Towards a research agenda for water, sanitation and antimicrobial resistance
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ABSTRACT
Clinically relevant antimicrobial resistant bacteria, genetic resistance elements, and antibiotic residues (so-called AMR) from human and animal waste are abundantly present in environmental samples. This presence could lead to human exposure to AMR. In 2015, the World Health Organization (WHO) developed a Global Action Plan for Antimicrobial Resistance with one of its strategic objectives being to strengthen knowledge through surveillance and research. With respect to a strategic research agenda on water, sanitation and hygiene and AMR, WHO organized a workshop to solicit input by scientists and other stakeholders. The workshop resulted in three main conclusions. The first conclusion was that guidance is needed on how to reduce the spread of AMR to humans via the environment and to introduce effective intervention measures. Second, human exposure to AMR via water and its health impact should be investigated and quantified, in order to compare with other human exposure routes, such as direct transmission or via food consumption. Finally, a uniform and global surveillance strategy that complements existing strategies and includes analytical methods that can be used in low-income countries too, is needed to monitor the magnitude and dissemination of AMR.

Key words | antibiotics, antimicrobial resistance (AMR), risk assessment, risk management, sanitation, water

INTRODUCTION

‘Without urgent, coordinated action, the world is headed for a post-antibiotic era, in which common infections which have been treatable for decades can once again kill.’ Dr Keiji Fukuda, World Health Organization (WHO) Assistant Director-General for Health Security

In May 2015 the World Health Assembly of the WHO approved the Global Action Plan on Antimicrobial Resistance (GAP on AMR) (WHO 2015a). AMR elements, including resistant bacteria and antibiotic resistance genes (ARGs or AMR genes), as well as antibiotic residues, are common in water, wastewater, and feces. Therefore, understanding and addressing the role of water, sanitation and hygiene (WaSH) in combating AMR, including antibiotic resistance, is a critical element of the GAP on AMR. Box 1 summarizes the strategic objectives of the GAP on
AMR. The role of WaSH in combatting AMR focuses on improved awareness and understanding through surveillance and research, in order to reduce the incidence of AMR infection. WaSH thus contributes to objectives 1–3 of the GAP on AMR (Box 1).

Current WHO Guidelines for Drinking-Water, Recreational Water, and Safe Use of Wastewater do not yet contain information on antibiotics and other antimicrobial agents, their metabolites, AMR bacteria, or AMR genes. Occurrence and trend data for these elements are needed for risk assessment and risk management strategies for health-related AMR in the environment to be developed and implemented.

A WHO workshop was organized as a side event of the IWA Health Related Water Microbiology Symposium on September 18, 2015, in Lisbon, to identify knowledge gaps and to develop a research agenda for WaSH aspects of AMR. This paper describes the presentations, input, discussions, discussion and results of this workshop as building blocks for a research agenda.

**METHODS**

During the workshop ‘Developing a Research Agenda for Water, Sanitation and Hygiene (WaSH) and Antimicrobial resistance (AMR)’, input from the scientific community of water professionals was solicited on the role of the environment in the spread of AMR, mostly antibiotic resistance, and possible adverse health outcomes of environmental exposures in order to identify knowledge gaps and to develop a research agenda for WaSH aspects of AMR. Key issues in risk assessment, risk management, and monitoring and surveillance were discussed. This research agenda aims to identify knowledge gaps that need to be addressed in order to achieve the WaSH-related objectives (objectives 1–3, see Box 1) of the GAP on AMR and can thus be a foundation for future global guidance and action on WaSH and AMR.

The program started with several presentations on the latest insights regarding AMR and WaSH from both health and environmental perspectives. During breakout sessions, the participants were divided into three groups to consider questions on the following topics:

- risk assessment
- risk management
- monitoring and surveillance.

