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Systems Biology of Drug Resistance Evolution

Briefs

Lytic phages obscure the cost of antibiotic resistance in Escherichia coli

Alex Hall (ETH-Zurich)

We used a model and experiments with Escherichia coli to show that lytic phages strongly affect the incidence of antibiotic resistance in drug-free conditions. Under phage parasitism, the likelihood that antibiotic-resistant genetic backgrounds spread depends on their initial frequency, mutation rate and intrinsic growth rate relative to drug-susceptible genotypes, because these parameters determine relative rates of phage-resistance evolution on different genetic backgrounds. The average cost of antibiotic resistance in terms of intrinsic growth in the abiotic experimental environment was small relative to the benefits of an increased mutation rate in the presence of phages. This is consistent with our theoretical work indicating that, under phage selection, typical costs of antibiotic resistance can be outweighed by realistic increases in mutability if drug resistance and hypermutability are genetically linked.

Finding mutant–selection concentrations and coexistence windows for tetracycline antibiotics.

Carlos Reding (University of Exeter)

Since the discovery of antibiotics, drug-resistant bacteria have steadily proliferated in clinical environments. The frequency of isolation of multi-drug resistant (MDR) pathogens is increasing over time and it risks attaining a level beyond which we can exert no control over important pathogenic infections. However, in nature, resistant populations often only represent some fraction of the total bacterial population density, seemingly maintaining steady coexistence with their drug-susceptible counterparts. We need to understand how a healthy balance can be maintained between sensitive and resistant bacteria. What are the molecular, genetic and ecological mechanisms underlying this balance? Could we even exploit some of these to reduce the load of MDR pathogens in hospitals?

Seeking to address the theory behind some of these issues, we use in vitro evolutionary/competition experiments and population genetics models to examine the question of what drug dosages support competitive exclusion and which support coexistence. We found that two strains of E. coli with differential sensitivity to tetracycline appeared able to co-exist in a narrow window of concentrations for about, 21 human days. Importantly, the ‘MIC’ (a pharmacological measure of drug efficacy) of the drug-susceptible strain was lower in the presence of its competitor than in its absence. Finally, we asked whether the cost of resistance was removed by evolution if the drug were absent. This will be discussed during my talk.

A population level study of multi-drug resistance in Salmonella Typhimurium from Mexico

Claudia Silva (Institute of Biotechnology, UNAM)

We found two main genotypes in the Salmonella enterica serovar Typhimurium population from Mexico. ST19, considered as the founder genotype world-wide, carried the Salmonella virulence plasmid (pSTV) and the Salmonella genomic island 1 (SGI-1). Among the ST19 strains, few integrons were detected, and presented a high level of susceptibility for all ten antibiotics tested. The most prevalent genotype was ST213, which carried a multi-drug resistance IncA/C plasmid and integrons. ST213 strains were more resistant than ST19 strains; some of them were resistant to nine of the ten antibiotics tested. Transformation experiments showed that the IncA/C plasmid accounted for most of the multi-drug resistance phenotype of ST213 strains. Interestingly, the ST213 strains did not carry the virulence plasmid; and thus the pSTV has not been found together with the IncA/C plasmid.

Recently, we have discovered that all ST213 strains carry a lysogenic phage. Although this phage does not carry antibiotic resistance determinants, phage-encoded proteins could be enhancing the antimicrobial resistance ability. Comparative genomic studies with ten representative strains are underway to determine other genomic regions that distinguish the ST213 from the ST19 strains. ST213 strains are considered as a public health problem in Mexico, since they have been associated with human systemic infections. We believe that antibiotic resistance could be considered as a virulence factor, since this phenotype allows the permanence of the strains in the environment, such as in food-animals, and could make them more successful in colonizing host tissues.

Using experimental evolution to evaluate combination therapy: antibiotics + phages for the control of Pseudomonas aeruginosa.

