



Swimming Pools: A Favourable Environment for the Transfer of Bacterial Resistance

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Abstract

Bacterial resistance to diverse antimicrobials and biocides is a growing public health concern of global implications, which is why resistant bacteria are considered emerging environmental contaminants. Due to the selective pressure exerted by antimicrobials such as chlorine and other strong oxidants, this problem tends to increase, especially in environments such as swimming pools, where antimicrobials are being used in strong but non-lethal concentrations. Some bacteria report, in addition to a marked resistance to disinfection, the ability to live for prolonged periods in the presence of chlorine, which has important implications for public health. Through a review in different databases, the most relevant and updated articles on microbiological analysis in swimming pool water were chosen. The results showed that most of the studies are focused on the analysis of water quality, only considering indicator groups, but few studies focus on antibiotic-resistant bacteria capable of withstanding chlorination while retaining antibiotic resistance genes in the environment.

All this considered, the main objective of this review is to present updated information and delve into the role of swimming pools as an environment conducive to the harboring of antibiotic-resistant bacteria.

Keywords: Antibiotic Resistance; Bacterial Resistance; Swimming Pools; Health Risks

Abbreviations

ARB: Antimicrobial Resistance Bacteria; ARGs: Antibiotic Resistance Genes; MRGs: Multiresistance Genes

Introduction

According to the World Health Organization (WHO), bacterial resistance is a major threat to global health [1]. An estimated 700,000 deaths occur each year from infections caused by resistant microorganisms, and the problem is exacerbated by anthropogenic activities that contribute to their spread through the environment [2]. The inappropriate use of antibiotics, their indiscriminate use in subtherapeutic treatments or to increase the growth rate of animals [3], poor treatment of industrial and hospital effluents [4], climate change [5], and the Covid-19 pandemic [6,7], are counted as the main causes increasing the problem, so that antimicrobial resistant bacteria (ARB) are now considered as emerging environmen-

tal contaminants [8]. The economic impact of bacterial resistance is growing, since related infections are associated with increased morbidity, mortality, length of hospitalization, and financial costs. According to the review of bacterial resistance, carried out by Jim O'Neill in 2014, it is estimated that a continuous increase in resistance would lead to the death of 10 million people each year and a reduction of to 3.5% in Gross Domestic Product that would cost the world up to 100 billion dollars, all this by the year 2050 [9].

Aquatic ecosystems are recognized reservoirs for ARB and antibiotic resistance genes (ARGs), which are pressing public health problems worldwide. Due to the constant supply of microorganisms associated with human disease from bathers, recreational waters, especially swimming pools, are one of the most common means for the transmission of waterborne diseases. Recent characterizations of the human skin resistome show that ARGs and sev-

eral bacteria of epidemiological interest exist in the human dermal microbiome [10]. Researchers found that the load of bathers in swimming pools contributes to the increase in ARGs, and that the absolute abundance of ARGs increases by almost two orders of magnitude when the load of bathers exceeds 0.1 person/m²·h. [11].

To control the presence of any potentially harmful organism in recreational waters, highly oxidizing chemical treatments are usually applied under planned concentrations and time of exposure. At appropriate concentrations, chlorine can kill most bacteria and viruses, making chlorination by far the most widely used treatment for the disinfection of water [12]. However, it has been suggested that chlorination induce increased resistance in surviving organisms, making treated water an important reservoir for the spread of antibiotic resistance to opportunistic pathogens. This of course has strong implications for public health [13]. All this considered, the skin is the most exposed way of entry for resistant pathogens, due to the extended microbial exchange with water, turning into a considerable risk for swimmers and pool users.

Little is known about which ARB can withstand disinfection and persist in pool water. This paper reviews the available information on ARGs, ARB, and factors that influence the selection pressure of resistant organisms.

In recent years, the role of the environment as an important source and route of dissemination of resistance has been increasingly recognized. Antibiotic resistance genes being transferred from environmental bacteria to human pathogens through water, including chlorinated water and/or swimming pools, is one of the main risks to public health today [8,13]. There is evidence that bacteria surviving residual chlorine have higher resistance to common antibiotics [14]. Thanks to co-selection factors, the pressure exerted by chlorine activates antibiotic resistance genes [15], which means that these bacteria not only adapt to a hostile environment, but also generate resistance factors to antibiotics that can be transferred horizontally, linking resistant bacteria in the natural environment with the human microbiota.

