

Antibiotics and Antibiotic resistance determinants: an undesired element in the environment

E.O. Igbinosa*¹ and E.E. Odjajare²

¹ Applied Microbial Processes & Environmental Health Research Group, Department of Microbiology, Faculty of Life Sciences, University of Benin, Private Mail Bag 1154, Benin City, Nigeria

² Department of Basic Sciences, Benson Idahosa University, Private Mail Bag 1100, GRA, Benin City, Nigeria

* Corresponding author: email: etinosa.igbinosa@uniben.edu

The rapid development of resistance in bacteria is an evolutionary response to the selective pressure of antibiotics and results from natural selection. It is established that microbial adaptation evolves, and the cause for the development of antibiotic resistance is the widespread misuse of antibiotics. Pollution of the environment by antibiotic resistance genes can increase the chances of human pathogens for acquiring resistance. The release of residues containing human microbiota into environments containing bacteria enriched in resistance elements increases the possibility of acquiring novel resistance determinants by human-linked bacteria. The spread of resistance genes in natural ecosystems can challenge the population dynamics and the physiology of natural microbial populations. Active stewardship is needed to avoid gene flow to and from environmental resistance reservoirs. In this chapter, we discuss the dissemination of antibiotics resistance genes (ARGs) and antibiotics resistance bacteria (ARB) in the environment, with emphasis on how these elements interact with and affect the health and general wellbeing of human and natural ecosystems.

Keywords: antibiotics, antimicrobial resistance; environment; public health; pollution

1. Introduction

Antibiotics are biologically active compounds and are widely used in humans and animals to prevent or treat microbial diseases. They are utilized as growth promoters and feed additives in livestock, poultry and fish farming. Consequently, there are ample opportunities for antibiotics to be released into natural ecosystems, where they impact the structure and activity of microbial inhabitants (Martinez 2009). The growing use of antibiotics for therapeutic and non-therapeutic purposes has led to the development and dissemination of microbial resistance determinants both in clinical and non-clinical settings. The occurrence and dissemination of antimicrobial-resistant bacteria (ARB) and antimicrobial resistance genes (ARGs) are recognized as a major public health concern. The effect of clinically relevant ARGs and ARB that are released from anthropogenic sources, along with the excessive use of antibiotics in both human and veterinary settings, is currently considered to be a serious environmental and ecological hazard (Cantón 2009; Allen *et al.*, 2010; D'Costa *et al.*, 2011; Bush *et al.*, 2011). The resistant bacteria in the environment can lead to structural changes in the composition of microbial communities, with potential toxic effects on the balance of natural ecosystems. Previous studies have highlighted soil and water environments as recipients, reservoirs, and sources of ARGs of clinical concern (Martinez 2009; Wright 2010). Similarly, soil and water environments receive inputs of antibiotics and antimicrobials, which can serve to amplify ARGs (Chee-Sanford *et al.*, 2009; Heuer *et al.*, 2011). The ARGs carried by these bacterial contaminants can multiply in their hosts, transfer to other bacterial populations and be subject to further development and progression in the bacterial community. As such, ARB that occur in the environment represents potentially serious risks for human health and the sanctity of the environment.

Bacterial resistance to antibiotics has assumed an increasing importance with regard to its impact on both public and environmental health. The primary problem is represented by the emergence of antimicrobial resistance among pathogenic bacteria to humans and animals, which makes treatment difficult for some life-threatening infections. The global spread of ARGs and their acquisition by clinically relevant bacteria is associated with the increased environmental pollution, which constitutes a serious challenge for health and wellbeing of humans. The increasing spread of ARGs among environmental bacteria has led environmental scientist to consider ARB and ARGs as emerging pollutants or contaminant in the natural environment (Pruden *et al.*, 2006; Pruden *et al.*, 2013). These resistance determinants (ARB and ARGs) possess atypical characteristic features and uniqueness compared to other contaminants or pollutants; their ability to amplify and spread, persisting in the environment has been recognized (Pruden *et al.*, 2006; Tacão *et al.*, 2012; Pruden *et al.*, 2013; Tacão *et al.*, 2014).

Antibiotic resistance is a threat to human and animal health globally, and key measures are required to reduce the risks posed by antibiotic resistance genes that occur in the environment. The World Health Organization has stated that emergence of antimicrobial resistance is a complex problem driven by many interconnected factors and variables. However, environmental pathways of antimicrobial resistance have not yet been directly addressed by the World Health Organization (WHO 2012a). Research was initially focused on clinical settings as the main site of the spread and evolution of antimicrobial resistance. Over the course of the past two decades, scientists have increasingly broadened their focus to include the environment as a source of resistance genes and as a site of antimicrobial-resistance evolution

(Kümmerer 2004; Pruden *et al.*, 2014). These serious health issues make it imperative not only to control ARB and ARGs in the environment but also to assess and understand their dissemination in the environment with a view to protecting the environment and preserving the public health.

2. Antibiotics resistance in clinically relevant bacteria

The indiscriminate misuse and overuse of antibiotics in humans, animals and agriculture settings is the leading cause of increasing trend in microbial resistance. Resistance to antibiotics first appeared in hospitals with the emergence of sulfonamide-resistant *Streptococcus pyogenes* in 1930s followed by penicillin-resistant *Staphylococcus aureus* in the 1940s (Levy and Marshal 2004). However the prevalent threat is posed by multiple drug resistance (MDR) and some pathogens such as *Mycobacterium tuberculosis*, methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Staphylococcus aureus* (VISA), Vancomycin-resistant enterococci (VRE), *Klebsiella pneumoniae*, drug-resistant *Escherichia coli*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are globally notable examples in the hospital community (Levy and Marshal 2004). Penicillin-resistant *Streptococcus pneumoniae* (PRSP), a common pathogen of children, is also mainly community-acquired (File 2006). This has resulted in an increased frequency and duration of hospitalization, with increased exposure to multidrug-resistant pathogens that are present in healthcare settings (Wright 2010). In the past, most pathogens resistant to multiple antibiotics were isolated from healthcare settings, where antibiotics use was prevalent. Currently, MRSA is routinely isolated from the community and, worryingly, antibiotics resistance is associated with increased virulence (Robinson *et al.*, 2005). Opportunistic pathogens including *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, *Acinetobacter baumannii* and *Burkholderia cepacia* are frequently intrinsically resistant to multiple antibiotics (Wright, 2010). Resistance to penicillin, cephalosporins and other β -lactam antibiotics has increased the use of erythromycin and new macrolides such as azithromycin and clarithromycin (Gay *et al.*, 2000). For this reason bacterial infections resistant to antibiotics are becoming increasingly common in clinical settings (Zoutman and Ford 2005). Many pathogenic agents have become resistant to various classes of antibiotics since the 1960s. This fact has become clinically, epidemiologically and socioeconomically important, because infections caused by resistant bacteria can be especially difficult and costly to treat.

