Natural recreational waters and the risk that exposure to antibiotic resistant bacteria poses to human health

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Abstract

Antimicrobial resistance (AMR) is widely recognised as a considerable threat to human health, wellbeing and prosperity. Many clinically important antibiotic resistance genes are understood to have originated in the natural environment. However, the complex interactions between humans, animals and the environment makes the health implications of environmental AMR difficult to quantify. This narrative review focuses on the current state of knowledge regarding antibiotic resistant bacteria (ARB) in natural bathing waters and implications for human health. It considers the latest research in understanding the transmission of ARB from bathing waters to humans. The limitations of existing evidence are discussed, as well as research priorities. The authors are of the opinion that future studies should include faecally-contaminated bathing waters and people exposed to these environments to accurately parameterise environment-to-human transmission.

Keywords

Antibiotic resistance, bathing, swimming, recreation

Introduction

Antimicrobial resistance (AMR) poses a considerable threat to human health, wellbeing and prosperity globally. The ability of microorganisms to survive and reproduce in the presence of drugs produced to suppress microbial pathogens limits our ability to control infections. Several publications have made concerning quantitative estimates of the future impacts of AMR on human morbidity, mortality and the economy if nothing is done to curb the spread of AMR [1, 2]. Antibiotic resistant bacteria (ARB) are an important group of AMR organisms, and the transmission dynamics of ARB, including the resistance determinants and mobile genetic elements they harbour, are complex to elucidate. This complexity is partly due to antimicrobial resistance genes (ARGs) being borne on broad host range mobile genetic elements that can move between bacterial species, and the ubiquity, and adaptability of bacteria to survive in various environments under multiple stressors. Another layer of complexity comes from the acquisition of human-associated bacteria from sewage-contaminated environments, which may erroneously be classified as human-to-human transmission using traditional epidemiological methodologies that are based on differences in strain characteristics.

While AMR in bacterial pathogens has been a focus of concern for a considerable time, more recently the evolution and ecology of AMR in natural environments has received greater attention. Evidence suggests that clinically important ARGs originated in the natural environment and have emerged in human pathogens via horizontal gene transfer [3, 4]. Natural surface waters are reservoirs of diverse ARB globally, including clinically important bacteria harbouring extended-spectrum beta-lactamase (ESBL) and carbapenemase genes conferring resistance to critically important antibiotics [5-11]. Furthermore, aquatic environments are affected by many types of pollution, and are places where humans come into close contact with waterborne microorganisms [12, 13]. Some research on the transmission dynamics of ARB between humans, animals, and the environment seem to suggest that transmission of ARB from environments to humans is only sporadic and contributes a small proportion of all transmission events [14, 15], but these studies may underestimate risk through exposure to human faeces and human-adapted pathogen strains in contaminated surface and coastal waters. In this review, we briefly summarise the evidence relating to ARB in bathing waters and the current evidence of the health effects associated with exposure to ARB in bathing waters. We consider the latest research in understanding the role of bathing waters in the transmission of ARB and ARGs to the human microbiome, its limitations, and consider research priorities.

Why the environmental dimension of antimicrobial resistance is important

Mechanisms conferring AMR have evolved in environmental bacteria over evolutionary time to protect organisms from antimicrobials naturally produced by bacteria and fungi [16]. Bacteria possessing diverse ARGs are present in all natural environments. However, the environment receives faecal waste from humans and animals treated with antimicrobials, resulting in the dissemination of ARB, ARGs and antimicrobials themselves with approximately 70% of antimicrobial drugs (up to 90%) excreted in an active form [17, 18]. These waste streams contain a high prevalence and diversity of ARB, including clinically important pathogens harbouring ARGs conferring resistance to critically important antibiotics. Wastewater treatment processes are not currently designed to remove ARB, but still reduce bacterial and antimicrobial discharges to receiving waters. In high-income countries with established sewer networks and treatment facilities, many studies have shown that municipal wastewater contains significant numbers of clinically important ARB and ARGs [19], even after treatment [20]. Efficacy of treatment, where present, is variable depending on treatment type [20]. Studies in river catchments show strong associations between environmental ARB load and distance from, size and type of wastewater treatment plant [21]. In addition, agricultural land use correlates