Bacteria of fecal or potentially pathogenic origin, such as those from the Enterobacteriaceae family [16], *Pseudomonas aeruginosa* [17], and *Legionella pneumophila* [18], can be transmitted through swimming pools and are implicated in a wide variety of acute or chronic diseases, including respiratory, urinary tract, and gastrointestinal infections [19]. Most of these bacteria are biofilm-

formers, which enables them to settle on surfaces, resist antimicrobial agents, and survive for extended periods, thereby becoming potential opportunistic agents [20].

The routes of antibiotic resistance dissemination towards human pathogens through person-to-person contact have been extensively described in hospital and community settings [21]. However, the role that human commensals play in transferring resistance from environmental bacteria to pathogens, and the routes of dissemination for resistance genes carried by environmental bacteria that are exposed to selective pressure, such as the bacteria found in swimming pools, are not well understood. Deepening our understanding of the selection pressures that chlorination conditions exert on environmental and opportunistic bacteria is of vital importance in contributing to minimizing the problem of bacterial resistance.

Based on the premise of addressing the issue of bacterial resistance to antimicrobials, Colombia became one of the pioneering countries towards regulating the administration of antibiotics in the world with its “National Plan for Response to Antimicrobial Resistance” [22], this “Plan” adheres to the five strategic objectives of the Global Plan and encompasses not only the human and animal health sectors, but also the phytosanitary and environmental sectors. The latter is considered a strategic sector in combating the transfer routes of bacterial resistance. Within the framework of this Plan, understanding the potential role of swimming pools as a reservoir or source of transfer of antibiotic resistance to commensal, opportunistic, or human pathogenic bacteria would contribute to achieving the following strategic objectives: 1. improving awareness of antimicrobial resistance through effective communication, education, and training, and awareness raising. 2. Strengthening knowledge and the scientific base through surveillance and research. 3. Reducing the incidence of infections with effective sanitation, hygiene, and infection prevention measures [23].

The main objective of this review is to better understand the role of swimming pools as an environment conducive to the proliferation of antibiotic-resistant bacteria.

Bacterial resistance

Resistance to antimicrobial agents is a mechanism developed by microorganisms to survive and compete against other organisms in the environment. In the case of bacteria, it is possibly as old as the bacteria themselves, but it has spread remarkably in recent de-

cedes. Evidence of the importance of the environment as a transmission route is supported by the detection of resistant bacteria in the gastrointestinal microbiota of indigenous people from the Amazon who have never been exposed to antibiotics [24]. Furthermore, the discovery of bacteria that are over 30,000 years old and contain anti-biotic resistance factors [25] also provides evidence for the importance of the environment in the transmission of antibiotic resistance. It is believed that the human host is where a higher rate of horizontal transfer occurs, although the environment (water or soil) is considered the place where most mobile gene elements originate. Antimicrobial substances reach the environment through various routes, including clinical settings, human and animal waste, the use of antibacterial products, and foods supplemented with antimicrobials. Even surfaces in contact with food and handlers can facilitate dis-semination [26].

The emergence of antibiotic resistance can be attributed to either intrinsic or inherent mechanisms and acquired mechanisms. Intrinsic mechanisms are specific and located in genes present in the chromosome and efflux systems, while acquired mechanisms involve mutated genes and those horizontally transferred through mobile gene elements. These elements provide bacteria with the ability to develop mechanisms of resistance to antibiotics, which include I. production of bacterial enzymes that inactivate antibiotics through hydrolysis, acetylation, adenylation, or phosphorylation. II. Modifications in the target site, such as mutations in the gene encoding the antibiotic target, acquisition of genes encoding substitutes for the original targets, modifications that prevent the drug from reaching the target site, and even alterations in the target site. III. Alterations in membrane permeability, energy-dependent changes in antibiotic entry or increased antibiotic exit through efflux systems [27]. Knowing the routes by which these bacteria containing resistance genes or the genes themselves reach and transfer within the human microbiota is therefore crucial.