**RESULTS**

Prof. Dr Ana Maria de Roda Husman of the National Institute of Public Health and the Environment (RIVM) of the Netherlands opened the discussion on the importance of AMR in the environment. The complex interaction of the natural environment, i.e., water, soil, and air (Huijbers et al. 2015), and the interplay of AMR bacteria, AMR genes, and antibiotic residues in the environment were highlighted. AMR originates from humans and animals exposed to antibiotics, and from the environment itself; thus, AMR should be approached as a ‘One Health’ problem. Sources for AMR include, for example, wastewater and manure, as was shown during her presentation. There needs to be recognition that there are multiple uses of water, such as washing, irrigation, recreation, and drinking, that contribute to the increasing risk of exposure to AMR. Existing safety plans, such as Water Safety Plans and Sanitary Safety Plans, do not specifically address AMR yet and De Roda Husman suggested that greater attention should be afforded on AMR in such Safety Plans.

Kate Medlicott of the WHO went on to summarize the WHO GAP on AMR. The World Health Assembly at its
67th session adopted resolution WHA 67.25 on combating antimicrobial resistance (WHO 2015a). Through this resolution, the Health Assembly requested the development of a draft GAP to combat AMR, including antibiotic resistance. The WHO has led the development of a GAP that reflects the commitment, perspectives, and roles of all relevant stakeholders, and in which everyone has clear and shared ownership and responsibilities. The action plan is built on six guiding principles: public and stakeholder engagement, actions based on best available knowledge and evidence, ‘prevention first’, ‘access not excess’, sustainability, and incremental targets for implementation.

Furthermore, a briefing note on AMR in the environment was prepared (WHO 2015b).

Prof. Dr Antoine Andremont, of Diderot Medical School and Bichat Hospital Bacteriology Laboratory (France), explained the role of sanitation in the development and spread of AMR. In his presentation, AMR was addressed from a medical perspective and he indicated the need to consider the roles of the environment and agriculture in addition to clinical contributions to the development of resistance (Allen et al. 2010; Graham et al. 2014; Zhang et al. 2015). He demonstrated that, in France, single-antibiotic resistance was predominantly of hospital origin, but this has evolved to community-borne (food and environment) transmission whereby multi-drug resistant bacteria return from the environment back into the hospital.

Two examples of major AMR genes impacting human health and coming from environmental sources were presented:

- ESBL genes confer antibiotic resistance to all beta-lactams except carbapenems (plus multi-resistance) (Humeniuk et al. 2002);
- NDM-1 genes confer the same phenotype plus resistance to carbapenems (Kumarasamy et al. 2010).

These examples show that there is a feedback loop, whereby more infection results in more antibiotic use, which results in more antibiotic resistance. More ESBL infection leads to more use of carbapenems, which leads to the rise in carbapenem (NDM-1) resistance (Rossolini et al. 2008).

The contribution of ‘medical tourism’ to AMR was shown by Kumarasamy et al. (2010), with the spread of NDM-1 from India to the UK. Andremont stressed the importance of sanitation in his presentation. Poor sanitation or the lack of sanitation is an important pressure on AMR (Andremont & Walsh 2015). The One Health concept links the environment, agriculture/food, and sanitation/community health in an integrated risk-based approach.

The next speaker, Lydia Abebe of the University of North Carolina (USA), presented the preliminary findings of an ongoing systematic literature review of WaSH and AMR that focused on the current status and gaps in knowledge. The literature review focused on AMR bacteria in the environment and associated human health implications. Abebe discussed the purpose of the review, which is to evaluate the role of environmental exposure to AMR bacteria and human health outcomes through evaluating the methods used to create the linkages. Expected outcomes from the review will be an assessment of methods used to create environmental linkages between transmission of AMR bacteria in environmental and human reservoirs to human health outcomes to identify gaps, and thereby make recommendations for establishing stronger evidence for links between environmental exposure to AMR bacteria and adverse human health outcomes. This work will lead to a literature review that focuses on AMR and WaSH from an integrated One Health perspective, and it is envisaged that this will serve to stimulate a research agenda on AMR and WaSH.

Prof. Dr Mark Sobsey of the University of North Carolina (USA) continued with an overview of the research topics from the Joint Programming Initiative on Antimicrobial Resistance (JPI-AMR) agenda for the European Union (JPI-AMR 2013). The JPI-AMR programme seeks to harmonize AMR priorities and research initiatives to address research gaps. There are six priority topics that will be targeted to reduce AMR: therapeutics (alternatives to antibiotics), diagnostics (treatment and prevention of infection), surveillance (monitoring, including of environmental reservoirs), transmission, environment (including sources, selection and dissemination mechanisms), and interventions (for example, treatment technologies). One of the priority topics, selection and dissemination mechanisms in the environment, emphasizes the assessment of the contribution of pollution of the environment with antibiotics, antibiotic residues, and AMR bacteria on the sources, occurrence,
and spread of AMR and the development of strategies to minimize environmental contamination by antibiotics and AMR bacteria.