with AMR in aquatic sediment, with grassland used for grazing showing a positive association [21]. Whilst there is no doubt that human activity impacts the levels and types of ARB and ARGs in aquatic environments, there is less certainty on the role of antimicrobials, including antibiotics, at environmental concentrations. However, there is a growing body of evidence that some antibiotics at high measured environmental concentrations are capable of driving the evolution of resistance [22-26]. This means that in addition to bathing waters constituting an exposure risk to existing clinical AMR pathogens, there is also the risk that mixing of human, animal and environmental bacteria in the presence of antibiotic residues may drive emergence of novel ARGs in human commensals or pathogens. It is possible that ingestion of environmental bacteria harbouring previously unknown ARGs could result in acquisition of novel ARGs by commensal and opportunistic pathogens resident in the human microbiome (Figure 1).

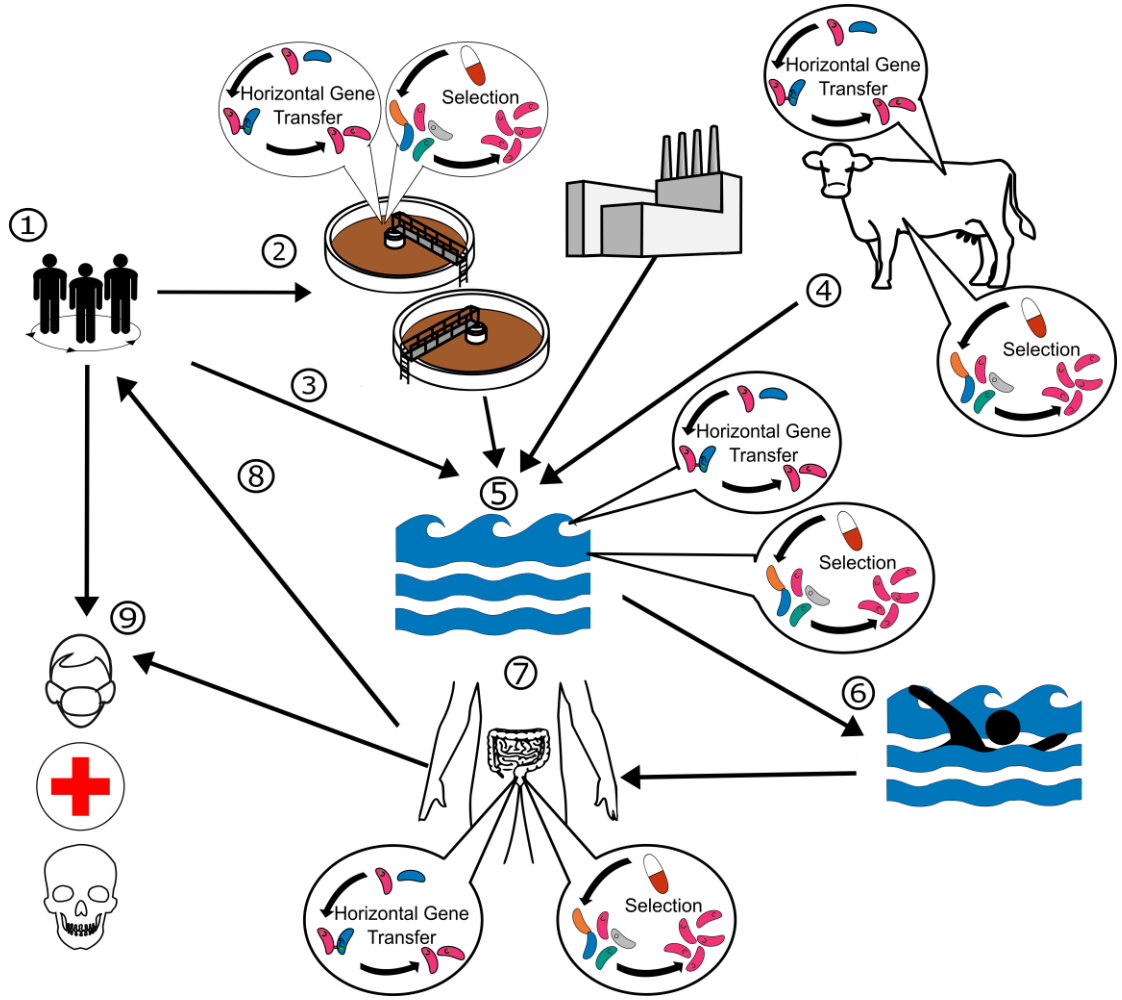

Figure 1. Sources and transmission routes of antibiotic resistant bacteria (ARB) in natural surface waters used for bathing. 1) ARB circulate in the community, spread via person-to-person contact. 2) Wastewater treatment plants (WWTPs) collect wastewater for treatment prior to discharge. High prevalence of diverse ARB and antimicrobial residues make WWTPs a potential hotspot for the exchange of mobile genetic elements, and selection for resistance genes. 3) Poor sanitation and flooding can lead to the contamination of natural surface waters by human excreta containing ARB and antibiotic residues. 4) Livestock are administered antibiotics, and resistance can proliferate through selection and horizontal gene transfer. ARB in animal microbiota enter surface waters run off after rainfall. 5) In surface waters, environmental microorganisms are subject to selection for resistance in response to exposure to antibiotic residues introduced via pollution. Resistance genes may spread through the environmental microbial community via horizontal gene transfer. 6) Bathers using

natural surfaces water for recreation may ingest water containing ARB. 7) ARB may colonise the gut and undergo genetic exchange with resident microbiota, and/or transient pathogens. Use of antibiotics in human medicine may select for resistance in the gut microbiome. 8) Asymptomatic carriers can transmit ARB to members of the wider community. 9) Vulnerable individuals exposed to ARB may develop symptomatic infections. Severe infections may require hospitalisation, and possibly result in death.

