

THE ROLE OF SANITATION IN THE DEVELOPMENT AND SPREAD OF ANTIMICROBIAL RESISTANCE



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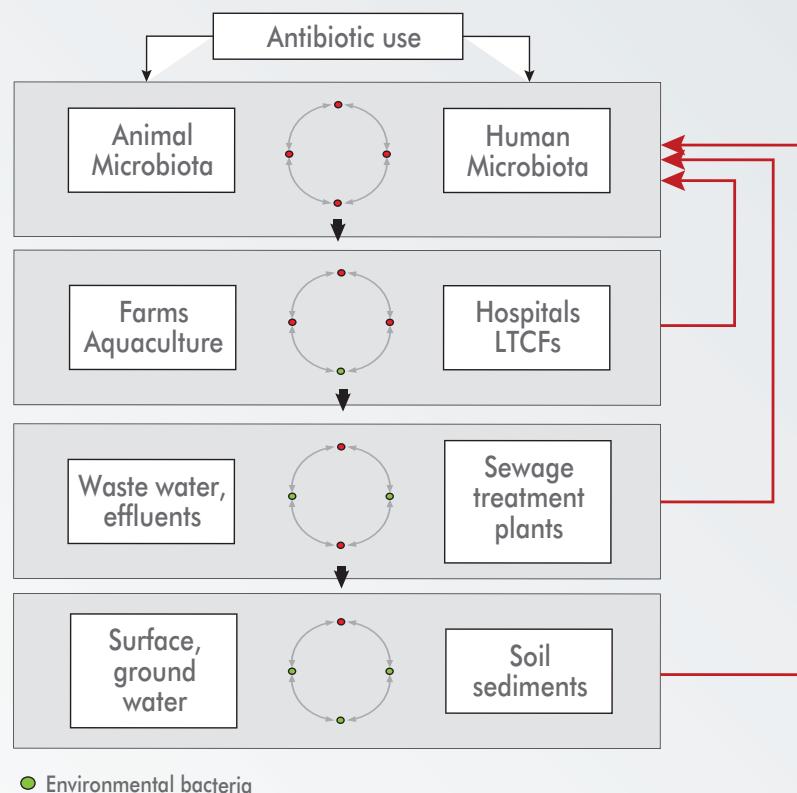
Many studies on antibiotic resistance (AR) focus on hospital infections yet in developing countries where sanitation is so poor, the continuous recycling of antibiotic resistant bacteria in poor communities invariably impacts on the health of those communities, the life span of the individual and represents a financial burden. More studies are urgently needed examining the risk factors for carriage of AR bacteria and their impact on human infections and wellbeing. Greater political commitment is required and a global awareness campaign encapsulating a "one world health" message, as, invariably, it is an issue that has global ramifications.

The link between sanitation, or lack thereof, and antimicrobial resistance (AMR) is primarily to do with two factors: the level of antibiotic resistant bacteria in a person's gut, and the level of AMR in the environment. The argument that resistance starts in a hospital and then spreads into the community or environment is often inaccurate and most certainly naïve. There is little evidence that few, if any, new antibiotic resistance mechanisms (ARMs) were acquired by bacteria in health settings yet there is some substantial evidence that clinically relevant bacteria have acquired ARMs from environmental bacteria typified by the sequestration of the CTM-X-15 gene by *E. coli* from environmental *Kluyvera* spp. and has now become the globally dominant extended spectrum β-lactamase (1–4).

There is a significant documented problem of AMR occurring between animal, human and environmental sectors, including water bodies and soil (Fig. 1). Antibiotic resistant bacteria (and the antibiotics themselves) are excreted with effluents and sewage into the environment, and from there re-contaminate humans and animals via drinking water or food. This does not only concern antibiotic resistant bacteria but also antibiotics entering the ecosystem mediating direct resistance and collateral damage. Once antibiotics enter the ecosystems, they can influence bacterial populations (5), and correspondingly influence potable water (6, 7). Accordingly, critics have commented on the long-term impact of antibiotic remnants in aquatic and