Finally, Prof. Dr Mark Sobsey discussed the urgency of new research into AMR surveillance. Many studies have been carried out to detect AMR in environmental samples. Nevertheless, there is no organized, harmonized and functional system for AMR surveillance in the environment. Globally used monitoring methods for environmental microbial surveillance are the detection of *Escherichia coli* and intestinal enterococci. These methods are based on the detection of fecal contamination, but no global or national surveillance systems are in place for the detection of environmental AMR. Dr Sobsey presented the need for the establishment of an international, standardized surveillance programme for AMR and antibiotic use in human and agriculture settings that includes targeted environmental monitoring relevant to human exposures. Potential approaches to environmental surveillance for AMR bacteria were discussed.

**Risk assessment**

During this breakout session, facilitated by Prof. Dr Antoine Andremont, the following questions were asked of the participants:

- What are the needs to identify and quantify the sources, occurrence, and transport of AMR bacteria and their genes?
- What are the needs to estimate risk of AMR bacteria to human health?

Multiple studies (references were made by participants to studies conducted in Germany, New Zealand, Australia, Denmark, the UK, the Netherlands, Thailand, and South Africa) have been carried out on the identification of antibiotic (AB) residues, and AMR bacteria and AMR genes throughout the water cycle as well as certain ‘hotspots’ such as hospital wastewater systems, biogas plants, wastewater treatment plants, and livestock such as poultry (New Zealand). The monitoring data now available are insufficient to identify occurrence status and trends in the appearance of AMR. It is clear, however, that AMR is found throughout the pathways from human and animal to excreta and manure, but it is more difficult to identify and quantify these conditions in the other domains of the water cycle. Research is needed on the selection of bacteria, AMR properties, locations, sample matrices, and standardized analytical methods for monitoring. The HACCP approach (Hazard Analysis and Critical Control Points) could be a useful method to identify the ‘critical control points’, bacterial analytical methods, and matrices. Water Safety Plans and Sanitary Safety Plans are primarily based on this approach. It is important to find the most important pathways, whether these be drinking water or other environmental exposure pathways and media, e.g., irrigation with wastewater. This knowledge would help to inform the public and other stakeholders on effective measures.

There is discussion on the importance of water as a pathway for AMR compared to other exposure routes, such as food or from person to person. However, this does not mean that inadequate sanitation and fecally contaminated water could not be important routes for AMR transmission. Little is known about exposure to AMR through WaSH routes and the resulting effects on public health. Therefore, research is needed in this field. So far, experiments to transfer genes in laboratory simulations have not succeeded.

The potential risk of infection by AMR bacteria through the consumption of drinking water gives rise to the public’s questions and concern. This is especially a concern in areas in which water reuse projects are being developed and implemented. Governments and water companies need to address these questions supported by scientific data that are based on actual evidence of exposure and observed health risks. It is important that these data are collected in a transparent way with good study design and methods, allowing for easy comparison with studies in other countries or regions.

Following this inventory, the discussion shifted towards the needs of resource-limited countries. One of the participants sketched the situation in India, where generic antibiotics are very inexpensive and readily available for a large population, and there is often poor sanitation. A worldwide analysis (Woerther et al. 2013) demonstrated that Asia is one of the continents with the highest incidence of ESBL-enterobacteria fecal carriage. From the African region, the aspect of the susceptibility of a resource-limited population to infections from WaSH exposures was mentioned in relation to the increased
risk of AMR infections. The limited resources in low resource settings call for a pragmatic approach with a baseline surveillance strategy supported by appropriate monitoring and further strengthening of water and sanitation conditions. Research is needed to develop these approaches.

Risk management

During this breakout session, facilitated by Prof. Dr Ana Maria De Roda Husman, the following questions were asked of the participants:

- What are the needs with regard to water and wastewater treatment technologies?
- What are the needs with regard to practical risk management systems to identify policies, practices, and tools to minimize human exposure?

