysulphone, cellulose acetate (60–100 psi)	1.9–3	tryptic soy broth	[75]
		Various configurations <100 nm, including carbon nanotubes	0.5[b]–9	drinking water	[77]
	ARGs	polyamide (2.0 MPa) <500 Da	4.9–8.1	swine wastewater	[62]

		15–300 or 400 Da, 38-40 bar, polyamide	3–3.6	filtered secondary wastewater, distilled water	[63]
Reverse Osmosis (~100 Da or less)	Phages	Polyamide, cellulose acetate membranes, pore: 3–4 nm up to 23 nm (100–160psi)	3.5–4.4	tryptic soya broth	[75]
	ARGs	polyamide (3.6MPa) (ARG increase after treatment in wetlands)	5.2–9.5	wastewater, wetlands	[62]
		200 Da, 40 bars, polyamide	4	filtered secondary wastewater, distilled water	[63]

Table 1 shows the overall efficiency of removal of "true phages" (or "infectious phages") and ARGs (primarily in extracellular form) through classic and novel disinfection treatments, in a range of aquatic settings.

[a]Log reduction: ARG=Log gene copies, Phage=Log.

[b]Log disinfection values lesser than 1 and greater than 0 Log are possible when the count of gene copies (in the case or ARGs) or PFU/ml (in the case of phage plaques) are between 1 and 10 gene copies or PFU/ml, respectively. Note that while phage cultivation requires a cultivation method on agar through bacterial infection to quantify plaques, generally gene copies will be determined by a suitable molecular method, such as qPCR. Accessibility of working with molecular methods, however, is not straight-forward for most water monitoring microbiology labs.

[c] Data were collected from studies in WWTPs, drinking water treatment, and lab-scale and buffered water matrices, with the latter being the most frequent source.

[d]=iARG= intracellular ARGs

614 NR=Not reported.

615 Observation: Detailed reviews on the disinfection and removal treatment of ARB have
616 been covered extensively elsewhere [22–24].

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618