semi-aquatic environments (8–10). India produces about 40% of the world's antibiotics and has been commended by WHO for supplying the world with miracle drugs but the contamination in the Indian (and generally throughout South Asia) environment with antibiotics is immense. Antibiotics can enter sewerage treatment plants (STPs) through human excretion, farm animals and the direct disposal of medical and industrial wastes. Some antibiotics are removed through the degradation and sorption to biosolids during treatment plant processes, such as the secondary and tertiary stages (11), but not all antibiotics are completely removed (12). Studies by Al-Ahmad et al. (2009) and Wiethan et al. (2000) suggest that bacteria, which have already shown resistance to antibiotics, will not necessarily have a selective advantage in sewage treatment (13, 14).

Of greatest concern is the production and gross environmental contamination with fluoroquinolones (e.g. ciprofloxacin), once considered the perfect antibiotic. Studies have shown therapeutic levels of ciprofloxacin in Indian rivers and if the elegant studies of Beaber and colleagues are extrapolated to South Asia, this would indicate a colossal level of gene transfer happening in and between bacteria in this part of the world (15). This antibiotic load or pressure is further exacerbated by the levels of poorly degraded antibiotics contained in peoples normal flora that either enter sewerage treatment plants or worryingly, through open defecation.

Figure 1: The main digestive or environmental reservoirs of AMR to which the worldwide human community belongs and is also exposed

The major interrelations between human and animal activities and the water system are obvious in many parts of the world. Above is a representation of the main digestive or environmental reservoirs of AMR to which the worldwide human community belongs and is also exposed. Each independent reservoir is included in a dashed black outline, inside which cross-transmission may occur. Arrows show the flux of ESBL-E from one reservoir to another. Environmental niches comprise mainly water, soils and plants, where genetic material exchanges between bacteria of digestive and/or environmental origin occur. From Woerther PL et al. Clin. Microb. Rev. 2013 (3).