Emergence of multi-drug resistance in community acquired pathogens such as *Mycobacterium tuberculosis* and *Streptococcus pneumoniae* demonstrate a worrisome situation in which the effectiveness of the antibiotics that previously successfully treated the infections has deteriorated over time. Nosocomial pathogens associated with hospital acquired infections, such as methicillin-resistant *Staphylococcus aureus* (Börjesson *et al.*, 2010), *Pseudomonas aeruginosa* (Fuentefria *et al.*, 2011; Igbinsola *et al.*, 2012; Odjadjare *et al.*, 2012) and Vancomycin-resistant *Enterococcus faecium* (Araújo *et al.*, 2010; Iweriebor *et al.*, 2015), occur with high frequency of drug-resistance characteristics and have been detected in wastewater treatment plants that receive hospital effluent discharge. The discharge of effluents from wastewater treatment plants provides the pathway for introduction of contaminants into the receiving environment. Multi-drug resistant *Acinetobacter baumannii* is a rapidly emerging pathogen in health care settings where antimicrobial resistance has seriously limited treatment options (Eliopoulos *et al.*, 2008). As a last alternative for multi-drug infections, carbapenems are used to treat *Acinetobacter baumannii* associated infections however, carbapenem-resistant strains of the pathogen are being reported at an alarmingly high rate (Poirel and Nordmann 2006; Pezzo *et al.*, 2012). Environmental pollution plays a critical role as an important reservoir in outbreaks of *A. baumannii*, thereby underscoring the need for increased use of antibiotics with higher potency.

Antibiotics as human and animal excretory products (active, metabolites and conjugates) enter into water bodies either directly or after passage through wastewater treatment plants (WWTPs) (Segura *et al.*, 2009). The introduction of antibiotics into the environment causes a selective pressure which results in an increase in the proportion of bacteria that are resistant to antibiotics (Tacão *et al.*, 2014; Igbinsola and Obuekwe 2014; Igbinsola and Oviasogie 2014). Increased resistance to a wide variety of antibiotics has been found in bacteria located in water bodies receiving runoff from agricultural and abattoir environments (Igbinsola *et al.*, 2012; Igbinsola *et al.*, 2013; Igbinsola, 2015) and higher frequencies of resistant bacteria have been found in watercourses receiving waste water from hospitals and sewage from peri-urban and rural communities (Okoh and Igbinsola, 2010; Igbinsola *et al.*, 2011; Odjadjare *et al.*, 2012). In addition to this antibiotics are also released into environments as inappropriately disposed unused drugs and as part of effluent from drug production facilities (Larsson *et al.*, 2007). The processes that lead to the development of antibiotic resistance have probably occurred throughout all of microbial evolutionary descent. Phylogenetic analyses showed that the OXA genes which encode a class of β -lactamases that confers resistance to a broad range of β -lactam antibiotics have existed on plasmids for millions of years (Barlow and Hall 2002). Resistant bacteria, and antibiotic resistance genes that work to inactivate antibiotics, and antibiotic molecules are present in the environment at all times and thus distinguishing naturally occurring resistance in organisms from resistance as a result of environmental pollution (Wright 2010).

3. Dissemination of antibiotic resistant bacteria in the natural environment

The existence of a natural environmental resistance gene pool, which includes all known clinical resistance mechanisms, has been discovered (D'Costa *et al.*, 2006; Aminov 2009). Aquatic systems are main recipient and reservoirs of antibiotics and other compounds from anthropogenic sources (e.g., disinfectants, metals) that accumulate and persist over time. A large fraction of antibiotics reach the environmental milieu in an active form (Baquero *et al.*, 2008; Martinez 2009; Martinez 2009; Taylor *et al.*, 2011; Lupo *et al.*, 2012). However antibiotics are introduced into the environment through various pathways that include effluents from disposal of human waste, waste from agricultural food animal production and aquaculture, direct application to some plants, industrial effluents from pharmaceutical facilities, agricultural run-off and disposal of ethanol production waste products. The discharge of effluents from wastewater treatment plants represents important point sources of contaminants in the environment. Wastewater treatment plants (WWTP) have been described as "hot spots" for antibiotics and for antimicrobial resistance; although some treatment options appear promising for reducing the load of antibiotic residues that could be distributed to the receiving environment (Michael *et al.*, 2013; Rizzo *et al.*, 2013). Treatment options and removal pathways of antibiotic residues include adsorption, biodegradation, disinfection, membrane separation, hydrolysis, photolysis, and volatilization depending on antibiotics properties (Zhang and Li 2011). Tetracyclines are removed mainly by adsorption onto the biomass flocs; beta-lactams are largely degraded by hydrolysis reactions driven by bacteria or physical chemical processes; and erythromycin and ciprofloxacin are recalcitrant toward biodegradation in activated sludge (Li and Zhang 2010).

