Survival and growth of antibiotic resistant bacteria in treated wastewater and water distribution system and their implication in human health: A review

Abstract

Multiple antibiotic resistant bacteria (MARB) and antimicrobial drugs enter the environment via wastewater, especially from hospitals and pharmaceutical plants, and through agricultural runoff, leading to contamination of surface and groundwater. This is a serious problem in arid regions such as Oman where wastewater is recycled for irrigation and recharging aquifers. Treatment with chlorine does not completely remove bacteria from wastewater or prevent their re-growth in downstream distribution systems. MARB can infect humans via contaminated food and drinking water, or directly from the environment. Agricultural runoff and sewage, either treated or untreated, are also the main sources of MARB in coastal seawater. It is necessary to use antibiotics more prudently in medicine, treat wastewater more effectively, eliminate the discharge of untreated waste into the environment, and curtail the profligate use of antibiotics as growth promoters for livestock.

Keywords: Antibiotic resistance, bacteria, survival and growth, human health.
Wastewater treatment and survival of antibiotic resistant bacteria

It is clear that antibiotic-resistant bacteria can be detected in wastewater treatment plants. Treatment of sewage can reduce the number of both sensitive and resistant bacteria significantly. However, as will be discussed in the next section, not even chlorination is sufficient to eliminate these bacteria completely [1-2]. In short, the current methods used for treatment of sewage effluent do not remove all organic matter and pathogenic microbes. Sewage treatment plants are the main sources of antibiotics and MARB released to the environment, is a serious public health concern [3].

The occurrence of multiple antibiotic-resistant bacteria in treated wastewater has been reported in other studies. Antibiotic-resistant strains of intestinal microflora, originating not only in humans but also in domestic and agricultural animals, find their way to sewers and sewage treatment plants [1-2, 4]. The highest resistance to various drugs has been found in E. coli strains isolated from the main sewage treatment plants receiving effluents from three sewage sources in southern Austria. The most frequent resistance rates were found in E. coli isolates from a sewage treatment plant receiving municipal sewage and sewage from a hospital [5]. The susceptibility of Acinetobacter spp isolates from upstream and downstream of the hospital and pharmaceutical sewage treatment plant discharge points was studied. The results revealed that hospital effluents affected the prevalence of oxytetracycline resistance only. On the other hand, the wastewater discharge from pharmaceutical sewage treatment plant was increased both single- and multiple-antibiotic resistance among Acinetobacter spp isolates [6].

A major problem facing water suppliers is the deterioration of bacteriological quality of the water being distributed [7]. Since wastewater treatment processes and disinfection systems do not inactivate the whole microbial population, the surviving microorganisms can adapt to the conditions in the distribution system and start multiplying [1, 8-9]. Hence, it is meaningful to analyze treated water in terms of microbial populations and the factors influencing their growth.

The microorganisms found in a water distribution network can either be indigenous, including those growing in pipelines [10], or exogenous bacteria such as fecal coliforms, which are introduced and transported in water from the treatment plant [9-10, 11]. The latter group may potentially include pathogenic bacteria carrying resistance genes. The viability of coliform bacteria in water distribution systems is used as an indicator for the presence of potential pathogens, including opportunistic microbes [8]. The presence of these bacteria in water occurs as a result of complex interactions among various environmental factors [12]. These factors include disinfection effectiveness, physicochemical parameters and the quality of the water source [1, 9, 13]. Re-growth in the distribution system is stimulated by high content of organic matter in the water, as shown by the linear relationship between bacterial level and organic concentration [12].

Antibiotic resistance, heavy metals and chlorine disinfection

Some sewage treatment plants in Oman receive industrial wastes that contain high concentrations of heavy metals. In many cases, these heavy metals are found in the treated sewage effluent and sludge at concentrations exceeding maximum permissible levels [14-16]. It is noteworthy that bacterial isolates from waste streams contaminated with heavy metals have also been found to be resistant to antibiotics. For example, microbial isolates from pig manure containing...
high concentrations of zinc and copper were found to be highly resistant to antibiotics including piperacillin and doxycycline [17]. Other investigators have reported that MARB have common survival mechanisms for resistance to antibiotics and heavy metals [18-20]. Some antibiotic resistant bacteria such as Enterobacter, Pseudomonas, Aeromonas, Klyvera, Klebsiella, Pasteurella, Serratia and Salmonell spp isolated from treated sewage effluents use essential heavy metals for their survival, and that these bacteria are also resistant to chemical disinfection [1].