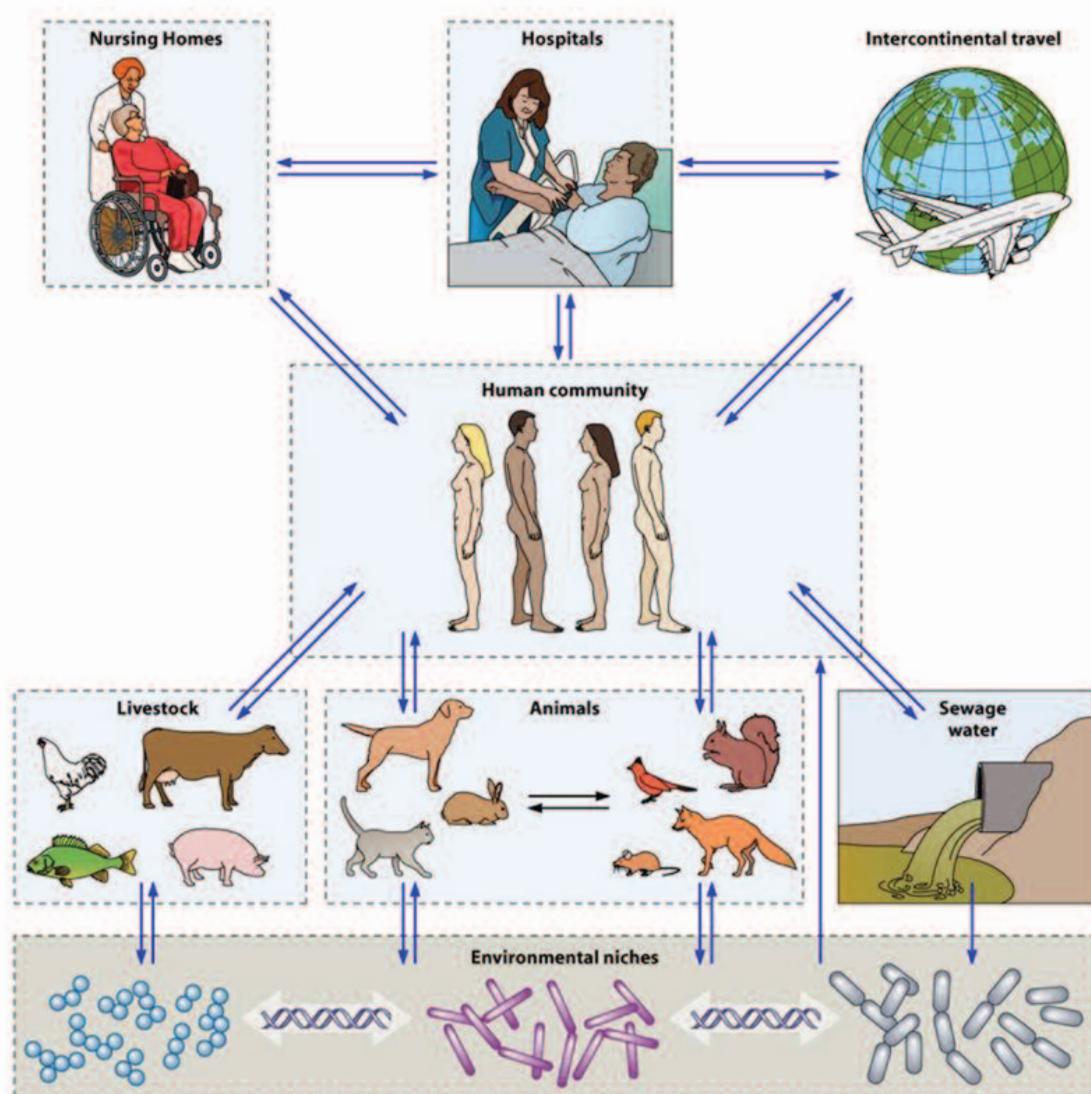
Aside from the chemical pollution caused by antibiotics, invariably they will increase the development and subsequent spread of antibiotic resistant bacteria (ARB) and antibiotic resistant genes (ARGs) – particularly those associated with mobile genetic elements i.e. DNA structures that mediate the transfer of resistance genes between bacteria e.g. plasmids (6, 17). (Fig. 2). Large amounts of antibiotics are released into municipal waste due to the incomplete metabolism (e.g. fluoroquinolones) of humans or due to disposal of unused antibiotics resulting in the detection of ARBs and ARGs (18-23). In India a simple point of prevalence study involving 171 seepage samples and 50 tap water samples from New Delhi detected ^{bla}NDM-1 in two of 50 drinking-water samples and 51 of 171 seepage samples. Bacteria containing ^{bla}NDM-1 included *Shigella boydii* and *Vibrio cholera* and the gene was shown to be carried on plasmids that can be easily transmissible between unrelated bacteria. Worryingly, the transfer of the plasmids was more common at 30°C than at 25°C or 37°C which

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corresponded to the mean annual temperature of New Delhi indicating that transfer of resistance genes can readily transfer in human waste and treatment plants in South Asia (16).

The spread of resistance in an eco-system occurs proportionally to the lack of sanitation, as shown by the contamination in the South Asian environment with not only

Figure 2: The four reactors for the antibiotic-promoted genetic evolution



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extended-spectrum beta-lactamases but also carbapenemase-producing Enterobacteriaceae. But Western countries are also affected by the problem, as shown by the cases of MRSA on pig farms in Holland and neighbouring countries or in the deadly cases in Germany involving the multi-resistant *E. coli* O104:H4 strain. It is also highly suspected that ESBL *E. coli* that cause infections in

humans are acquired, at least in part, through the food chain. However, there is very little known as to the true level of contamination with antibiotic resistant bacteria between humans and animals via the surrounding environment or the food chain. Environmental contamination in developing countries directly affects the level of cleanliness in food products and therefore treatment of both animal and human

Figure 3: World Bank map of countries with critically high sanitation needs – the larger the circle, the greater the need



sewerage becomes critical.