Biological waste treatment processes rely on complex ecological interactions among the microbial species present in the system reactors. The understanding of these ecological factors is growing and processes to treat wastewater have been shown to influence the contribution of antimicrobial resistance elements and resistant strains of bacteria released into the environment (Novo and Manaia 2010). Microbial communities react with drastic changes in ecosystem functioning, species composition and abundance (Vieira *et al.*, 2008; Nogales *et al.*, 2011). Several consequences may arise from aquatic pollution. The connection between these impacts and potentially pathogenic bacteria is of particular relevance for human wellbeing (Nogales *et al.*, 2011). High amounts of organic matter deposited in water bodies lead to nutrient enrichment. This promotes growth of heterotrophic bacteria, which include various pathogens such as *Shigella* sp., *Salmonella enterica* and *Vibrio cholerae*. These are etiological agents of waterborne diseases, like cholera and diarrhea, which affect millions of people worldwide. These illnesses are most frequent in developing countries, where access to treated water and sanitation is limited (WHO, 2000). Also, pathogenic bacteria originating from human feces are released directly into the environment through wastewater discharges, thereby compromising water quality (Igbiosa *et al.*, 2009; Nogales *et al.*, 2011). Aquatic systems can be highly impacted by human activities, receiving contaminants and pollution from different sources and thereby encouraging the exchange and mixture of genes as genetic platforms.

Globally, there has been growing concern regarding increased presence of antibiotic residues in the aquatic and terrestrial environments. Of particular concern is the potential development of microbial resistance in the presence of antibiotic concentrations and further promotion and spread of antibiotic resistance genes (ARGs) to human pathogens by means of horizontal gene transfer (HGT) facilitated by mobile genetic elements such as plasmids, transposons and integrons. The pathogens carrying ARGs could end up in the food web and subsequently carried to humans and animals thus causing diseases and treatment failure. When antibiotics are present, resistance traits tend to rapidly spread among microbial populations (Zhang *et al.*, 2011). These characteristics of bacterial evolution contribute to a rapid development of multi-resistant pathogenic strains. Consequently, once a new antibiotic is put into use, it does not take long before it is challenged by novel resistant microorganisms (Costa *et al.*, 2006).

4. The spread of resistance among environmental bacteria

Antibiotic resistance is not only found in pathogenic bacteria but also in environmental organisms inhabiting terrestrial and aquatic habitats. Freshwater environments are well-recognized systems for the dissemination and evolution of antibiotic resistance (Baquero *et al.*, 2008; Figueira *et al.*, 2011; Tacão *et al.*, 2012). *Escherichia coli* have been recognized as a major player in the dissemination of antibiotic resistance (Henriques *et al.*, 2006; Zhao and Dang 2012). Resistance genotypes so far characterized include genes for resistance to last-line antibiotics such as third generation cephalosporins and fluoroquinolones (Alouache *et al.*, 2012; Wellington *et al.*, 2013; Tacão *et al.*, 2014). To some extent, similar resistance phenotypes and genotypes are shared by strains exposed to similar environmental pressures (Wicki *et al.*, 2011). Considering this fact, some animal hosts may support the preferential development of certain genotypes. Water bodies are sites of genetic exchange where environmental bacteria interact with microbes originated from humans and other animal sources, through horizontal gene transfer (HGT). Opportunistic pathogens often have large and versatile genomes, prone to sharing genetic materials. This peculiarity helps these organisms to colonize a more diverse set of environments. As consequence, aquatic ecosystems may become a threat to human health when they are affected by pollutants carrying resistant bacteria (Baquero *et al.*, 2008). Genetic transfer can be performed between

pathogenic and environmental bacteria and even between very phylogenetically distant organisms such as species of Gram-positive and Gram negative bacteria (Courvalin 1994; Kruse and Sørum 1994).

The main risk for public health is that resistance genes are transferred from environmental bacteria to human pathogens. The potential for resistant bacteria and resistance genes to move from one ecosystem to another has been demonstrated between animals and humans (Wegener *et al.*, 1999; Kruse 1999). The inclusion of certain growth promoters in animal feed has been recognized as a cause for the selection of resistance genes in the commensal microflora of animals and their transmission to humans by means of the food chain (Wegener *et al.*, 1999; Kruse 1999). There is increasing evidence to show that repeated exposure of the environment to anthropogenically generated ARGs (for example, in soil manure) correlated with the emergence and proliferation of ARGs in indigenous microbiota (Jechalke *et al.*, 2013; Udikovic-Kolic *et al.*, 2014; Jechalke, *et al.*, 2014). The impact of animal production on the propagation of antibiotic resistance was also demonstrated by some zoonotic species of the genera *Salmonella*, *Campylobacter*, *Listeria*, *Staphylococcus*, *Enterococcus* and *Escherichia*, which are known to exhibit high levels of acquired antibiotic resistance (de Jong *et al.*, 2013; Garcia-Migura *et al.*, 2014; Brooks *et al.*, 2014). Although some animal production facilities implement systems that decontaminate liquid and solid wastes, such treatments are not intended to remove ARB and might even promote resistance (Moura *et al.*, 2007; Pei *et al.*, 2007).

5. Impact of antibiotics resistance determinant on human and environmental health

Wastewater discharges from domestic sources affect the diversity of resistant bacteria (Czekalski *et al.*, 2012; Thevenon *et al.*, 2012; Vignesh *et al.*, 2012). These impacts also shape the genetic pool of water bodies by increasing abundance of antibiotic resistance genes within the habitats (Tacão *et al.*, 2012). Thus, hospital effluents have been shown to be rich in resistance genes (Schwartz *et al.*, 2003) and resistant bacteria (Santoro *et al.*, 2012). Contamination in aquatic environments contributes to the spread of human pathogens along with the dissemination of antibiotic-resistant bacteria. The overexposure to antibiotics is leading to increasing levels of resistance in the human (and other animals) commensal microbiota (Austin *et al.*, 1999). The resistomes of fecal bacteria, once in environmental locale, contribute antibiotic resistance genes to non-resistant indigenous microorganisms (Aminov 2011; Tacão *et al.*, 2014). In aquatic systems the cycle may include subsequent transmission of antibiotic resistant to human-associated bacteria (Devirgiliis *et al.*, 2011; Figueira *et al.*, 2011). At the same time, enrichment in antibiotic resistant bacteria is promoted by the presence of antimicrobials or other contaminants in the environment. Bacterial communities in aquatic systems comprise antibiotic producers and bacteria intrinsically resistant to several antibiotics. These two groups are natural carriers of genetic determinants of resistance. Taking these factors together, aquatic environments can be seen as reactors system where drug resistance characteristics spread and recombine. Under such conditions, multidrug resistance features may emerge and transmission to pathogenic bacteria is facilitated (Taylor *et al.*, 2011; Lupo *et al.*, 2012). Several studies have shown that in natural systems, pollution promotes antibiotic resistance spread, for which mobile genetic elements have an important contribution. (Taylor *et al.*, 2011; Tacão *et al.*, 2012; Tacão *et al.*, 2014).