Chlorine as an activating agent of bacterial resistance

Water is usually treated with chlorine to minimize microbial growth, an effective, fast and inexpensive method [12]. When chlorine gas and water react, hypochlorous acid (HOCl) is formed, which dissociates to produce hydrogen ions (H⁺) and hypochlorite ion (OCl⁻). The multiple hit action of HOCl and OCl⁻ destroys microorganisms by attacking the cell from different sides, primarily causing physiological damage to the bacterial cell membrane, including the sulfhydryl groups of proteins, cytochromes, iron-sulfur proteins, and nucleotides, which are highly vulnerable to oxidative degradation by HOCl [28].

Chlorine significantly inhibits oxidative phosphorylation, oxygen uptake, and affects succinate dehydrogenase involved in the Krebs Cycle and glucose transport, leading to decreased ATP levels in bacteria treated with chlorine. Chlorination also disrupts metabolism and protein synthesis, and modifies purines and pyrimidines, potentially causing mutations [29]. However, during disinfection, both the disinfectant agent and its by-products can cause oxidative stress, stimulating bacteria to generate an adaptive response to the disinfectant agent that allows them to survive these conditions [30].

Most genes involved in antimicrobial resistance in bacteria are often found alongside other genes that promote resistance to different harmful chemicals, including disinfectants like Chlorine. This phenomenon is called co-selection, which occurs due to the selective pressure exerted by the biocide that maintains mobile gene elements and increases their transfer, resulting in bacteria being enriched with plasmids and integrons involved in the transfer of resistance markers [13,31,32]. Coselection can occur as co-resistance or cross-resistance. Cross-resistance happens when a single gene provides resistance or protection against multiple biocidal agents, while co-resistance, occurs when genetic information encoding several unrelated resistance mechanisms is transmitted on a single occasion/process and is expressed in the new bacterial hosts, promoting the maintenance of other resistance genes [33]. This raises concerns for bacteria resistant to chlorination and the selection pressure they face for the maintenance or transmission of antibiotic resistance genes.

Although there is no consensus on the relationship between Chlorine and antibiotic resistance, it is essential to consider the issue as the increase in resistance to antibiotics has become a major public health concern. While some authors suggest that disinfection does not contribute to the selection of resistant strains, others argue that it induces the development of antimicrobial resistance. This debate highlights the need for more evidence-based research to substantiate the precise links between chlorination and antibiotic resistance. Armstrong, *et al.* suggested back in the eighties that disinfection, purification, and distribution of water could increase the emergence of antibiotic-resistant bacteria [34]. Afterward, Murray, *et al.* 1984 evaluated 1900 bacterial isolates obtained from raw sewage influent and chlorinated sewage effluent from a sewage treatment plant. Of the isolates obtained 84% were resistant to at least one of the antibiotics tested, several presented multiple resistances. After chlorination, the number of resistant to

ampicillin and cephalothin increased. The authors conclude that chlorination, while initially reducing the total number of bacteria in wastewater, can substantially increase the proportions of antibiotic-resistant potentially pathogenic organisms [35]. Later other researchers have shown the presence and increase of ARBs (See

Table 1) and ARGs (See Table 2) in water treatment and distribution systems, and even in tap water [8,36-44]. Based on the above information, this review starts from the evidence that chlorination increases the proportion of ARBs.