Chlorine is the most widely used chemical disinfectant in wastewater treatment [21] because of its potency in its uncombined form and because it is effective against both bacteria and viruses, even at low concentrations [8]. Chlorine acts on bacterial cells by disrupting cell membranes, coagulating enzymes and nucleic acids, subsequently leading to a reduction or cessation in metabolic activity [8, 22]. Chlorine has been shown to cause fatal physiological injury to coliforms [23].

Tertiary treatment of wastewater with chlorine contributes greatly to the removal of most bacteria from treated effluent, including fecal coliforms [5]. Tertiary treatment should be able to eliminate both antibiotic-resistant and -sensitive pathogens, but this is not usually the case. As has been demonstrated in a number of studies, some bacteria overcome the chlorine treatment and survive to create subsequent public health problems. In particular, MARB isolated in Oman have been shown to resist the chlorination process and remain viable in tertiary treated sewage effluents from hospitals and industries [2,24]. It has been shown that MARB from sewage treatment plants treating waste from industrial sources are more resistant to higher chlorine concentrations than those from hospital sources [25]. It has been reported that water taken from the Gomti River in India used for human consumption still has MARB, even after chlorination [26].

The disinfection efficiency during treatment plays an important role in the maintenance of water quality in the effluent [7]. However, even if the majority of bacteria are eliminated, those that survive re-grow in the downstream distribution system. MARB were isolated from drinking water in distribution systems and household storage tanks [13]. Other investigators reported extensive growth in chlorinated sewage effluents of fecal and non-fecal coliforms [27]. It has been shown that coliforms survived chlorination in a treated water distribution system and that the maintenance of 1-2 mg/mL free chlorine was insufficient to eradicate the growth of coliforms in the distributed water [7]. Enumeration of bacteria in effluent from distribution lines used for irrigation showed that treated sewage had the lowest counts immediately after chlorination, but that microbial re-grow increased significantly while chlorine concentration decreased drastically at the end of the line and that isolates were resistant to several antibiotics [1].

The mechanisms by which bacteria develop resistance to chlorine include: (i) modification of cell surface structures which may lead to increased aggregation or clumping of cells in situ, (ii) formation of resistant endospores, (iii) microbial adhesion to pipe surfaces or to suspended particulate matter such as detritus or clay particles, and (iv) extrusion of protective extracellular capsular or slime layers [12]. Another factor in the survival and growth of bacteria downstream from disinfection is the loss of potency of the chlorine. The effect of chlorine as a function of time has been examined [21] and shows that immediately following chlorination, there is a rapid inactivation of E. coli found in the treated wastewater sample, but that five minutes after addition of chlorine there was little or no inactivation of E. coli. This rapid change results from the fact that chlorine is highly reactive against organic matter [12] and is rapidly converted from a free to a combined form due to the presence of exogenous organic matter in most water treatment systems [21]. Temperature also affects the biocidal activity of chlorine. At low temperature chlorine activity decreases [28].
Spread of resistant bacteria through recycling and reuse of wastewater

The process of wastewater treatment ideally removes pathogenic organisms from the water and either removes organics and solids or changes them into forms that are compatible with the environment. Recycled treated wastewater is either returned to surface waters or reused and this has the benefit of reducing water demand, specifically in arid regions [12, 16, 24, 29-30].

Coliform bacteria including *E. coli* are used as indicators of pathogenic bacteria of enteric origin in treated water used for irrigation. Routine microbiological analyses of treated wastewater in Oman have revealed that *E. coli* is present in numbers exceeding the permitted Omani standards. Therefore this wastewater poses a threat of contamination to both groundwater and coastal water. *E. coli* isolates from the wastewater treatment plant were resistant to several antibiotics commonly used to treat infections in humans. This presents a public health problem due to the potential difficulty in treating infections caused by this bacterium and other antibiotic-resistant pathogens that might be present [1]. Although antibiotic resistance may be present in bacteria which are not considered primary pathogens, they remain a threat to public health due to possibilities of transmittance of resistance to other microorganisms, especially human pathogens [31].