Environmental and pathogenic bacteria harbor antibiotic resistance genes; the regulations of these genetic units differ with the source. Pathogens usually carry these genes on mobile genetic elements and express them constitutively. Meanwhile, antibiotic resistance in environmental bacteria is usually chromosomally encoded and expression is initiated following exposure to antibiotics (Wright 2007). Antibiotic resistance can reach the environment with the potential of adversely affecting aquatic and terrestrial organisms which eventually might reach humans through drinking water and the food chain (Prior, 2008; Aarestrup *et al.*, 2008). The emergence of resistance is a highly complex process which is not yet fully understood with respect to the significance of the interaction of bacterial populations and antibiotics, even in a medicinal environment (Martinez and Baquero 2000; Alanis 2005). The transfer of resistant bacteria to humans could occur via water or food if plants are watered with surface water or sewage sludge, if manure is used as a fertilizer, or if resistant bacteria are present in meat (Dolliver and Gupta 2008). The significance of the transfer of antibiotic resistance from animals to humans is not clearly understood. However, to minimize this route and the unwanted intake of antibiotics, the antibiotic content of fishery products is monitored by authorities in many countries (FAAIR, 2002, WHO, 2003). World Health Organization estimated that globally 2.6 billion people lack access to basic sanitation, which likely results in direct releases of ARBs and pathogens into the environment and ambient waters (WHO 2012b). The detection of the New Delhi metallo-beta-lactamase-1-gene (*NDM-1* gene) in polluted surface waters and chlorinated tap water in India has important implications for people living in the city who are reliant on public water and sanitation facilities (Walsh *et al.*, 2011). *NDM-1* provides bacteria with resistance to a large number of antibiotics; it is highly mobile and is found in multiple waterborne pathogens, including *Vibrio cholerae* and *E. coli* (Walsh *et al.*, 2011; Kumarasamy *et al.*, 2010).

6. Future outlook

Antibiotics resistance genes are recognized as important environmental contaminants (Kümmerer 2004). Resistance can spread as a result of distribution of resistant bacterial strains or genetic elements of resistance throughout the receiving

environment; evolution and selection of new resistant strains; or the amplification of pre-existing resistant strains of bacteria. The introduction of antibiotics as environmental contaminants has important influence on processes that alter abundance of resistance genes in the environment in multiple ways. As a result, these systems may promote the spread of ARB and ARGs and even the emergence of novel resistance mechanisms and pathogens (Baquero *et al.*, 2008; Zhang *et al.*, 2009).

The recurrent detection and dissemination of ARGs and ARB in the environment constitutes a serious public health problem and suggests that ARGs should now be considered emerging environmental contaminants (Pruden *et al.*, 2006; Martinez 2009). Such consideration would ensure the need to constantly monitor the usage and possible routes of dissemination of antibiotics with a view to reducing associated environmental risks as much as possible. Emissions from sewage treatment plants (Karthikeyan and Meyer 2006; Lindberg *et al.*, 2007), hospitals (Lindberg *et al.*, 2004), drug production facilities (Larsson *et al.*, 2007; Fick *et al.*, 2009) and the food industry (Davis *et al.*, 2011) are obvious starting points.

The pool of extracellular DNA remains vastly uncharacterized, and some studies suggest that it is more extensive than previously thought (Corinaldesi *et al.*, 2005; Wu *et al.*, 2009). Other studies have specifically demonstrated the capability of extracellular ARGs to persist under certain environmental conditions and maintain its integrity for host uptake (Cai *et al.*, 2007). While focusing attention on individual resistant strains of bacteria has merit in some instances, this approach is also greatly limited by the unculturability of the vast majority of environmental microbes. As we have now entered the period of metagenomics the tools to tackle the complexity of resistance elements in the environment and precisely define the human influence is gradually becoming more accessible. Distribution of ARGs may also be considered in parallel with key genetic elements driving their horizontal gene transfer, such as plasmids, transposons, and integrons. However, environmentally-relevant concentrations driving selection of resistant strains are largely unknown. Indeed, some studies have noted correlations between antibiotics and ARGs in environmental matrices while others have noted an absence of such a correlation. In either case, it appears that ARGs persist and are transported further along pathways than antibiotics; suggesting distinct factors governing their transport (Peak *et al.*, 2007; McKinney *et al.*, 2010). Nevertheless, a reasonable approach for mitigating risk seems to be focusing attention on developing appropriate technologies for eliminating both antibiotics and genetic materials from waste watercourse.

The persistence of resistance genes in bacterial genomes is alarming (Andersson and Hughes 2011), as such it means that removal of selection pressures may have less effect on resistance gene abundance than anticipated. However, the potential control measure and intervention is to:

1. Minimize the selection pressure for resistance genes in the clinical setting.
2. Immediately reduce environmental release of antibiotics, both from manufacturing and use. This primarily has to be done using better treatment technologies.
3. Find the routes that enable environmental bacteria to disseminate resistance genes to clinically relevant species and close them.
4. Develop antibiotics exploiting new mechanisms to eliminate bacteria.

Finally, it is important to acquire a more comprehensive understanding of the molecular, evolutionary and ecological mechanisms associated with the acquisition and spread of antibiotic resistance. In parallel, the implementation of effective management options, mainly seeking to impose barriers against the dissemination of resistance from well-established resistance reservoirs, is urgently needed to combat the evolution and spread of antibiotic resistance, while protecting human health and the environment.