Source	Resistant Microorganisms	Resistance to ¹	
Drinking water Systems	1) <i>Escherichia coli</i> 2) <i>Acinetobacter spp.</i> 3) <i>Klebsiella pneumoniae</i> 4) <i>Staphylococcus aureus</i> 5) <i>Salmonella spp.</i> 6) <i>Streptococcus pneumoniae</i> 7) <i>Shigella spp.</i>	1) SXT, CRO, CAZ, AM, FEP 2) TET, GEN, AMK, COL 3) SXT, CIP, CRO, CTX 4) FOX 5) CIP, LVX, CRO, CTX, CAZ, IPM, MEM 6) OXA, PEN, SXT, CRO, CTX 7) CIP, LVX, CRO, CTX, CAZ, AZM	[8]
Tap Water	1) <i>Chryseobacterium spp.</i> 2) <i>Bosea massiliensis</i> 3) <i>Mycobacterium frederiksbergense</i> 4) <i>Brevundimonas mediterranea</i> 5) <i>Sphingomonas sanxanigenens</i> 6) – 7) <i>Sphingomonas spp.</i> 8) <i>Dyadobacter spp.</i> 9) <i>Microbacterium spp.</i> 10) <i>Afipia sp.</i> 11) <i>Bosea massiliensis</i> 12) – 13) <i>Brevundimonas mediterranea</i> 14) <i>Nocardia asteroides</i> 15) <i>Sphingobium abikonense</i> 16) <i>Achromobacter spp.</i> 17) <i>Pedobacter spp.</i> 18) <i>Flavobacterium spp.</i> 19) <i>Bacillus zhangzhouensis</i> 20) <i>Achromobacter spp.</i> 21. <i>Caulobacter spp.</i> 22. <i>Brevundimonas spp.</i> 23. <i>Mycobacterium frederiksbergense</i> 24. <i>Methylobacterium spp.</i>	1) AM, ATM, ETP, MEM, PB 2) ATM, VA 3) ATM 4) AM, ATM, ETP, OFX, CTX, FEP, SXT 5) ATM, ETP, OFX, CTX, FEP, S, RA, C 6) AM, ATM, ETP, OFX, CTX, CN, S, SXT, RA 7) AM, ATM, ETP, OFX, CTX, S, SXT, RA 8) ATM, OFX, CTX, FEP, VA, CN 9) ATM, OFX, CTX, FEP, OT, SXT, RA, C, PB 10) AM, ATM, MEM, CTX, DO, T, VA, CN, SXT, RA, C, PB 11) ATM 12) AM, ATM, OFX, CTX, FEP, 13) AM, ATM, OFX, CTX, FEP, SXT 14) CTX, FEP, RA, C 15) RA, PB 16) AM, CTX, VA, RA 17) AM, ATM, OFX, CTX, VA, CN, PB; 19) CTX 18) without resistance 20. AM, CTX, CN, RA 22. ATM, OFX, CTX, STX 23. CI 24. AM, ATM, ETP, MEM, OFX, CTX, VA, CN, SXT, E, C, PB	[36]

Origin	Resistant gene	Reference
Water distribution system	<i>strA, strB, dfrB1, dfrB2, aadA, blaCTX-M, tetA</i>	[42]
Tap Water	<i>arma, blaCMY, qnrS, blaCTX-M, ereB, qnrA, ereA, tetX, sul3, qnrB, tetA, sul2, mph, tetM, sul1 strB, aadA, blaTEM</i>	[43]
Water treatment and distribution system	<i>cat, cmr, blaTEM, blaSHV, sul1, sul2</i>	[44]
Drinking Water Treatment System after chlorination	MRGs (multidrug transporter, <i>mexE, smeB, smeC, smeD, smeE, and smeF</i>), aminoglycoside [<i>aadA, aph(6)-1, aph(3')-IIb</i>], beta-lactam (metallo-beta-lactamase, TEM-1, TEM-117, TEM-157, TEM-187, TEM-205, TEM-75, TEM-91, and TEM-118), and rifamycin (rifampin monooxygenase) were enriched	[40]
Drinking Water Treatment System E (Disinfection strategy UV light, NaOCl)	Resistance genes increased in waterworks treating surface water with disinfection. The majority (>80%) of reads in the multidrug resistance gene group were represented by the genes <i>rpoB2, Multidrug ABC transporter, mexF, and ompR</i>	[41]

Table 2: Antibiotic resistant genes reported in treated water.

Pool water as a medium for the transfer of potentially pathogenic and antibiotic-resistant bacteria

Water is an ideal environment for the growth and survival of most microorganisms, making it the primary transmitter of microbial diseases, and waterborne diseases cause over 1.5 million deaths per year, especially in low-income countries [45]. In general, microorganisms that are transmitted through water enter the human body through ingestion via oral route. However, exposure and contact with poorly treated recreational waters and contaminated water sources are also significant modes of transmission for infectious diseases. In 2012, Barna and Kádár published a review highlighting the risk of contracting infectious diseases in public swimming pools. They cited data from the "Centers of Disease Control" (CDC) in the United States, which reported 399 outbreaks of waterborne diseases between 1999 and 2008, resulting in over 5,000 cases. Shockingly, 9.6% of these cases were attributed to treated recreational waters [19]. Swimming pools are a breeding ground for various infectious bacteria, some of which can be resistant to disinfectant agents. *Pseudomonas aeruginosa*, a highly versatile and opportunistic pathogen that can easily adapt to various environmental conditions, including chlorinated water and the human body, is frequently isolated from these pools [17,46]. Various infections can be caused by *P. aeruginosa*, including external otitis [46], folliculitis [47], hot foot syndrome [48], and occasionally corneal ulcers, respiratory system diseases, and urinary tract infections [49]. Additionally, other isolated bacteria, such as *Escherichia coli* O57:H7 and *Shigella sonnei* are less commonly found but can still cause serious health issues like bloody diarrhea, thrombocytopenia, and hemolytic uremic syndrome [50]. *Legionella pneumoniae*, which can cause Legionellosis and Pontiac Fever [51], and