Whilst it has been known for some time that Enterococci, particular VRE, from the environment has impacted on the fecal carriage and ultimately infections in humans; the notion that human waste could impact significantly on human health has been particularly enhanced by multi-drug resistant Enterobacteriaceae. The fact that all humans and animals usually carry *E. coli* as part of their fecal flora and that this organism causes the majority of community acquired disease; mainly arising from endogenous sources, demonstrates their significance. Humans and animals are walking microcosms and can carry between 10–100 trillion bacteria and 10^{10} - 10^{12} bacteria/gram of feces. Thus the management of waste at a human and animal level is essential in lessening the burden of environmental AMR bacteria that can cause untreatable infections in the community.

Although animal waste is a constant concern, there is a lack of adequate sewage systems in countries such as India, Bangladesh, Pakistan, China, African and South American countries to deal with human and animal's waste. Moreover, in South Asia (India, Pakistan and Bangladesh) and many parts of Africa the vast majority of people eat with their fingers and are reliant on domestic cleaning agents to prevent cross-contamination. In many households in these countries there are precious few cleaning agents and the eating surfaces are possibly contaminated with fecal

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bacteria and thus the constant recycling from humans to the environment and visa versa is destined to continue ad perpetuum. In the "West" and in SE Asia where chopsticks are used to consume food there is at least a physical barrier between the environment and the oral cavity. Few studies have studied the impact of eating with your hands and its subsequent impact on the AMR load in the gut compared with the immediate environment.

The WHO-UNICEF Joint Monitoring Program (JMP) for Water and Sanitation, which tracks progress towards the water and sanitation targets of the Millennium Development Goals, estimates that 36% of the world's population, or 2.5 billion people, lack access to an improved sanitation facility, defined by the JMP as "one that hygienically separates human excreta from human contact" (24). This situation

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means that a large proportion of the world's people live at risk of contamination of their environment by human fecal matter (Fig. 3). UN figures show that approximately 700 million Indian people alone lack sanitation and that by 2020 China will have to process 500 billion tons/year of human waste. In Europe we have very little data on environmental contamination and its effects on antibiotic resistance particularly from the newer regions. Despite these worrying facts, sewerage treatment struggles to meet demands in rural areas in China, the Pakistani government have announced cut-backs in public funding for sanitation, and the Indian sewage system will continually lag behind through serious under-investment (Fig. 3). Currently, it is public funding or charitable funds that support such schemes and it is a sad fact that invariably demand outweighs the financial resources – often national governments do not see this issue as a priority and almost certainly fail to understand the indelible link between sanitation and the containment of human pathogens. Environmental risk factors for the dissemination of AMR bacteria have not been adequately assessed and while ad-hoc studies have examined the effects of antibiotic contamination on AMR, these are small-point prevalence surveys and lack structure to identify risk factors e.g. flooding. Food contamination, particularly in relation with environmental contamination, is another area that has lacked a systematic analysis particularly with regard to local markets and exported meat into Europe. The role of animals in AMR in these countries also remains underexplored.

Data from small studies and preliminary data from larger studies estimate that in the India/Pakistan alone over 200 million people carry carbapenemase-positive as normal flora and thus the potential for highly resistant strains to be continually recycled throughout and inter-communities is immense (Walsh, unpublished data). Whilst portable

sewerage treatments are being piloted in these areas lacking sanitation, there is not a systematic publicly funded programme seriously addressing these issues or the impact this will have on the environment, animals, food contamination and potable water with regard to AMR. Reducing pathogen load or destroying plasmid DNA in the environment will significantly lessen the burden of MDR in communities and correspondingly in hospitals, and reduce the health and financial burden in developing countries.

Sewerage treatment generally involves three stages: primary, secondary and tertiary treatment. In primary treatment, solids are removed by physical operations. In secondary treatment, biological and chemical processes are used to remove most of the organic matter. In tertiary or advanced treatment, additional processes (e.g. nutrient removal, removal of toxic materials, additional organic and suspended solids removal) are used to remove components. During all these processes, considerable changes occur in the distribution of the bacterial population (25). The general observation in literature is that treatment determines a significant reduction in the bacterial numbers, including the total numbers of resistant bacteria (26, 27). It has also been suggested that STPs concentrate AMR bacteria. Currently, no controlled studies have been undertaken comparing STPs to open defecation in countries where sanitation is pitifully poor. However, reports state that wastewater, or even treated wastewater, contain higher proportions of various resistant bacterial populations in relation to the respective proportions contained in surface water (27).