7. Conclusion

The indiscriminate use of antibiotics to treat diseases in humans, as well as environmental exposure to antibiotics and ARGs via agricultural and animal production, wastewater treatment, pharmaceuticals, hospital environment and aquaculture are important points of dissemination that contributes to the spread and perpetuity of antibiotics resistance determinants in the environment. The incidence of multidrug-resistant pathogenic bacteria is on the rise. More than 70% of the bacterial species that cause infections are likely to be resistant to at least one commonly used antibiotic. This trend will continue as long as the resistance problem remains unchecked. Although antibiotic resistance is clearly a global challenge, local action is necessary to reduce its spread via environmental exposure. Given the public and environmental health threat posed by antibiotics resistance, the development and implementation of national and international guidelines for the biological risk assessment of the emergence and propagation of ARB in the environment is a strategic priority. The generation of reliable comparisons and evaluation of temporal trends in antibiotic resistance in the environment are currently seriously limited owing to the disparity of surveillance strategies.

Acknowledgments The authors thank the "The World Academy of Science" (TWAS), Italy (Grant No. 14-091 RG/BIO/AF/AC_1-UNESCOFR: 324028575) for providing financial support for this research.

References

- [1] Aarestrup FM, Wegener HC, Collignon, P. 2008. Resistance in bacteria of the food chain: epidemiology and control strategies. *Expert Review of Anti-Infective Therapy* 6: 733-750.
- [2] Alanis AJ. 2005. Resistance to antibiotics: Are We in the Post-Antibiotic Era? *Arch Med Res* 36:697-705.
- [3] Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. 2010. Call of the wild: antibiotic resistance genes in natural environments. *Nature Rev. Microbiol.* 8: 251-259.
- [4] Alouache S, Kada M, Messai Y, Estepa V, Torres C, Bakour R. 2012. Antibiotic resistance and extended-spectrum beta-lactamases in isolated bacteria from seawater of Algiers beaches (Algeria). *Microb. Environ.* 27, 80-86.
- [5] Aminov RI. 2009. The role of antibiotics and antibiotic resistance in nature. *Environ Microbiol* 11: 2970-2988.
- [6] Aminov RI. 2011. Horizontal gene exchange in environmental microbiota. *Front. Microbiol.* 2:158. doi: 10.3389/fmicb.2011.00158
- [7] Andersson DI, Hughes D. 2011. Persistence of antibiotic resistance in bacterial populations. *FEMS Microbiology Reviews*, 35:901-911.
- [8] Araújo C, Torres C, Silva N, Carneiro C, Gonçalves A, Radhouani H, Correia S, da Costa PM, Paccheco R, Zarazaga M, Ruiz-Larrea F, Poeta P, Igrejas G. 2010. Vancomycin-resistant enterococci from Portuguese wastewater treatment plants. *J Basic Microbiol* 50:605-609.
- [9] Austin DJ, Kristinsson KG, Anderson RM. 1999. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. *Proc. Natl. Acad. Sci. U.S.A.* 96, 1152-1156.
- [10] Baquero F, Martínez JL, Cantón, R. 2008. Antibiotic and antibiotic resistance in water environments. *Current Opinion in Biotechnology* 19: 260-265.
- [11] Barlow M, Hall BG. 2002. Phylogenetic analysis shows that the OXA beta-lactamase genes have been on plasmids for millions of years. *J Mol Evol* 5:314-321.
- [12] Björklund K, Rendahl P, Johansson MI, Tysklind M, Andersson BA. 2007. Environmental risk assessment of antibiotics in the Swedish environment with emphasis on sewage treatment plants. *Water research*, 41:613-619.
- [13] Börjesson S, Matussek A, Melin S, Löfgren S, Lindgren PE. 2010. Methicillin-resistant *Staphylococcus aureus* (MRSA) in municipal wastewater: An uncharted threat? *J Appl Microbiol* 108:1244-1251.
- [14] Brooks JP, Adeli A, McLaughlin MR. 2014. Microbial ecology, bacterial pathogens, and antibiotic resistant genes in swine manure wastewater as influenced by three swine management systems. *Water Res* 57: 96-103.
- [15] Bush K, Courvalin P, Dantas G, Davies J, Eisenstein B, Huovinen P, Jacoby GA, Kishony R, Kreiswirth BN, Kutter E, Lerner SA, Levy S, Lewis K, Lomovskaya O, Miller JH, Mobashery S, Piddock LJ, Projan S, Thomas CM, Tomasz A, Tulkens PM, Walsh TR, Watson JD, Witkowski J, Witte W, Wright G, Yeh P, Zgurskaya HI. 2011. Tackling antibiotic resistance. *Nature Rev. Microbiol.* 9, 894-896.
- [16] Cantón R. 2009. Antibiotic resistance genes from the environment: a perspective through newly identified antibiotic resistance mechanisms in the clinical setting. *Clin. Microbiol. Infect.* 15(Suppl. 1), 20-25.
- [17] Chee-Sanford JC, Aminov RI, Krapac IJ, Garrigues-JeanJean N, Mackie RI. 2001. Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. *Appl Environ Microbiol* 67:1494-1502.
- [18] Chee-Sanford JC, Mackie RI, Koike S, Krapac IG, Lin YF, Yannarell AC, Maxwell S, Aminov RI. 2009. Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J Environ Qual* 38:1086-1108.
- [19] Costa D, McGrann VM, Hughes KM, Wright DW. 2006. Sampling the antibiotic resistome. *Science* 311: 374-377.
- [20] Courvalin, P. 1994. Transfer of antibiotic resistance genes between Gram-positive and Gram-negative bacteria. *Antimicrobial Agents and Chemotherapy* 38:1447-1451.
- [21] Czekalski N, Berthold T, Caucci S, Egli A, Bürgmann H. 2012. Increased levels of multiresistant bacteria and resistance genes after wastewater treatment and their dissemination into Lake Geneva, Switzerland. *Frontiers in Microbiology* doi:fmicb.2012.00106.
- [22] Davis MF, Price LB, Liu CM, Silbergeld EK. 2011. An ecological perspective on U.S. industrial poultry production: the role of anthropogenic ecosystems on the emergence of drug-resistant bacteria from agricultural environments. *Current Opinion in Microbiology*, 14:244-250.
- [23] D'Costa VM, McGrann KM, Hughes DW, Wright GD. 2006. Sampling the antibiotic resistome. *Science* 311: 374-377.
- [24] D'Costa VM, King CE, Kalan L, Morar M, Sung WW, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD. 2011. Antibiotic resistance is ancient. *Nature* 477:457-461.
- [25] de Jong A, Thomas V, Klein U, Marion H, Moyaert H, Simjee S, Vallé M. 2013. Pan-European resistance monitoring programmes encompassing food-borne bacteria and target pathogens of food-producing and companion animals. *Int. J. Antimicrob. Agents* 41:403-409 (2013).
- [26] Devirgiliis C, Barile S, Perozzi G. 2011. Antibiotic resistance determinants in the interplay between food and gut microbiota. *Genes Nutr.* 6, 275-284. doi:10.1007/s12263-011-0226-x
- [27] Dolliver HA, Gupta SC. 2008. Antibiotic losses from unprotected manure stockpiles. *J Environl Quality* 37:1238-1244.
- [28] Eliopoulos GM, Maragakis LL, Perl TM. 2008. *Acinetobacter baumannii*: Epidemiology, antimicrobial resistance, and treatment options. *Clin Infect Dis* 46: 1254-1263.
- [29] FAAIR. 2002. Policy recommendations. *Clin Infect Dis* 34 Suppl 3:76-87.
- [30] Fick J, Söderström H, Lindberg RH, Tysklind M, Joakim Larsson DG. 2009. Contamination of surface, ground, and drinking water from pharmaceutical production. *Environmental toxicology and chemistry / SETAC*, 28:2522-2527.
- [31] Figueira V., Serra E, Manaia CM. 2011. Differential patterns of antimicrobial resistance in population subsets of *Escherichia coli* isolated from waste- and surface waters. *Sci. Total Environ.* 409, 1017-1023.
- [32] File TM. Jr. 2006. Clinical implications and treatment of multiresistant *Streptococcus pneumoniae* pneumonia. *Clin. Microbiol. Infect.* 12: 31-41.