Antibiotic resistance in the aquatic environment

As discussed above, there is strong evidence that antibiotics and antibiotic-resistant bacteria find their way into the environment. Indeed, resistance to antibiotics in bacteria from different environments is increasing substantially and becoming a worldwide concern [1, 32-33]. The profile of antibiotic-sensitive bacteria differs significantly from that of the pre-antibiotic era, with more and more environmental samples being reported to contain bacteria resistant to antibiotics [34]. Animals from the wild are exposed to antibiotic residues and bacteria with antibiotic resistance genes and consequently multiple antibiotic resistances have been found in almost all species of bacteria that inhabit humans, domestic animals and wildlife. Antibiotic-resistant bacteria from sewage effluents have been found to contaminate underground water and this has had a great impact on terrestrial and aquatic wild life [2, 32-36]. Antibiotic-resistance in the aquatic environment has received little attention, despite the fact it is a major problem, with large numbers of resistant bacteria being found in these ecosystems. Antibiotic-resistant bacteria have been reported in mammals, birds, turtles and fish. High-level resistance to aminoglycosides in environmental isolates of enterococci from aquatic habitats was reported [34]. The overuse of antibiotics, agriculture runoff and coastal development has resulted in an increase in antibiotic-resistant bacteria isolated from marine habitats. Unfortunately, the coastal environment has been used as a dumping ground for sewage and other waste products [36]. As a result, the environment has become a reservoir for resistant bacteria and provides a return path for antibiotic resistance genes in bacteria that can infect both humans and animals [37]. In particular, marine flora and fauna are exposed to antibiotic residues and to MARB [1, 32-33, 36, 38-39]. Bacterial isolates from fish in Chile, contained significantly high rate of MARB. In Oman, antibiotic-resistant bacteria were isolated from fish feeding near the dumping sites of sewage effluent, strongly suggesting that infection of fish by antibiotic-resistant bacteria is directly related to the treated wastewater exposure [2].

Polluted sewage effluents though agricultural runoff may also be the source of antibiotic-resistant bacteria isolated from wild sea turtles. MARB have been isolated from eggs and cloacal fluid of the green
turtles, *Chelonia mydas* [36, 40]. Microbial isolates from cloacae of the loggerhead turtle (*Caretta caretta*), had similar high level of MARB [38]. MARB were isolated from eggs and oviductal fluid, an indication of contaminated effluents encountered by the sea turtles during migration and in the feeding areas near their nesting grounds [24, 36, 38, 40].

Antibiotic resistant bacteria are also associated with aquaculture. The release of sewage effluents into seawater is one possible route for transferring antibiotic-resistant bacteria to aquaculture. It was demonstrated high levels of individual and multiple antimicrobial resistance within several groups of bacteria, including flavobacteria and aeromonads, associated with aquacultural environments [39].

**Antibiotic resistant bacteria as bio-indicators of aquatic environmental pollution**

The spread of MARB into the aquatic environment is a serious issue. Antibiotics and MARB enter the environment via routes as discussed above. Therefore, effluent pollution can be detected by the presence of MARB or antimicrobial agents. The presence of MARB in the aquatic environment has been investigated by many workers [2, 24, 32, 36, 38-42]. These studies have shown that many aquatic and marine habitats exposed to antibiotic residues have developed a significant MARB presence. In short, the presence of MARB in aquatic habitats can be used as a bioindicator of pollution [2, 24, 36, 42]. In one application of this concept MARB were isolated from soil and snails in a pond contaminated with treated sewage effluent from industrial and residential sources [40]. In another example, the presence of MARB in tissues of fish caught near the outlet of a sewage treatment plant, which is a clear indication of treated sewage effluent contaminating coastal areas [2].

MARB as bioindicators of pollution from contaminated effluents is an attractive and valuable point, particularly in the case of ocean pollution. Due to the low concentrations of pharmaceutical pollutants in seawater, they are difficult and expensive to detect using current methodologies, whereas detection of MARB is comparatively easy. In addition, it may not be feasible to survey for the presence of pharmaceutical compounds in many localities. The use of MARB in marine animals, such as sea turtles, as bio-indicators is probably more practical and can provide an integrated assessment of the extent of contamination along the turtles’ migratory routes [24, 41].

In summary, these investigations induce deep concerns about the dissemination of resistance to antibiotics in marine wildlife. However, they also suggest that the presence of antibiotic-resistant bacteria in marine animals such as the green turtle, can be used for monitoring the degree of pollution in the turtles’ feeding grounds along the seashore and in the wide geographical regions where they migrate.