There is very little doubt that the exchange of genetic information between bacteria does occur in sewerage treatment plants (STPs) and some commentaries have suggested STPs as epicentres of antibiotic resistance exchange (27). According to these studies, the conditions in STPs are favourable for the exchange of resistance genes from AMR bacteria to susceptible bacteria. Several studies

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indicated that the environmental conditions in wastewater treatment plants may enhance the likelihood of gene transfer (28). However, links are not yet well established between the presence of antibiotics in STPs and the favouring of resistant bacteria as well as the transfer of resistance at concentrations as low as those found for antibiotics in the environment. There is also very little data on whether the mechanics applied in STPs accelerates the exchange of genetic material between bacteria and at what stage of the treatment process if any, would this happen. What is also not well understood is the role of bacteriophages in controlling bacteria populations, and thus AMR bacteria populations, in STPs or the environment per se which is an area that warrants significant attention.

The published information about water and sewage decontamination procedures with respect to antibiotic resistance remains extremely scarce, and it is urgent to design actions to fill this critical gap. Specifically, the activities should provide a meta-analysis of current national and international activities concerning the situation of waste disposal including the availability of suitably clean potable water, and its relation with the spread of antibiotic resistant organism among humans and animals. Related to this meta-analysis, is information on antimicrobial resistance and whether community carried AR/community acquired infections is a key concern.

Decontamination procedures of antibiotic resistant organisms and resistance resistant platforms, as well as antibiotic detoxification in water and soil should be explored. Currently, there are a number of technologies to purify wastewater but do not necessarily address the issue of environmental decontamination with AMR populations. Eventually, a number of these interventions might even contribute to the selection of resistant bacteria. Thus we should explore the merits of these technologies and whether they can contribute to a holistic initiative that will potentially eradicate antibiotic resistant populations and avoid re-colonization. Currently, international studies are on an ad-hoc basis with very little international collaboration.

The overriding issue addressing AMR is a sociopolitical one and sanitation is but one of many factors that contribute to the creation and dissemination of AMR in communities particularly developing communities. Health systems in South and SE Asia are a mixture of public and private and therefore implementing a national system for AMR surveillance and infection control practices will be extremely challenging. Many of these countries do not commit to a national health system and seemingly public expenditure on sanitation is also pitifully inadequate. Regrettably, these

countries also have rapidly growing economies (Brazil, China, India and Thailand) and are vying for the global export market in poultry by 2030 and some are, bizarrely, promoting medical tourism as an unmet global medical need. And thus, we are faced with a paradox whereby the environment is carelessly being continually contaminated and yet international trade is being promoted that has direct contact with that environment. When Indian Prime Minister Modi came into power his mantra was "a clean India" – an admirable notion, but as *Time Magazine* pointed out in 2013 (29), there is still a vast amount to do – not least change the mind set of citizens and plan for the long term. Regrettably, such noble intentions cost money and thus often lack political traction which is the core root of this entire problem. •

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Professor Timothy R Walsh is currently Professor of Medical Microbiology and Antibiotic Resistance at Cardiff University, Cardiff, Wales, and leads an active research in antibiotic resistance. He has published/presented over 400 papers in particular on the characterization of β -lactamases within Gram-negative bacteria, in journals such as Clinical Microbiological Reviews, Microbiology and Molecular Biology Reviews, Lancet Infectious Diseases, Nature and Lancet. His research has been supported by a wide variety of funding bodies including the Wellcome Trust, MRC, European Union, IMI, British Society of Antimicrobial Chemotherapy, BBSRC, and Gates Foundation. He acts as an advisor to the WHO and MSF, and is also Director to the South Asian Antibiotic Resistance Program.