Epidemiological evidence of the impact of recreational exposure to ARB on human health

Three main health outcomes can be considered when investigating the human health impacts of bathing related to ARB: Exposure, colonisation, and infection. Exposure to ARB in bathing waters presents a public health risk through gut colonisation, subsequent symptomatic infection in exposed individuals, and dissemination of ARB to the wider community (Figure 1).

Several studies investigating exposure to bacterial pathogens or faecal indicator bacteria (FIB) in bathing waters have established that ARB, including human epidemic clones with resistance to critically important antibiotics, are present at levels likely to result in ingestion by water users. All used different methods to quantify resistance among *Escherichia coli* cultured from recreational waters around high-income countries in Europe and the United States. These include phenotypic resistance to clinically important antibiotics [9, 27-29], detection of specific ARGs, such as $bla_{\text{CTX-M}}$ or pathogenic *E. coil* [13, 28, 30], and *E. coli* resistant to multiple antibiotics [28, 31]. All studies conclude that exposure to resistant *E. coli* is likely among various water users studied, but particularly high among surfers and children, who tend to ingest larger volumes of water.

Prior to infection, ARB and/or ARGs must become established in the human microbiome, either through colonisation or genetic exchange between ingested bacteria and those present in the gut. Asymptomatic colonisation and transmission is likely to be an important driver of community-associated carriage of AMR *E. coli* [32], however few studies have directly measured the risk of colonisation among people exposed to recreational waters. The first study to do so [13] found that people who surf frequently were approximately three times more likely than non-bathers to be faecal carriers of ESBL-*E. coli*, and more than 4 times as likely to carry *E. coli* harbouring the resistance gene $bla_{\text{CTX-M}}$. Recently, a Dutch study quantified ESBL-*E. coli* carriage in open-water swimming contestants and found that these individuals had significantly elevated rates of carriage compared to the general population [33]. While exposure in a single swimming event did not change the probability of ESBL-*E. coli* carriage, regular exposure to surface waters in this population of swimmers was associated with increased carriage. The relationship between degree of exposure and colonisation status (dose-response relationship), as well as duration of colonisation are not known, nor is the risk of subsequent infections in water users.