several non-tuberculous *Mycobacterium* like *M. marinum* that have been linked to outbreaks of subcutaneous granulomas [52].

Maintaining an effective concentration of residual chlorine in swimming pool water is a challenging task, despite chlorination being an effective disinfection procedure. Chlorine concentration is influenced by various factors, including pH, temperature, organic load, contact time, and the microorganisms present. Therefore, in warm or tropical regions, swimming pools may have residual chlorine concentrations below the required range, making it difficult to achieve lethal action and increasing the bacteria's ability to resist and persist in water.

The risk of contracting infectious diseases in swimming pools has been widely described [53,54], however, few studies on antibiotic resistance bacteria and associated genes isolated from swimming pools have been published. In 2008, Chrissanthy Papadopoulou, *et al.* published a study evaluating the bacterial resistance of 107 isolates obtained from 462 pool water samples in Greece between 1997 and 2005. The results showed that 35.5% of the isolates were resistant to antibiotics, and among them were identified as multiresistant bacteria such as *P. aeruginosa, P. alcaligenes, P. fluorescens, Leuconostoc, Staphylococcus aureus, Staphylococcus wernerii, Chryseobacterium indologenes, Ochrobactrum anthropi, Aeromonas hydrophila, Enterobacter cloacae, and Klebsiella pneumoniae* [55]. A more recent study, where the surrounding surfaces and therapeutic pool waters of 11 health centers in Bavaria, Germany were analyzed, showed that 102 isolates were resistant to antibiotics. Most isolates were Gram-negative non-fermenting bacteria such as: *Burkholderia spp., Moraxella spp., Pseudomonas spp.,*

Stenotrophomonas spp., and *Sphingomonas* spp. or Gram positive such as *Staphylococcus epidermidis* and *Bacillus subtilis*. The results indicated a relationship between the number of isolates in water samples and the number of patients using the pools in combination with poor water treatment [56]. Likewise, it is worth noting the study carried out in Guangzhou, China in 2018, in which 39 outdoor swimming pools were evaluated, in which bacteria such as *P. aeruginosa*, *E. coli*, *Shigella* and *Salmonella* were isolated. It was also reported that of the *P. aeruginosa* isolates, 27 presented resistance to different antibiotics of medical importance, including piperacillin, piperacillin-tazobactam, ticarcillin-clavulanic acid, ceftazidime, cefepime, imipenem, gentamicin, tobramycin, amikacin, levofloxacin and ciprofloxacin; and of these isolates, 17 were positive for housekeeping genes [57]. Schiavano and collaborators demonstrated the high prevalence of *P. aeruginosa* in swimming pools in the same year, along with its resistance to various antibiotics. Imipenem resistant strains were identified as carbapenems negative, and resistance was associated with inactivating mutations of the *oprD* gene, confirming the role of porins as a contributing factor in carbapenem resistance in Gram-negative bacteria. These results confirm that swimming pools can be a route for the transmission of antibiotic resistant bacteria and genes, which poses an additional risk for their users [58].

Antibiotic resistance genes detected in bacteria isolated from swimming pools and other aquatic environments