Research in relation to the establishment of risk management systems should focus on prevention and treatment. Educational materials to increase awareness of appropriate use of antibiotics and proper antibiotic disposal is required in order to reduce the release of antibiotics to wastewater and the environment. The group suggested that a literature review of the drug/pharmaceutical management practices of various countries would generate ideas of policy and waste management systems for the safe and environmentally protective disposal of antibiotics. The literature review should include veterinary (animal) and agriculture, household, and hospital/healthcare practices for antibiotic use and the disposal of unused antibiotics and the governance structure in place for this.

In terms of water and wastewater technology applications, three dominant questions centered on identifying basic mass balance inputs:

- What levels of AMR bacteria, genetic material, or antibiotic residues are entering the treatment system?
- What levels are removed or could potentially be removed?
- What levels in effluent and biosolids are necessary to protect the receiving environment and ultimately benefit the clinical settings?

With regard to answering these questions, the group noted that studies have been undertaken on the occurrence of AMR bacteria, AMR genes, and antibiotic residues in raw sewage, although, not using a standardized methodology. Moreover, quantitative information is needed on potential environmental concentrations; loading mass/volume; and total load in animals, agriculture, wildlife, household, and hospital settings. Loading and concentration data should include those related to antibiotic metabolites (i.e., excreted forms and potential environmental transformation products) as well as parent compounds (direct disposal via toilet flushing). These research questions may be challenging and efforts will be required to ensure that analytical methods and detection limits are adequate and standardized between samples.

With respect to treatment, there are serious knowledge gaps around fate (persistence and survival), interactions, and treatment efficiency (removal, log reductions) of antibiotic compounds, metabolites, AMR bacteria, and AMR genes in water and wastewater technologies. Treatment studies should consider both AMR bacteria as well as AMR genes because DNA may persist despite death of the cell or biological entity. Alternatively, there may be other treatment markers or indicators of AMR bacteria or genes present as indicators of removal or reduction. ‘Critical control points’ should be identified.

Research into treatment technologies should also consider low-cost technologies, appropriate developing world technologies, conventional treatment (water and wastewater), non-standard techniques such as solar/sunlight, and septic systems with/without reticulated water supply. There is a need for criteria and guidelines to assess technologies in order to assist policymakers and utility managers in identifying appropriate technologies and their performance capabilities.

To address the third dominant question, the group noted that treatment requires a goal. How much removal is necessary to make a difference in controlling ARM impacts on the environment and human health? What levels in the environment are acceptable with regard to public health protection? How can we work to quantify health impacts associated with reductions in drug usage or drug concentrations (will reducing drug usage have negative health impacts)? What are the health impacts associated with AMR bacteria or genes and loading to the environment, that is, will reducing AMR bacteria or genes loading to the environment improve...
clinical health outcomes? A potential method of analysis is a Quantitative Microbial Risk Assessment (QMRA) as suggested by Ashbolt et al. (2015). An example QMRA study for exposure to ESBL in recreational waters was published recently (Schijven et al. 2015), but further studies on this subject are necessary.

**Monitoring and surveillance**

During this breakout session, Prof. Dr Mark Sobsey initiated the discussion with the following questions to the participants:

- What are the needs with regard to monitoring?
- What are the needs with regard to surveillance?
- What are the needs with regard to regulatory activities and agents?

Numerous studies have detected AMR in environmental samples through a variety of culture and molecular methods (Huijbers et al. 2015). Nevertheless, there is no organized, harmonized, and functional system for AMR surveillance in the environment.

There are different reasons to perform environmental surveillance of AMR, such as identifying emerging genes and a potential genetic relationship; characterization of pharmaceutical waste; identifying AMR bacteria, AMR genes, and antibiotic residues entering the environment; and, identifying other hot spots compared to sewage (source tracking). Appropriate methods should be established depending on the purpose of AMR surveillance in the environment.