Flor Inés Arias-Sánchez & Michael Hochberg (Université Montpellier 2)

The genetically based resistance of bacteria to antibiotics is a growing problem in animal farming and in public health. One possible solution for the evolution of bacteriophages in combination with antibiotics. However, many aspects related to the significance of combination therapy in terms of ecological and evolutionary interactions remain unknown.
In this study we investigated how single or multiple mortality factors influence population persistence and genetic adaptations in terms of resistance evolution. We established 96 experimental populations of the Gram-negative opportunistic bacterium Pseudomonas aeruginosa strain PA01. We tested 2 Streptomycin doses alone and in combination with the lytic bacteriophage LUZ7. 3 relative timings of streptomycin introduction were assayed.
Our results showed that all populations in single treatments survived, but in combined treatments 50% of the populations went extinct. We observed that higher antibiotic dose leads to higher proportion of mortality, and we discovered that delaying antibiotic addition leads to higher extinction rates.
To our knowledge, this is the first study that addresses the importance of implementing different antibiotic schedules in the use of phages and antibiotics for the treatment of Pseudomonas aeruginosa. Our work shows that combination therapy has great potential for infection clearance.

Klebsiella pneumoniae ST258 and Escherichia coli ST410: CTX-M-15 and KPC-2 producers in a Mexican Hospital

Jesús Silva Sánchez[1], H. Barrios-Camacho[1], L. Poirel[2], P. Nordmann[2], J. Salazar Salinas[3], U. Garza Ramos[1], R. Núñez-Ceballos[4]

[1] Departamento de Diagnóstico Epidemiológico, Centro de Investigaciones Sobre Enfermedades Infecciosas, Instituto Nacional de Salud Pública.
[2] Medical and Molecular Microbiology Unit. Dept of Medicine, University of Fribourg, Switzerland.
[3] Laboratorio de Vigilancia Epidemiológica, ISSTE.
[4] Departamento de Vigilancia y Control Epidemiológico, ISSTE.

Objective: To investigate the molecular characteristics of carbapenemase-producing enterobacterial clinical isolates recovered in a Mexican hospital.

Methods: Three non-duplicate K. pneumoniae and a single E. coli isolates were obtained in March 2013. The phenotypic screening of carbapenemase activity was carried out using the CarbaNP test. Positive isolates were then further investigated at the molecular level by searching for genes encoding IMP, VIM, KPC, NDM carbapenemases, and genes encoding extended-spectrum beta-lactamases. MICs were determined by using E-test strips following CLSI guidelines. Genotyping was performed by PFGE and MLST using the Pasteur website (http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html).

Results: The four isolates were resistant to all beta-lactams, quinolones, and aminoglycosides, and showed reduced susceptibility to carbapenems, remaining susceptible only to tigecycline and colistin. PFGE analysis showed that the three K. pneumoniae isolates were clonally-related, these isolates harbored three plasmids being respectively 80-, 30- and 20-kb in-size, and the blaKPC-2 gene was located onto the 80 kb IncY-type plasmid group. The E. coli isolate co-produced the carbapenemase KPC-2 together with the ESBL CTX-M-15, both genes being located onto self-transferable IncI1- and IncF-type plasmids, respectively. MLST analysis showed that the three K. pneumoniae isolates belonged to Sequence Type ST258 and the E. coli isolate belonged to ST410. * Conclusion: That study highlights the emergence of multidrug-resistant and KPC-2-producing enterobacterial isolates in Mexico. That observation further underlines the needs for systematic surveillance of carbapenemase producers in this country.

The non-robustness of pharmacological measures of antibiotic efficacy: treatment with antagonistic combinations

Mark Hewlett (University of Exeter)

The purpose of this study is to demonstrate that the standard pharmacologically accepted definitions of drug interaction are potentially limited if they fail to capture changing drug interaction or non-robust synergystic or antagonistic behaviour. We show that dependent on dose, time and resource background, a treatment combination that initially appears synergistic, may in fact be antagonistic later, or vice versa.

Adaptive landscapes of variant mutant alleles change as concentration of antibiotic treatment change

Portia Mira (UC Merced)

Most of what we know today about the evolution of antibiotic-resistance is focused on the selections of lethal drug concentrations that allow the detection of rare mutants with strong phenotypes (4). However, this is just the tip of the iceberg when it comes to the evolution of antibiotic-resistance genes. In fact, high-resolution competition assays show that selection of resistant bacteria occurs at extremely low antibiotic concentrations (3). In natural and clinical settings bacterial pathogens are exposed to a wide range of antibiotic concentrations, often associated with non-medical use of antibiotics. It is critical to ensure that the correct concentrations are used on order to kill the bacterial infections, but in many situations, this is not the case. It is very important that we begin to focus on a variety of concentrations of antibiotics rather than just the lethal concentrations that we thought had the biggest impact on the evolution of resistance genes.