Recently, in 2021, Shuai X., *et al.* evaluated the distribution of antibiotic ARGs in 16 swimming pools, both before and after the facilities were closed, taking into account varying numbers of users. The study involved collecting water samples and sampling the skin surfaces of bathers. The results indicated that the load of bathers contributed to an increase in ARGs, and that the ARG pool was maintained in different pools with similar transfer dynamics. The study found 18 ARGs that were not detected before swimming on human skin, which remained after showering, suggesting that swimming could lead to the exchange of ARGs and bacteria between the skin and water [11]. This fact has also been verified after swimming in the ocean, so it is advisable to bathe before and after aquatic exercise [59]. Regarding resistance genes, it is worth highlighting the review by Zhang, X.X., *et al.* 2009 where a summary of the distribution, types, and horizontal transfer of antibiotic resistance genes in different aquatic environments, including animal production, domestic and hospital wastewater, surface water, groundwater, wastewater treatment plants, and drinking water. However, and despite the potential risk of co-selection that chlorine could exert in swimming pool water, this type of water was

not included. The tetracycline resistance genes (*tetA*, *tetB*, *tetD*), aminoglycosides (*aphA1* and *aphA2*), chloramphenicol and vancomycin (*floR* and *vanA*), sulfonamide and trimethoprim (*dfrA*, *dfrA7*, *sul1*, *sul2*), and β -lactams and penicillin (*ampC*, *blaTEM-*, *mecA*, *penA*) are among the characterized genes isolated from chlorinated water samples [60].

The aim of numerous studies on swimming pools is to identify and understand *P. aeruginosa*, a highly adaptable bacterium with intrinsic tolerance to various disinfectants and antimicrobial agents that has been deemed harmless in this manmade environment. In 2017, a study of 41 swimming pools in central Italy's Marches Region, looking to assess the resistance of this bacterium to the β -lactam antibiotic imipenem (part of the carbapenem group), found that resistance factors for imipenem involve low permeability, inducible β -lactamase activity, and multidrug delivery systems, but the most common factor is the loss of OprD outer membrane porins. This study showed that the highest incidence of imipenem-resistant *P. aeruginosa* was found in samples from swimming pools. The isolates did not have carbapenemase, and their resistance was associated with mutations in the *oprD* gene, underscoring the role of porins in the bacteria's resistance to the drug [58]. These findings reinforce the critical importance of ongoing assessment of not only the microbial purity of swimming pool water, but also its potential as a repository or conduit for transmitting antibiotic resistance and associated genes, among commensal, opportunistic, or human pathogenic organisms.

Resistant residual bacteria disinfection in swimming pools after the pandemic

Several reports have described an increase in multiresistant organisms after the Covid 19 Pandemic [61]. However, research focused specifically on the issue of COVID-9 disinfection and resistant residual bacteria in swimming pools was limited. There is widespread concern that increased use of disinfectants during and after the pandemic may contribute to increased antimicrobial resistance in bacteria, including those found in swimming pools. Since 2020, various reports have highlighted the emergence of residual bacteria that are resistant to disinfection, particularly with Chlorine. In a review by Hao-Bin Wang in 2021, 13 of the 15 genera of residual bacteria identified were pathogens potentially multidrug-resistant, among them *Pseudomonas* and *Acinetobacter* [62]. The COVID-19 pandemic has caused a significant setback in the progress made in combating antimicrobial resistance, as stated by the Center for Disease Control (CDC). Furthermore, the threat of infections caused by antimicrobial-resistant bacteria has escalated. The United Na-

tions environment program report has acknowledged the potential catastrophic consequences of the continuous growth and dissemination of antimicrobial resistance. The report also cautions that another pandemic is looming, as the incapability of treatment systems to fully eradicate dangerous microorganisms can lead to the emergence of superbugs that can resist modern medicine and trigger another pandemic [63].

Conclusion

The studies evaluated suggest that the adaptive response generated by chlorine disinfection is closely linked to the persistence of resistant bacteria in recreational waters, turning swimming pools into an important reservoir of ARB and ARGs, as well as the activation of cross-resistance between disinfectants such as chlorine and antibiotics. Thus, the risk of exposure to ARB and ARG appears to be higher in chlorinated recreational water (swimming pools).

Most studies found on microbial resistance to antibiotics focus on water quality and the use of conventional quality indicators. There is little information on microbial species capable of persisting in recreational waters subjected to disinfection, and which of them may be of epidemiological importance. Finally, the COVID-19 pandemic has increased the threat of antibiotic resistance and its spread in environments that promote exchange and closeness between people.

This review highlights the need to deepen the study of molecular epidemiology and the characterization of ARB and ARGs that can persist in recreational waters, and the efficiency of disinfectant treatments in the removal of ARB and ARG efficiency of disinfectant treatments for their removal

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Conflict of Interest

The authors declare no conflict of interest.

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