- [33] Fuentefria DB, Ferreira AE, Corção G. 2011. Antibiotic-resistant *Pseudomonas aeruginosa* from hospital wastewater and superficial water: Are they genetically related? *J. Environ. Manag.* 92:250-255.
- [34] Garcia-Migura L, Hendriksen RS, Fraile L, Aarestrup FM. 2014. Antimicrobial resistance of zoonotic and commensal bacteria in Europe: the missing link between consumption and resistance in veterinary medicine. *Vet Microbiol* 170: 1-9.
- [35] Gay K, Baughman W, Miller Y, Jackson D, Whitney CG, Schuchat A, Farley MM, Tenover F, Stephens DS. 2000. The emergence of *Streptococcus pneumoniae* resistant to macrolide antimicrobial agents: a 6-year population-based assessment. *J Infect Dis.*182:1417-1424.
- [36] Henriques I, Fonseca F, Alves A, Saavedra MJ, Correia A. 2006. Occurrence and diversity of integrons and beta-lactamase genes among ampicillin-resistant isolates from estuarine waters. *Res. Microbiol.* 157, 938-947.
- [37] Heuer H, Schmitt H, Smalla K. 2011. Antibiotic resistance gene spread due to manure application on agricultural fields. *Curr Opin Microbiol* 14:236-243.
- [38] Igbinsola EO and Obuekwe IS (2014). Evaluation of antibiotic resistant gene in abattoir environment: A concern to public health. *Journal of Applied Sciences & Environmental Management* 18:165-171.
- [39] Igbinsola EO and Oviasogie FE (2014). Multiple antibiotics resistant among environmental isolates of *Stenotrophomonas maltophilia*. *Journal of Applied Sciences and Environmental Management* 18: 255-261.
- [40] Igbinsola EO, Obi LC, Tom M and Okoh AI (2011) Detection of potential risk of wastewater effluents for transmission of antibiotic resistance from *Vibrio* species as a reservoir in a peri-urban community in South Africa. *International Journal of Environmental Health Research* 2011, 1-13 doi: 10.1080/09603123.2011.572278
- [41] Igbinsola EO, Odadjare EE, Igbinsola IH, Orhue PO, Omoigberale MMO, Amhanre NI. 2012. Antibiotic synergy interaction against multidrug resistant *Pseudomonas aeruginosa* isolated from an abattoir effluent environment. *The Scientific World Journal* Article ID 308034, 5 pages. doi:10.1100/2012/308034
- [42] Igbinsola IH, Igbinsola EO and Okoh AI. 2014. Molecular detection of metallo- β -lactamase and putative virulence genes in environmental isolates of *Pseudomonas* species. *Polish Journal of Environmental Science* 23:415-421.
- [43] Igbinsola IH, Nwodo UU, Sosa A, Tom M, Okoh AI. 2012. Commensal *Pseudomonas* species isolated from wastewater and freshwater milieus in the Eastern Cape Province, South Africa as reservoir of antibiotic resistant determinants. *Int J Environ Res Public Health* 9:2537-2549.
- [44] Igbinsola IH, Okoh AI. 2012. Antibiotic susceptibility profile of *Aeromonas* species isolated from wastewater treatment plant. *The Scientific World Journal*, vol. 2012, Article ID 764563, 6 pages, 2012. doi:10.1100/2012/764563
- [45] Igbinsola IH, Okoh AI. 2013. Detection and distribution of putative virulence associated genes in *Aeromonas* species from freshwater and wastewater treatment plant. *Journal of Basic Microbiology* 52, 1-7. doi: 10.1002/jobm.201200351
- [46] Igbinsola IH. 2014. Antibigram profiling and pathogenic status of *Aeromonas* species recovered from Chicken, *Saudi Journal of Biological Sciences*. 21: 481-485.
- [47] Igbinsola IH. 2015. Prevalence and detection of antibiotic resistant determinant in *Salmonella* isolated from food producing animals. *Tropical Animal Health and Production* 47:37-43.
- [48] Iweriebor BC, Gaqavu S, Obi LC, Nwodo UU and Okoh AI. 2015. Antibiotic susceptibilities of *Enterococcus* Species Isolated from hospital and domestic wastewater effluents in Alice, Eastern Cape Province of South Africa. *Int J Environ Res Public Health* 12:4231-4246.
- [49] Jechalke S, Heuer H, Siemens J, Amelung W, Smalla K. 2014. Fate and effects of veterinary antibiotics in soil. *Trends Microbiol.* 22, 536-545.
- [50] Jechalke S, Kopmann C, Rosendahl I, Groeneweg J, Weichelt V, Krögerrecklenfort E, Brandes N, Nordwig M, Ding GC, Siemens J, Heuer H, Smalla K. 2013. Increased abundance and transferability of resistance genes after field application of manure from sulfadiazine-treated pigs. *Appl. Environ. Microbiol.* 79:1704-1711.
- [51] Karthikeyan KG, Meyer MT. 2006. Occurrence of antibiotics in wastewater treatment facilities in Wisconsin, USA. *The Science of the total environment*, 361:196-207.
- [52] Kruse H, Sørum H. 1994. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. *Applied and Environmental Microbiology* 60: 4015-4021.
- [53] Kruse, H. 1999. Indirect transfer of antibiotic resistance genes to man. *Acta Vet. Scand.* 92: 59-65.
- [54] Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. *Lancet Infect Dis* 10:597-602.
- [55] Kümmerer K. 2004. Resistance in the environment. *J Antimicrob Chemother* 54: 311-320.
- [56] Lambiase A, Piazza O, Rossano F, Del Pezzo M, Tufano R, Catania MR. 2012. Persistence of carbapenem-resistant *Acinetobacter baumannii* strains in an Italian intensive care unit during a forty-six month study period. *New Microbiol* 35: 199-206.
- [57] Larsson DGJ, de Pedro C, Paxeus N. 2007. Effluent from drug manufactures contains extremely high levels of pharmaceuticals. *Journal of hazardous materials*, 148:751-755.
- [58] Levy SB, Marshall B. 2004 Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med*, 10(12 Suppl):S122-9.
- [59] Li B, Zhang T. 2010. Biodegradation and adsorption of antibiotics in the activated sludge process. *Environ Sci Technol* 44:3468-3473.
- [60] Lindberg R, Jarnheimer PA, Olsen B, Johansson M, Tysklind M. 2004. Determination of antibiotic substances in hospital sewage water using solid phase extraction and liquid chromatography/mass spectrometry and group analogue internal standards. *Chemosphere*, 57:1479-1488.
- [61] Lupo A, Coyne S, Berendonk TU. 2012. Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in water bodies. *Front. Microbiol.* 3:18. doi: 458 10.3389/fmicb.2012.00018
- [62] Martinez JL, Baquero F. 2000. Mutation frequencies and antibiotic resistance. *Antimicrob Agents Chemother* 44: 1771-1777.