Also, because of the widespread use of beta-lactam antibiotics, there have been additional approaches that utilize a combination of mechanisms based on activators for beta-lactamases such as clavulanic acid, sulbactam and tazobactam (5). These inactivators destroy the β-lactamase activity, therefore enhancing the ability of the drug to destroy the cell wall. Here we will show that there are many evolutionary pathways within the resistance gene blaTEM-50 depending on the concentrations of antibiotic treatment. We will be focusing on the treatments that combine the β-lactam antibiotic, specifically penicillin, plus one of the inhibitors. An Inhibitor-resistant TEM (IRT) is a bacterial strain that produces an inhibitor-resistant enzyme that break down these beta-lactamase inhibitors. Within the TEM-50 gene we are looking at, there are two substitutions that contribute to the IRT phenotype, M69L (IRT-5) and N276D (not yet isolated). To study the effects of epistatic interactions within these IRT's, we need to look at the adaptive landscapes and only focus on the pathways that show preference to a mutant containing an inhibitor-resistant mutation (IRM), i.e. arrows pointing toward a mutant containing an IRM. If an allele had a mutation in the first location (1000) or last location (0001) with an arrow pointing toward it, this signifies the mutant that contains one additional mutation is preferred under the specific conditions, over the ancestral mutant. This indicates that those inhibitor resistant mutations are beneficial to the cell rather than detrimental.

Resistance of Pseudomonas aeruginosa against quorum sensing inhibitors

Rodolfo García Contreras (UNAM)

Pseudomonas aeruginosa is an opportunistic pathogen that causes approximately 10 % of nosocomial infections; in addition, it is resistant to several antibiotics and is able to generate resistance against new antimicrobials such as the ones that target cell-cell communication (quorum sensing or QS), a process that coordinates the expression of several virulence factors in this and other important pathogens. In my presentation I will explain how nutritional and environmental factors select for those bacteria with active QS systems and the resistance mechanisms against quorum quencher that are currently known.

Exploring the environmental resistome of Mexican soils and water bodies in Morelos with contrasting degree of anthropogenic perturbation

Pablo Vinuesa, Luz Edith Ochoa Sánchez, Perla Tinoco, Jazmín Ramos Madrigal, Pablo Rodríguez Bucheli, Javier Rivera *(Center for Genomic Sciences, UNAM)*

Morelos is a small State in Central Mexico rich in eco-geographic diversity and environmental problems. There are three main rivers that cross the State from the Northern uplands to the Southern planes. All of them get heavily loaded with sewage and other residual waters which are used to irrigate different crops. Since the Revolution, the largest extension of cropland in Central and Southern Morelos is devoted to the cultivation of sugar cane. We have recently initiated a new research program at CCG-UNAM with the aim of gaining scientific insight into the problem of the spread of antibiotic resistant bacteria and their resistance genes at a landscape-scale. Our study focuses on diverse Proteobacteria, currently mainly members of the alpha and gamma subdivisions, including Acinetobacter, Achromobacter, diverse genera of the Enterobacteriaceae, Pseudomonas, Stenotrophomonas and Ochrobactrum. All these genera and families are known to contain opportunistic or specialized pathogens, which are also multidrug-resistant, making use of different resistance mechanisms. However, very little is known about the ecology, population genetics and environmental distribution of these bacteria. We hypothesized that due to the genetic proximity of environmental and human-associated strains of these bacteria, odds are high for horizontal transfer of resistance determinants between native and invading strains released to the environment with residual waters. It is not clear, however, in which direction the HGT might occur, a question that we are addressing in our study.

We are generating large collections of environmental isolates from these bacteria, sampled from rivers, sediments and agricultural soils across Morelos. The sampling sites have contrasting levels of residual contamination, thus allowing us to explore the native resistome as well as that of human-associated bacteria. Here we will present an overview of our current findings in this context, including a comparative analysis of the genetic diversity and resistance profiles of bacteria isolated from different habitats and sampling sites, with a discussion o the patterns found sofar.

Acknowledgements: This work is supported by CONACyT grant no.179133 and PAPIIT-UNAM grant no. IN211814 to PV.

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Maximino Aldana *(Institute for Physical Sciences, UNAM)*

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