

## Horizontal Gene Transfer as a Global Mediator of Antimicrobial Resistance

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### Abstract

Antimicrobial resistance in pathogenic bacteria has become a serious public health concern as infections with multi-drug resistant bacteria are becoming increasingly difficult to treat. In a recent publication by Hatosy and Martiny, the diversity of antimicrobial resistance genes in marine systems was explored using functional metagenomics. Work from these authors, as well as other studies of antimicrobial resistance in non-clinical environments, emphasize the need for a One Health approach to address problems associated with antimicrobial use and resistance.

**Keywords:** Antimicrobial resistance, Metagenomics, One health, Pathogenic multi-drug resistant bacteria

Antimicrobial resistance is an increasingly difficult obstacle to overcome in treating multi-drug resistant bacterial infections and is now a serious public health concern. The World Health Organization recently issued a report stating that a post-antibiotic era, where common infections are lethal and untreatable, is a likely possibility in the twenty-first century (WHO | Antimicrobial resistance: global report on surveillance 2014). Though antimicrobial resistance has traditionally been studied within a clinical context, it is increasingly clear that the global impact of antimicrobial use and resistance can only fully be appreciated through the study of non-clinical settings as well. Due to widespread use, antimicrobial compounds often unintentionally end up in soil, water, and our food supply. The native microbial communities in these environments are then altered by selecting for genes that confer resistance to these antimicrobials. Consequently, antimicrobial resistance genes are retained and spread among microbes in these environments.

Although antimicrobial resistance in soil and animals have been well studied, the same is not true for marine environments. The ocean serves

as a unique microbial reservoir as there is presumably little selection for resistance against sources of antimicrobials (such as those generated from humans or naturally produced from marine bacteria), as such compounds are rapidly diluted as they diffuse away from the source. Notably, the ocean covers 70% of the Earth's surface area and is home to approximately  $10^{29}$  bacterial cells. Due to its vastness, it is an ecosystem greatly impacted by anthropogenic influences (ex: coastal or river runoff) and by the potential for horizontal gene transfer among microorganisms. Conversely, any changes in marine ecosystems will in turn have significant impact on terrestrial systems and human health.

In a recent publication in *Applied and Environmental Microbiology*, Hatosy and Martiny presented three possible mechanisms contributing to the occurrence of antimicrobial resistance in marine environments: (1) Coastal runoff of antimicrobial resistant bacteria from terrestrial sources, (2) anthropogenic antibiotic runoff, and (3) selection for resistance in response to antimicrobial production from native marine species (Hatosy and Martiny, 2015). The

authors then characterized the distribution of antimicrobial resistance genes in the ocean using functional metagenomics (Hatosy and Martiny, 2015). Functional metagenomics is a method in which DNA is collected from the ecosystem of interest, then cloned and screened for a function of interest (reviewed in (Mullany, 2014)). Seawater was collected at five sample sites representing a diverse set of environments with different coastal proximities. DNA fragments from the sample sites were cloned into antibiotic-sensitive *E. coli* and the clone libraries were screened for antimicrobial activity against four antibiotics: ampicillin, tetracycline, sulfadimethoxine, and nitrofurantoin. Based upon the frequency of resistant clones in the clone library, the frequency of antimicrobial resistance genes against these antimicrobials in marine bacteria is approximated to reach up to 0.9% of marine bacteria. Of the genes that contributed to the antibiotic resistance phenotype in their experiments, 28% of the genes were previously known antimicrobial resistance genes (identified against the Antibiotic Resistance Genes Database ARDB and GenBank). Interestingly, the remaining 72% of genes that contributed to the antibiotic resistance phenotype were not previously classified as antimicrobial resistance genes.

Therefore, in addition to the three mechanisms of marine antimicrobial resistance originally posited, the authors introduce a fourth mechanism based on their findings: proteins mediating other physiological processes confer cross-resistance to antimicrobials. Their data support this hypothesis because many genes that conferred resistance to the antimicrobials tested were believed to have primary functions associated with other pathways that can potentially be co-opted for antimicrobial resistance (ex: transporters, oxidoreductases and

hydrolases). Notably, the antibiotics used for selection in their experiments all differed in their mechanism of action and two of these, sulfadimethoxine and nitrofurantoin, are synthetic compounds and are not produced by microorganisms. Therefore, they are unlikely to be encountered in the open-ocean sample sites from this study, supporting a pre-existing, fortuitous, cross-resistance to antimicrobials conferred by these genes rather than an evolutionary driver for resistance.

One caveat of functional metagenomics studies is that the DNA fragments must be assayed their non-native bacteria. This is particularly necessary as the majority of microorganisms are unculturable and environmental samples possess a vast diversity of microorganisms that makes it impossible to pursue such a study in the native bacterium. The DNA sequences were placed into an *E. coli* cloning strain and therefore the DNA sequences were expressed in a different genetic background. Though this method only identifies genes that are capable of being expressed and functional in *E. coli*, it also demonstrates the powerful contribution of horizontal gene transfer to antimicrobial resistance. Furthermore, the authors created a genome clone library from DNA fragments from an antibiotic-sensitive *Synechococcus* strain in antibiotic-sensitive *E. coli* and found numerous genes that conferred resistance to the antibiotic in the *E. coli* clone library. This illustrates that horizontal gene transfer between bacteria may produce a bacterium that is resistant to an antibiotic due to the unique combination of genes. Therefore, the antimicrobial resistance capabilities of an organism may easily be amplified by the acquisition of genes through horizontal gene transfer. Likewise, commensal bacteria may acquire antimicrobial resistance genes that are then passed on to pathogenic bacteria.

In addition to the acquisition of specific genes that render an organism resistant to antimicrobials, another form of horizontal gene transfer remains more dire: the transfer of multi-drug resistant plasmids from one organism to another. Plasmid mediated multi-drug resistant *E. coli* was detected in 5.4% of wild bird and mammals sampled in Ireland (Carroll *et al.*, 2015). Based on a comprehensive literature review, out of 1415 species of human pathogenic organisms (including viruses, prions, bacteria, rickettsia, fungi, protozoa and helminthes), 61% are zoonotic (Taylor *et al.*, 2001). The large number of microorganisms that can reside in and be transmitted from animals to humans make the prevalence of antimicrobial resistance genes in the microbial communities residing in animals alarming. Moreover, antibiotics are routinely used among farm animals to promote growth and prevent infections and statistical models estimate that the global average annual antimicrobial consumption is 45 mg/kg, 148 mg/kg and 172 mg/kg of cattle, chicken and pigs, respectively (Van Boeckel *et al.*, 2015). More recently, plasmid-mediated polymyxin resistance in China was found in 21% of livestock animals and 15% of raw meat samples tested, and in 16 human patients (Liu *et al.*, 2015). The overuse of antimicrobials in livestock provides a breeding ground for antimicrobial resistant bacteria by selecting for bacteria that have evolved resistance to the antimicrobials used, which in turn, may interact with pathogenic bacteria and pass on resistance elements.

Collectively, these data demonstrate that antimicrobial resistance is a global problem affecting any ecosystem due to the ubiquitous nature of microorganisms. Antimicrobial resistance is no longer just a clinical problem, and antibiotic use in any context affects the health of the oceans, soil ecosystems, animals

and humans. Therefore, a One Health approach to address antimicrobial resistance is needed to prevent or delay a time where once treatable bacterial infections become untreatable.

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