Mughini-Gras *et al*. (2019) performed source attribution modelling approaches in the Netherlands, demonstrating that recreational exposure to surface waters is an important driver of ESBL-*E. coli* and plasmid-mediated AmpC-producing *E. coli* community carriage. During summer months, bathing in natural waters accounted for approximately 6% of transmission of these AMR *E. coli*, comparable with consumption of chicken meat or carriage associated with foreign travel. This is a higher contribution to ARB transmission compared to two recent studies estimating transmission dynamics across the One Health spectrum based on prevalence of resistant bacteria in different ecosystem compartments (human, animal and environmental) [14, 15]. Thorpe *et al.* (2021) used data from 3482 carbapenem-resistant *Klebsiella* isolates collected across the One Health spectrum in Italy in ARB transmission modelling to estimate that the environment is responsible for 0.21% of human ARB infections. River

water samples were included in the environmental samples, but it was not specified whether the sampled water bodies were used for recreation, nor the extent to which these waters were affected by faecal pollution. In addition, the observed niche adaptation of different *Klebsiella* species into specific compartments (humans, animals, water) might also hamper the observation of clones relevant for human infection, simply because contamination of water with species that show the highest relevance for human infections (*K. pneumoniae*) might be "diluted" by environmentally-adapted *Klebsiella* species. Booton *et al.* (2021) used data obtained in surveys of ESBL-producing bacteria in Thailand to estimate that 1.8% of human colonisation events originated in the environment, and that antimicrobial usage (AMU) is a major explanatory variable relating to ESBL-producing bacteria transmission in Thailand. However, another study on drivers of ARB infections at a global scale illustrated the importance of sanitation in reducing antibiotic resistance, particularly in low and middle income settings [34]. Collignon et al (2018) concluded that unless sanitation is improved and dissemination reduced, limiting AMU is unlikely to mitigate the spread of antibiotic resistance.

Empirical evidence of infections caused by ARB associated with exposure to natural surface waters is derived from case-control, and case-studies. Søraas *et al.* (2013) reported recreational exposure to surface waters as an independent risk factor for community-acquired antibiotic resistant urinary tract infections in Norway [35]. Difficult-to-treat infections caused by ARB in aquatic environments have been reported, for example sepsis caused by a strain of carbapenemase-producing *Enterobacter asburiae* following a near-drowning event in a French river from which the same ARB was detected [36], and infections of fracture wounds by bacteria resistant to clinically important antibiotics following exposure to seawater have also been documented [37].

Limitations of the evidence

Many studies have reported the presence of clinically important ARBs and ARGs in recreational waters. However, the public health consequences of exposure to such ARB are difficult to establish. Classical epidemiological study designs struggle to disentangle the complexity of evolutionary trajectories of ARB and ARGs which in some cases are independent, and thus the causal nature of association is difficult to determine. If attributions are based on differing characteristics of strains from different exposure routes, human ARB strains in sewage will likely be attributed to direct person-to-person transmission. Furthermore, the opportunistic nature of these organisms means that infections may not occur at the time of exposure, so "outbreaks" and conventional public health strategies designed to attribute sources of transmission are unlikely to be effective in identifying transmission routes. Cross-sectional studies may overlook the original transmission or gene transfer events that lead to bacteria-gene combinations becoming widely established in the human microbiome at a population level. Further, the prevalence of specific ARGs/ARB in different ecosystem compartments may not be a true reflection of transmission dynamics, for the very reason that enrichment occurs in humans under antibiotic therapy, regardless of the origins of ARGs or ARBs. An analogy can be made with SARS-CoV-2, where cross-sectional studies focusing on humans, animals and the environment would conclude that this was primarily a human infection, masking the fact that the virus has a wildlife origin, without which there would be no pandemic despite the fact the original animal-human transmission events may have been rare or occurred only once.