During the discussion, different methods were mentioned to identify and quantify AMR bacteria, AMR genes, and antibiotic residues in the environment, such as culture methods and molecular detection methods. Initially, selection of an index parameter needs to be set. Different index parameters were discussed: antibiotics, detection of AMR in fecal indicators (such as *E. coli* and intestinal enterococci), other clinically relevant microorganisms (such as *Clostridium difficile*, *Staphylococcus aureus*, and bacteriophages), horizontal gene transfer and metagenomics. Then, standard methods should be prescribed to detect these index parameters. A tiered approach was recommended because of different resource settings, and specific guidance on this approach is greatly needed.

Studies on environmental surveillance of AMR do not always link to clinical relevance, e.g., studies do not focus on obtaining data relevant to human exposures from environmental pathways, AMR bacteria of human health/clinical concern, or human exposure media; studies do not focus on known major sources of AMR release to the environment. To improve the linkage to clinical relevance, the group discussed the importance of combining data from humans, animals, and the environment; the One Health approach; communication from the environmental domain to clinical and veterinary domains; and, linking human surveillance with environmental surveillance. To better address the links between human, animal, and environmental data, designing surveillance strategies with a harmonized and tiered approach was recommended.

As a result of monitoring and surveillance, data on AMR in the environment will be collected, which can form the evidence base to take actions to minimize exposure and human health risks. Therefore, a threshold (regarding risk level and safety) of AMR in the environment within a regulatory framework is needed. The threshold should answer the following questions: What is an acceptable level of AMR risk? When should management take action to further minimize risk? Furthermore, the gathered data should be communicated to other relevant fields and stakeholders, such as, for example, healthcare professionals, policy-makers, and water and sanitation experts, and provide advice on reduction or removal of AMR, including recommendations on cost-effectiveness.

**DISCUSSION**

The WHO workshop organized on September 18, 2015, in Lisbon, provided the opportunity for participants to contribute to a research agenda on WaSH and AMR. For each of the three key topics discussed, namely risk assessment, risk management, and monitoring and surveillance, it was evidently demonstrated that there are more open questions than answers at this time. Box 2 links research questions to GAP on AMR objectives. A research agenda should be consistent with and support the process of focusing on relevant questions and sharing best practices. Box 2 summarizes the output of the workshop discussion,
Identification and quantification of sources, occurrence, and transport (contributes to GAP-objective no. 2):

- Quantify concentrations and total loads of AMR bacteria, AMR genes, and antibiotic residues from humans and animals to the environment.
- Identify and quantify AMR bacteria, AMR genes, and antibiotic residues in different water-related exposure routes and determine the importance of each route in order to focus research capacity and to support guidance and advice to stakeholders and the public on adequate actions. Identify, quantify, and locate in time and space the sources that are hotspots of release such as health care facilities.
- Set up a uniform system and methodology for identification and quantification (HACCP as a suggested approach).
- Perform meta-analysis to determine and quantify the persistence of AMR bacteria, AMR genes, and antibiotic residues in different environmental matrices.
- Determine the occurrence, loads, and persistence of AMR bacteria, AMR genes, and antibiotic residues in the environment.

Understanding the risk to human health (contributes to GAP-objective no. 2):

- Identify health impacts associated with reductions in drug usage or drug concentrations.
- Identify health impacts associated with ARB/ARGAMR bacteria and gene concentrations and loading to the environment.
- Conduct risk assessment strategies to identify acceptable environmental concentrations or acceptable biological entities (i.e., certain bacteria or genes are acceptable whereas others are not) in exposure media such as water.
- Determine concentrations or loads (in wastewater effluent and biosolids) that are necessary to protect the receiving environment and ultimately benefit the clinical settings.

Efficiency of water and wastewater treatment technologies (contributes to GAP-objective no. 2 and 3):