**Implications for human health; Spread of antibiotic resistant bacteria to humans from the environment.**

The spread of MARB among pathogenic and commensal bacteria is a global health concern. Each year worldwide about 17 million people die from infectious diseases, most of which are caused by bacteria [43]. The Centers for Disease Control has stated that in the United States more than two million people are sickened and 23,000 die each year as a result of antibiotic-resistant infections [44]. Antibiotic resistance can lead to increases in human and domestic animal health care costs as well as increased mortality and morbidity [45]. Consumption of contaminated water or food consequently leads to gastrointestinal tract by various bacterial,
including *E. coli* [46]. Antimicrobial resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans, the most common cause of urinary tract infections and a common cause of both community and hospital-acquired bacteremia. Moreover, the transfer of antibiotic resistance determinants from *E. coli* to normal flora of gastrointestinal tract has been reported [47]. Due to the fact that consumption of contaminated food has been considered the main route of transmission of drug resistance, relatively little attention has been paid to other routes by which antibiotic resistance can be disseminated, such as natural water, wastewater and soil environments [48]. Nevertheless, it is clear that MARB in the environment have serious implications for human health. Not only can MARB spread to humans from animals, as previously described, they can also spread from the environment to humans. Once established in the environment, resistant bacteria can infect humans either directly, via re-infection by way of meat, crop or potable water [9, 49-51].

One major route for transmission of drug resistant strains to humans involves soil microorganisms, which are the main producers of antibiotics. The use of drugs in agriculture adds to their accumulation in the soil and contributes further to the selection of soil microbes that carry resistance genes. Such genes may subsequently be acquired by non-soil microbes, which can infect animals [52] and thence humans. Reservoirs of antibiotic-resistant bacteria are found not only in domesticated animals but also in wild animals. Those isolated from wild animals living in close proximity to human activities are more often resistant to antibiotics than those isolated from animals living in remote areas. This is a serious concern because in recent years about 75% of new human diseases have originated in wildlife (zoonoses) [53]. Surface water contamination with fecal-derived bacteria, is a health concern. However, groundwater contamination is also a serious health problem [45]. In Ontario, most rural families rely on about 500,000 private wells [54]. About 10% - 34% of these wells are not within the drinking water standards associated with a high risk of gastrointestinal illness [55]. Regardless the risks of antimicrobial-resistant *E. coli* in potable water, only a few studies have been conducted on this topic. MARB in soil or in water used for irrigation may cause crop contamination [51-56]. It was reported that fresh vegetable crops such as lettuce are important sources of *E. coli* outbreaks in humans, although outbreaks resulting from contaminated meat are more deadly [51]. A recent study has shown that in the Jordanian retail markets, fresh leafy vegetables were not contaminated with any type of common diarrheagenic pathogens, but they were associated with few percentage of multiple antibiotic-resistant *E. coli* [56]. In Jordan also, *Pseudomonas aeruginosa* with relatively similar common antimicrobial resistance pattern and serotypes were isolated form feces of patients and in drinking water sources [57].

**Antibiotics and MARB in the environment: Possible remedies**

In Oman, efforts are made to show why and how MARB arise and why MARB in the environment is a serious problem. It was reported that better health care practices and more stringent regulations should be implemented in order to control the usage of antibiotics and to stop the emergence and dissemination of multiple resistant strains which are comparatively similar to the global concerns [2, 4, 41-42].

We suggest the following four major strategies to reduce the spread of MARB:

1. Eliminate the release of untreated sewage into the environment;
(2) Use antibiotics more prudently in medicine;
(3) Improve sewage treatment technologies to more efficiently remove antibiotics and MARB and
(4) Curtail the use of antibiotics as growth promoters in food animals.

With regard to points 1 and 2, dumping of raw sewage into rivers, lakes and coastal areas is a serious health hazard, whether or not antibiotic-resistant bacteria are present. It should be self-evident that universal application of sewage treatment would be a very good thing, though obviously it may be politically and economically difficult to achieve, especially in less developed countries. Second, antibiotics need to be more carefully utilized in medicine and veterinary medicine. Greater awareness on the part of both health professionals and the public of the risks of improper use and disposal of antimicrobial drugs will be crucial to decrease the abuse of antibiotics and to minimize their release, and the release of resistant bacteria, into the environment. In the United States, it is promising that recent reports from the Centers for Disease Control and Prevention [44] and recent actions by the Food and Drug Administration [58] have gotten wide coverage in the news media which may help to make the public more aware of the seriousness of the problem.