- [63] Martinez JL. 2009a. Environmental pollution by antibiotics and by antibiotic resistance determinants. *Environ. Pollut.* 157:2893-2902.
- [64] Martinez JL. 2009b. The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proc Biol Sci* 276:2521-2530.
- [65] McKinney CW, Loftin KA, Meyer MT, Davis JG, Pruden A. 2010. tet and sul antibiotic resistance genes in livestock lagoons of various operation type, configuration, and antibiotic occurrence. *Environ. Sci. Technol.* 44: 6102-6109.
- [66] Michael I, Rizzo L, McArdell CS, Manaia CM, Merlin C, Schwartz T, Dagot C, Fatt-Kassino D. 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Res.* 47: 957-995.
- [67] Moura A, Henriques I, Ribeiro R, Correia A. 2007. Prevalence and characterization of integrons from bacteria isolated from a slaughterhouse wastewater treatment plant. *J. Antimicrob. Chemother.* 60: 1243-1250.
- [68] Nogales B, Lanfranconi MP, Piña-villalonga JM, Bosch R. 2011. Anthropogenic perturbations in marine microbial communities. *FEMS Microbiology Reviews* 35: 275-298.
- [69] Novo A, Manaia CM. 2010. Factors influencing antibiotic resistance burden in municipal wastewater treatment plants. *Appl Microbiol Biotechnol* 87: 1157-1166.
- [70] Odjadjare EE, Igbinsola EO, Mordi R, Igere B, Igeleke CL, Okoh AI. 2012. Prevalence of multiple antibiotic resistant (MAR) *Pseudomonas* species in the final effluents of three municipal wastewater treatment facilities in South Africa. *Int J Environ Res Public Health* 9:2092-2107.
- [71] Okoh AI, Igbinsola EO. 2010. Antibiotic susceptibility profiles of some *Vibrio* strains isolated from wastewater final effluents in a rural community of the Eastern Cape Province of South Africa. *BMC Microbiology* 10:143. doi:10.1186/1471-2180-10-143
- [72] Peak N, Knapp CW, Yang RK, Hanfelt MM, Smith MS, Aga DS, Graham DW. 2007. Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. *Environ. Microbiol.* 9: 143-151.
- [73] Pei R, Cha J, Carlson KH, Pruden A. 2007. Response of antibiotic resistance genes (ARG) to biological treatment in dairy lagoon water. *Environ. Sci. Technol.* 41:5108-5113.
- [74] Poirel L, Nordmann P. 2006. Carbapenem resistance in *Acinetobacter baumannii*: Mechanisms and epidemiology. *Clin Microbiol Infect* 12: 826-836.
- [75] Prior LSOS. 2008. The 2008 Garrod Lecture: Antimicrobial resistance-animals and the environment. *J Antimicrob Chemother* 62:229-233
- [76] Pruden A, Pei R, Storteboom H, Carlson KH. 2006. Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. *Environ. Sci. Technol.* 40:7445-7450.
- [77] Pruden A, Larsson DGJ, Amézquita A, Collignon P, Brandt KK, Graham DW, Lazorchak JM, Suzuki S, Silley P, Snape JR, Topp E, Zhang T, Zhu Y-G. 2013. Management options for reducing the release of antibiotics and antibiotic resistance genes to the environment. *Environ Health Perspect* 121:878-885.
- [78] Rizzo L, Manaia CM, Merlin C, Schwartz T, Dagot C, Ploy MC, Michael I, Fatta-Kassinou D. 2013. Urban wastewater treatment plants as hotspots for antibiotic resistance spreading into the environment. *Sci Total Environ* 447: 345-360.
- [79] Robinson DA, Kearns AM, Holmes A, Morrison D, Grundmann H, Edwards G, O'Brien FG, Tenover FC, McDougal LK, Monk AB, Enright MC. 2005. Re-emergence of early pandemic *Staphylococcus aureus* as a community-acquired methicillin-resistant clone. *Lancet* 365, 1256-1258.
- [80] Santoro DO, Romão CMCA, Clementino MM. 2012. Decreased aztreonam susceptibility among *Pseudomonas aeruginosa* isolates from hospital effluent treatment system and clinical samples. *International Journal of Environmental Health Research* 22:560-570
- [81] Schwartz T, Kohnen W, Jansen B, Obst U. 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water and drinking water biofilms. *FEMS Microbiology Ecology* 43:325-335.
- [82] Segura, PA., François M, Gagnon C, Sauvé S. 2009. Review of the occurrence of anti-infectives in contaminated wastewaters and natural and drinking waters. *Environ Health Perspect.* 117(5):675-84. doi: 10.1289/ehp.11776.
- [83] Tacão M, Correia A, Henriques I. 2012. Resistance to broad-spectrum antibiotics in aquatic systems: anthropogenic activities modulate the dissemination of *bla*CTX-M-like genes. *Appl. Environ. Microbiol.* 78, 4134-4140.
- [84] Tacão M, Moura A, Correia A, Henriques I. 2014. Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. *Water Res.* 48, 100-107. doi: 10.1016/j.watres.2013.09.021
- [85] Taylor NG, Verner-Jeffreys DW, Baker-Austin C. 2011. Aquatic systems: maintaining, mixing and mobilising antimicrobial resistance? *Trends Ecol. Evol.* 26:278-284.
- [86] Thevenon F, Adatte T, Wildi W, Poté J. 2012. Antibiotic resistant bacteria/genes dissemination in lacustrine sediments highly increased following cultural eutrophication of Lake Geneva (Switzerland). *Chemosphere* 86: 468-476.
- [87] Udikovic-Kolic N, Wichmann F, Broderick NA, Handelsman J. 2014. Bloom of resident antibiotic-resistant bacteria in soil following manure fertilization. *Proc. Natl Acad. Sci. USA* 111:15202-15207.
- [88] Vieira RP, Gonzalez AM, Cardoso AM, Oliveira DN, Albano RM, Clementino MM, Martins OB, Paranhos R. 2008. Relationships between bacterial diversity and environmental variables in a tropical marine environment, Rio de Janeiro. *Environmental Microbiology* 10:189-199.
- [89] Vignesh S, Muthukumar K. Arthur James R. 2012. Antibiotic resistant pathogens versus human impacts: A study from three eco-regions of the Chennai coast, southern India. *Marine Pollution Bulletin* 64: 790-800.
- [90] Walsh TR, Weeks J, Livermore DM, Toleman MA. 2011. Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. *Lancet Infect Dis* 11:355-362.
- [91] Wegener H, Aarestrup F, Gerner-Smidt P, Bager F. 1999. Transfer of resistant bacteria from animals to man. *Acta Vet. Scand.* 92: 51-58.
- [92] Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM, Williams AP. 2013. The role of natural environment in the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect. Dis.* 13, 155-165.