- Provide criteria and guidance on waste and wastewater management for the reduction of AMR bacteria, AMR genes, and antibiotic residues in relevant settings, e.g., clinical, agricultural, household, and the environment.
- Quantify the role of AMR horizontal gene transfer in waste and wastewater.
- Identify ways of optimizing onsite waste treatment for reduction of AMR bacteria, AMR genes, and antibiotic residues.
- Address exposure risks by determining reduction efficiencies of applied water, waste treatment and human and animal excreta treatment processes for AMR bacteria, AMR genes and antibiotic residues and other WaSH-related settings and facilities, including household water treatment processes, water reuse practices and alternative sanitation systems.
- Identify simple microbial measurements (such as bacterial indicators) for system verification.
- Incorporate AMR bacteria, AMR genes and antibiotic residues in WHO Water Safety Plans and Sanitation Safety Plans, programs and practices. Identify critical control points to utilize in such Safety Plans.
- Develop criteria and guidance to water utilities, wastewater treatment plants and other AMR-relevant treatment facilities (antimicrobial production and aquaculture facilities) on the reduction efficiencies of their applied treatment processes and overall treatment for AMR bacteria, AMR genes and antibiotic residues.
- Develop criteria and guidance to different communities such as rural communities on the reduction efficiencies of AMR bacteria, AMR genes and antibiotic residues by local waste and wastewater practices and processes.

(Continued.)
The abundance of research questions and ideas identified during this workshop naturally led to the topic of funding. The speakers encouraged the workshop participants to apply to open calls for research proposals from funding organizations, such as the Bill and Melinda Gates Foundation and European Joint Programming Initiatives (JPIAMR), in order to address the topic of AMR and WaSH.

From the discussion, three major themes could be identified, which are discussed here.

**Evidence of water as a pathway for AMR exposure**

The importance of water, wastewater, and wastes as pathways for AMR human exposure (compared to other routes such as food consumption) was identified as a research focus (see also the review by O’Neill (2015)). Little is known about the exposure to AMR through WaSH routes, drinking water consumption, and water reuse applications and resulting effects on public health. Data based on magnitudes of exposure and objectively measurable health outcomes should be collected in a consistent and transparent way, to achieve data comparability among countries or regions, respecting the basic principles for the ‘right to data’.
comparable for all countries throughout the world. Current prevention and control by existing WaSH guidance should be assessed, i.e., the efficacy of AMR reduction by specific wastewater, excreta, biosolids, and water treatment technologies. Guidelines on AMR and WaSH should be harmonized with and preferably incorporated into the GAP on AMR as well as in the risk-based approach developed in the Water Safety Plans (Bartram et al. 2009) and the Sanitation Safety Plans of the WHO (Jackson et al. 2015).

Uniform and global surveillance

The call for a consistent, pragmatic, and transparent approach is also reflected in the use and harmonization of surveillance and monitoring techniques and the call for criteria and guidelines. Methods that can be used in all countries, including those with limited resources, should be identified, validated, and implemented to monitor AMR in the environment. The sampling methods should be those already in use to survey the bacterial concentrations and loads in the environment. The simplest would be to add screening to quantify a single type of AMR bacteria as a minimal requirement in these samples. ESBL-E. coli appears to be a relevant candidate because: (i) it is easily detected using commercial media and screening plates used in human medicine; (ii) large variations in occurrence and densities that are to be expected between countries can be quantified; and (iii) it could be a monitoring system common across human medicine, the food chain, and water and wastewater management, thus allowing powerful geographical comparisons and follow-up over time.

CONCLUSIONS

Understanding and addressing the role of WaSH in combating AMR is a critical element of the GAP on AMR. Therefore, research and development of guidance under the three previously identified themes is needed:

- to identify and quantify the sources, occurrence and transport of antibiotics, other antimicrobial agents and their metabolites, AMR bacteria, and AMR genes in environmental media, especially water, wastewater, animal fecal wastes and biosolids and other media to which humans are exposed;
- to understand the human health outcome from environmental exposures;
- to identify water and waste treatment technologies that can remove antibiotics and other antimicrobial agents, their metabolites, AMR bacteria, and AMR genes in human animal wastes and in environmental media for which the WHO provides specific guidance;
- to identify options to minimize the release or introduction of antibiotics and other antimicrobial agents, AMR bacteria, and AMR genes into the environment by the establishment and use of policies, programs, and practices to eliminate and minimize use and to contain or destroy these contaminants at their source or ‘critical control points’;
- to identify policies, practices, and tools to minimize human exposure to AMR from the environment;
- to support and develop monitoring strategies, surveillance and regulatory activities for AMR prevention and control related to the environment and WaSH.

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DISCLAIMER

Kate Medlicott is an employee of WHO. The authors alone are responsible for the views expressed in this article and
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