USE OF BILE ACIDS AND BILE SALTS TO INHIBIT BACTERIAL CONJUGATION AND ACQUISITION OF ANTIBIOTIC RESISTANCE

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Background

Over the last half a century, human health has profoundly benefited from the discovery and use of antibiotics to treat and prevent infectious diseases. Nevertheless, after many years of extensive and uncontrolled use we witness a dramatic and worldwide increase in human-pathogenic bacteria that are resistant to one or multiple antibiotics. Worrisomely, infections caused by resistant microorganisms fail to respond to conventional treatment and even last-resort antibiotics have lost their power [1]. According to the World Health Organization (WHO), more and more bacteria have developed resistance against antibiotics, leading to persistent infections, which increases the risk of spreading to others [2]. Antimicrobial resistance in bacterial pathogens is a worldwide challenge associated with high morbidity and mortality and one of the most difficult challenges of the contemporary medicine. In September 2013, the Center for Diseases Control (CDC) issued a threat report, naming infections with resistant *Clostridium difficile*, Carbapenem-resistant *Enterobacteriaceae* (CRE), and drug-resistant *Neisseria gonorrhoeae* as among the most urgent clinical problems [3].

Antimicrobials are valuable therapeutics whose efficacy is seriously compromised by the emergence and spread of antimicrobial resistance. While the initial rate of bacterial resistance to new drugs is normally about 1%, modern usage of antibiotics has caused a massive increase in the number of resistant bacteria. Studies show that within 8-12 years after abundant usage, strains resistant to multiple drugs become widespread. Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment and their natural phenotype is a multi-drugs resistance [4]. Therefore, combating this problem requires solid understanding of the biological principles and factors

affecting the evolution, divergence, and spread of antibiotic resistance genes (ARGs) among bacterial pathogens.

Resistance to antibiotics can occur either by mutations or by acquisition of resistance-conferring genes via horizontal gene transfer (HGT), which is considered to be the most effective process in the current antibiotic resistance pandemic. Today, it is becoming increasingly clear that not only ARGs harbored directly by specific clinical pathogens are of relevance, but rather, all mobile genetic elements (MGEs) and bacteriophages, form a resistance genes pool (designated the resistome), circulating in pathogenic, commensal and environmental bacteria contribute to this troubling phenomenon. Such resistome is believed to compose an environmental reservoir, from which pathogenic bacteria can acquire resistance via HGT [5]. HGT has caused antibiotic resistance to spread from commensal and environmental species to pathogenic strains and *vice-versa*.

Of the three canonical mechanisms of HGT, namely transformation, transduction and conjugation, the latter is thought to have the greatest impact on the dissemination of antibiotic resistance genes. Bacterial conjugation is a process by which genetic material is transferred from a donor bacterial cell to a recipient bacterium through a bridge-like connection known as the conjugative pilus. Conjugation in Gram-negative bacteria is mediated by the type four secretion system (T4SS), comprising of 12 to 30 proteins that are assembled into a large exporting machinery, spanning the inner and outer membranes and involved in substrate transport and pili biogenesis [6]. The genes for the conjugative machinery are encoded by autonomously replicating plasmids or by chromosomally encoded integrative and conjugative elements (ICEs) [7,8].

The conjugation of MGEs conferring resistance has been observed in many types of ecosystems ranging from transfer between bacteria in insects, soil, and water environments to various food and healthcare associated pathogens [9]. Importantly, transfer of plasmids between unrelated bacteria over large taxonomic distances have been also reported [10-12]. The role of plasmid conjugation in ARGs dissemination is well demonstrated by the $bla_{CTX\ M}$ ESBL genes, which have disseminated to various narrow and broad host range plasmids within Enterobacteriaceae, as well as to other opportunistic human pathogens [13]. Similarly, the transfer of plasmids in pathogens has led to the worldwide spread of numerous ARGs conferring resistance to -lactams, quinolones, aminoglycosides, tetracyclines, sulfonamides, and many other drug classes [14]. Moreover, multiple ARGs are often co-localized on the same plasmid, which allows a relatively easy spread of multidrug resistance.

While pathogens are becoming more and more multidrug or extensively drug resistant, pharmaceutical companies have dramatically reduced their drug discovery programs, resulting in severe public health consequences and lack of suitable antibiotic therapy [15]. Due to this lag in the discovery of novel antibiotic chemotherapies and because of the increasing occurrence of resistant strains, public health is running out of treatment options for dealing with infectious

diseases.

The Need

Antimicrobials are valuable therapeutics whose efficacy is seriously compromised by the emergence and spread of antimicrobial resistance. Worrisomely, infections caused by resistant microorganisms fail to respond to conventional treatment and even last-resort antibiotics have lost their power. Antimicrobial resistance in bacterial pathogens is a worldwide challenge associated with high morbidity and mortality and one of the most difficult challenges of the contemporary medicine. Multiple drug resistant strains of some bacteria have reached the proportion that virtually no antibiotics are available for treatment and their natural appearance is resistant to antibiotics. Furthermore, studies have shown that within 8-12 years after abundant usage, strains resistant to multiple drugs become widespread. Therefore, the development of new antibacterial drugs, which directly kill the bacteria (put a strong selective pressure) cannot provide a sufficient solution for the long term. Thus, the identification of a specific compound that can inhibit bacterial conjugation, but not kill the bacteria has a promising potential to be used in health, food and agriculture infrastructures to fight the spread of resistant bacteria and pathogens. This compound can be added to different detergents, antiseptics and cleaning materials. It can be also used for washing wounds, hospital, food industry, and agriculture infrastructures (such as poultry enclosure or cowsheds).

The Technology

We have identified a novel composition that inhibits the conjugation process and gene transfer between bacteria that can therefore reduce the development of antibiotics resistant strains. This compound contains 2% (W/V) of Lithocholic acid, (3 -hydroxy-5 -cholan-24-oic acid) and Taurine (2-aminoethanesulfonic acid) and was found to significantly inhibit antibiotic resistant plasmid from various incompatibility groups between bacteria.

The Market

The pharmaceutical industry owes a great deal of its early prosperity to the development of antibacterial drugs, and as a consequence the market encompasses several of the oldest drug classes. The market is highly saturated and has significant generic penetration, yet still experiences continuous growth due to increasing sales volume, as well as the rise of premium-priced novel treatments for resistant bacteria (for example, Pfizer's Zyvox (linezolid)). According to a new

market report from Transparency Market Research, a global market intelligence company, the global antibacterial drugs market was valued at $43.55 billion in 2012 and is expected to grow at a CAGR of 0.3% from 2013 to 2019, to reach an estimated value of $45.09 billion in 2019. Our initial products target mainly the environment ecosystem of bacteria.

The antimicrobial coatings and environmental antimicrobial are targeted to prevent or inactivate microbes, such as bacteria, fungi (including molds), and viruses. The major additives that are considered today, are mainly silver, copper, zinc oxide, zirconium, titanium dioxide, and zinc omadine. The market is divided further on the basis of their major applications such as indoor heating, ventilation and air conditioning (HVAC), medical, mold remediation, building & construction, food & beverages, textiles, cleantech & others in which the indoor HVAC coatings dominate over all other coatings.

The market size for antimicrobial coatings was about $1.5 billion in 2012 by value and is estimated to grow with a compound annual growth rate of about 11.8% from 2013 to 2018. The data mentioned in the report are based on the overall demand for the major antimicrobial coating and environment categories. Our additive product aims to complement these products, to eliminate the development of acquired resistant in bacteria. We estimate this market to exceed 1.5 billion USD.

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