The third step toward solving the problem concerns the effectiveness of wastewater treatment. The main objective of sewage treatment is to remove pollutants and produce environmentally safe water for disposal or recycling and sludge that is suitable for reuse as fertilizer. The treatment procedures include physical, chemical, and biological processes. However, it is clear that current methods for the treatment of sewage are not adequate, since both antibiotics and resistant bacteria can be found in the effluents.

Some new technologies that have potential for more efficient waste treatment are essential. Note that an important part of sewage water purification in the future will be monitoring the influence of the process on the antimicrobial susceptibility profiles of bacterial indicators that could spread to the environment and to humans [59].

Reverse osmosis for the treatment of municipal sewage effluent has been available for several decades [60]. Reverse osmosis has the advantages that it can produce clean water from chlorinated, filtered, secondary sewage effluent on a large scale [61] and the recovery rate for reclaimed water can reach 98% [62]. However, even though reverse osmosis produces more environmentally acceptable water than conventional sewage treatment plants, several obstacles currently make its use impractical. These obstacles include membrane fouling, pH of the feed effluent, the high cost of reverse osmosis membranes and the economics related to high energy-input [61-62].

Nanotechnology for water reclamation may play an important role in the future for water and food security [63]. Several nanotechnology procedures and applications have been tested for their efficacy in treating contaminated effluents. New membranes are being introduced that involve the use of catalysts made of nanomaterials for the breakdown of toxic compounds in water. These nanomaterials include zeolites and carbon compounds, bimetallic nanoparticles and mixed oxides [64]. Emerging nanotechnology applications to wastewater treatment may therefore replace inefficient conventional methods. Photodialysis is one of the emerging technologies whereby light sensitive organic matter and microbes can be treated [32, 65].

Photo-catalysis was used effectively to degrade certain antibiotics and other pharmaceuticals in aqueous environment using combination of titanium oxide and ultraviolet light. However, the presence of organic compounds inhibits photo-catalytic degradation of some antimicrobial compounds such as sulfamethoxazole [65]. Also, frequency and light intensity are important factors in photodecomposition. Photodecomposition is not effective in turbid water, sewage, sewage pipes and soil [32]. This
technology may be improved by combining the composite photo-catalytic membranes and nano-membrane separation technologies [66]. The fourth major step needed to reduce MARB in the environment concerns the use of antibiotics as growth promoters in chickens, fish, cattle and other food animals. The use of antibiotics as feed additives for food animals should be phased out, because it is ultimately detrimental to human health. Another reason to curtail or eliminate the use of antibiotics in this way is that as resistant strains arise and are selected, the drugs will inevitably become less effective. A decline in the growth-promotion response of pigs to antimicrobial agents, and this decline was associated with the presence of bacteria having increased resistance to the agents that were used [67]. In Denmark, it has been possible to reduce the usage of antimicrobial agents for food-animals significantly and in general this has led to decreases in the prevalence of resistance [68]. It has been noted that when the antibiotics are not used at all, resistance is generally not observed. E. coli isolates from chickens in Jamaica were not resistant to gentamycin because it is not used in livestock there [69]. In general, Europe has been quicker to adopt limitations on the use of antibiotics as growth promoters than the United States [70]. However, in the United States some progress is being made. Though tenaciously opposed by farmers and pharmaceutical manufacturers, the Food and Drug Administration (FDA) prohibited the use of fluoroquinolones in poultry in 2005 and has recently adopted some restrictions on the use of cephalosporins [58, 70]. Although these steps have been criticized as inadequate [71], there is hope that with increased awareness of the seriousness of the problem [44], further progress will be forthcoming.

**Conclusion**

MARB and other pollutants are rapidly increasing in aquatic and terrestrial habitats at an alarming rate. It is clear that strategies must be developed and implemented internationally to avoid dumping residues of various antibiotics and other pharmaceuticals directly into the environment. Stringent sets of rules and regulations will be required to prevent direct sewage drainage into the aquatic and terrestrial habitats. Examination of these effluents for the presence of MARB, heavy metals and other contaminants would reflect the effectiveness of the waste treatment. Since MARB are relatively easily detected, this means that the presence of MARB in wildlife and environmental samples can be used as a biological indicator to monitor the degree of environmental pollution.
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