Many of the studies mentioned in this review focus on resistance among specific bacterial species such as *E. coli* and *Klebsiella* spp., that have been recovered from humans, animals and the environment and for which there are standardised protocols for their isolation and characterisation. However, the results may not hold true for other bacteria or genes. A further limitation of the evidence currently available is the lack of harmonisation of approaches to quantify and characterise ARB across

the One Health continuum, including bathing waters. Variations in methodologies include volumes of water collected and examined, and some studies use solely culture-based approaches whilst others apply molecular methods. This limits the ability to detect AMR in some instances, particularly rare and novel phenotypes and genotypes, and the ability to directly compare or meta-analyse findings generated even within the same country. Current activities to monitor water quality (e.g. 76/160/EEC, 2006/7/EC) focus on designated bathing waters, and data are collected at minimum once per month during the bathing season. This approach does not include examination of the characteristics of the microorganisms present which may have a major impact on public health, including resistance to antibiotics. Therefore a harmonised approach to surveillance of bathing waters for the presence of ARB/ARGs is needed. The European Union bathing water regulations are currently under review and consideration should be given to inclusion of determining characteristics such as antibiotic resistance.

There is also a paucity of available information on the quality of non-designated bathing waters and their role in the transmission of ARB. The nomination of natural waters as designated bathing areas relies on a number of parameters, not only microbiological water quality. Waters not designated officially as bathing waters under bathing water regulations are frequently used for recreational purposes [38]. To inform policy, further understanding of the levels, differences in geographical distribution, types, and persistence of ARB that are present in natural recreational waters are needed, both in designated and non-designated waters, in order to protect human health.

Climate change will result in an increased frequency of intensive precipitation events, and other conditions, such as higher regional temperature [39], that may promote the development and dissemination of ARB, and favour the survival of human-adapted ARB. Rainfall increases run-off, transporting bacteria, including ARB, and substances that may promote the development of resistance [40] from land-based sources into natural surface waters [41]. Further, increased wastewater flow rates will lead to less efficient wastewater treatment [42, 43], and ultimately, to a greater fluctuation in the concentrations of human- and animal-associated ARB in designated and non-designated sites. These fluctuations are insufficiently characterised by the current sampling strategy at designated sites. Therefore, attention should be paid to short-term variability in surface water concentrations after extreme precipitation events.

Outlook

Natural recreational waters contaminated by faeces are an important environment in which bathers are exposed to human-associated ARB as well as the environmental resistome from which ARGs associated with human pathogens have emerged. Research aiming to determine the relative contribution that the human, animal and environmental microbiome make to AMR transmission should consider natural recreational waters specifically, and the populations that use them for recreation. Based on the limitations of the current evidence and future pollution pressures on surface waters, research should be directed towards the extent of recreation in non-designated sites and their water quality, and at capturing variation in water quality, including after extreme precipitation events. For most low-and-middle-income countries, exposure to natural surface waters through recreation will be one of many environmental exposure routes. Therefore there is a need for more international studies devoted to exposure to surface water during different activities, as convincingly established in the SaniPath study that identified the contribution of multiple sources of fecal contamination in the environment to child exposure [44]. Finally, if specific locations and activities with an exceptionally high risk of exposure to ARB can be identified, sociological research can help determine effective interventions to inform water users and reduce risks. Moreover, risks of ARB exposure will often (but not always) follow similar pathways as for other water-transmitted pathogens. Thus epidemiological research on the risks of surface water should take a holistic approach by studying ARB and other pathogens [43, 45].

Conclusions

There is compelling evidence that natural bathing waters play a role in the transmission of resistance from natural environments to humans, with clinical implications. However, the complexity of ARB/ARG transmission (bi-directional movement between environments, bacterial genetics, multiple sources of pollution), along with inconsistent and limited data across different geographical regions, have made it difficult to quantify environmental transmission. Further research is needed to understand transmission in different environmental settings, including designated bathing waters, non-designated sites, and natural surface waters internationally. Routine surveillance of ARB in bathing waters would be an important step in increasing our understanding of the risks discussed. Additionally, establishing harmonised methods, including longitudinal temporal studies to determine probability of exposure and transmission in different geographical settings is recommended.

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Declaration of competing interests

None

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- •• of outstanding interest

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