- [93] WHO (World Health Organization). 2012a. Antimicrobial Resistance.
- [94] Available: <http://www.who.int/mediacentre/factsheets/fs194/en/> [accessed 22 April 2015].
- [95] WHO (World Health Organization). 2012b. Health through Safe Drinking Water and Basic Sanitation. Available: http://www.who.int/water_sanitation_health/mdg1/en/index.html [accessed 31 May 2015].
- [96] Wicki M, Karabulut F, Auckenthaler A, Felleisen R, Tanner M, Baumgartner A. 2011. Identification of faecal input sites in spring water by selection and genotyping of multiresistant *Escherichia coli*. *Appl. Environ. Microbiol.* 77, 8427-8433.
- [97] World Health Organization (WHO). 2000. Antimicrobial resistance: a global threat. Essential Drugs Monitor. World Health Organization: Geneva, Switzerland. <http://apps.who.int/medicinedocs/pdf/s2248e/s2248e.pdf> [Accessed 29 June 2015]
- [98] World Health Organization 2003. Dept. of Communicable Disease Prevention Control and Eradication., Danish Veterinary Institute., and Danmarks jordbrugs forskning, Impacts of antimicrobial growth promoter termination in Denmark : the WHO international review panel' s evaluation of the termination of the use of antimicrobial growth promoters in Denmark : Foulum, Denmark 6-9, Geneva: World Health Organization. 57 p.
- [99] Wright GD. 2007. The antibiotic resistome: the nexus of chemical genetic diversity. *Nature Reviews Microbiology* 5: 175-186.
- [100] Wright GD. 2010. Antibiotic resistance in the environment: a link to the clinic? *Curr Opin Microbiol* 13:589-594.
- [101] Zhang Q, Lambert G, Liao D, Kim H, Robin K, Tung CK, Pourmand N, Austin RH. 2011. Acceleration of emergence of bacterial antibiotic resistance in connected microenvironments. *Science* 333, 1764-1767.
- [102] Zhang T, Li B. 2011. Occurrence, transformation, and fate of antibiotics in municipal wastewater treatment plants. *Crit Rev Environ Sci Technol* 41:951-998.
- [103] Zhang XX, Zhang T, Fang HH. 2009. Antibiotic resistance genes in water environment. *Appl. Microbiol. Biotechnol.* 82:397-414.
- [104] Zhao JY, Dang H. 2012. Coastal seawater bacteria harbor a large reservoir of plasmid mediated quinolone resistance determinants in Jiaozhou Bay, China. *Microb. Ecol.* 64, 187-199.
- [105] Zoutman DE, Ford BD. 2005. The relationship between hospital infection surveillance and control activities and antibiotic-resistant pathogen rates. *Am J Infect Control.* 33:1-5.