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Use of phenotype microarrays to study the effect of acquisition of resistance to antimicrobials in bacterial physiology

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Abstract

It is widely accepted that the acquisition of resistance to antimicrobials confers a fitness cost. Different works have shown that the effect of acquiring resistance in bacterial physiology may be more specific than previously thought. Study of these specific changes may help to predict the outcome of resistant organisms in different ecosystems. In addition to changing bacterial physiology, acquisition of resistance either increases or reduces susceptibility to other antimicrobials. In the current article, we review recent information on the effect of acquiring resistance upon bacterial physiology, with a specific focus on studies using phenotype microarray technology.

Keywords: Antibiotic resistance; Fitness costs; Biocides; Bacterial virulence; Phenotype microarrays

1. Introduction

Acquisition of resistance by human bacterial pathogens is a relevant problem for human health that may not only compromise treatment of infectious diseases themselves, but also development of such advanced therapeutic procedures as anti-cancer chemotherapy, immunosuppression for transplantation and the use of prostheses, among others, that require accurate prevention of infection [1,2]. Acquisition of resistance may be due to mutations, or to acquisition, through horizontal gene transfer (HGT), of resistance genes frequently present in large gene mobile elements (GMEs) as plasmids, phages, or transposons [3–5]. For any of these mechanisms, it is generally accepted that acquisition of resistance confers a fitness cost, i.e. a general, rather non-specific metabolic burden that reduces the competitive ability of resistant organisms, thereby challenging their transmission rate and virulence [6]. It is believed that acquisition of antimicrobial resistance via HGT involves different costs besides the additional energy requirements needed for replication, transcription and translation of the acquired GMEs. These fitness costs include energy required for the transfer process itself [7], integration of foreign DNA into the bacterial genome and metabolic costs associated with replication of recently acquired DNA and expression of encoded genes.

In the case of mutation-driven antibiotic resistance, most mutations involved in acquisition of resistance occur in genes encoding key players in bacterial physiology, including essential bacterial proteins that are antibiotic targets, transporters or regulators that repress expression of resistance determinants such as efflux pumps (Fig. 1). It is then expected that mutations in these elements would cause de-adaptation in bacterial physiology and consequently, fitness cost [8]. If that holds true, the effects on bacterial metabolism of acquiring

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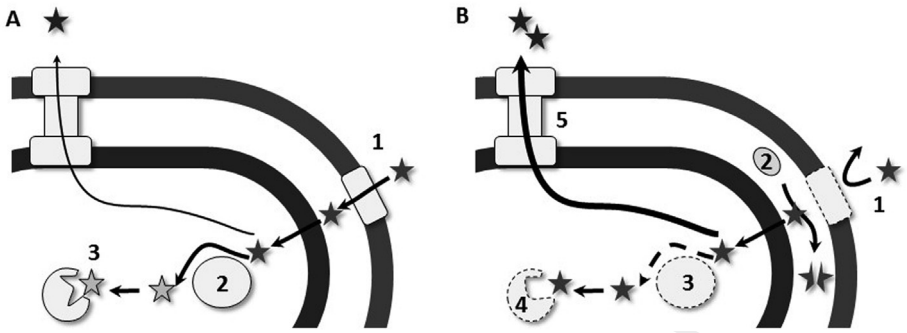

Fig. 1. General mechanisms of antibiotic resistance. To inhibit bacterial growth (panel A), an antibiotic must enter the bacterial cell, frequently through specific transporters (1). On some occasions, the antibiotic enters as a pro-drug and requires its activation by a bacterial enzyme (2); usually, intrinsically active efflux pumps reduce intracellular accumulation of the antimicrobial. Inhibition is achieved when the interaction of the antibiotic with its target is sufficiently proficient (3). Resistance can be achieved (panel B) by mutations in the target (or acquisition of target protection mechanisms) that impede a proficient antibiotic–target interaction (3) or by reducing the intracellular concentration of the antibiotic due to mutations in the transporters (or their regulators), impairing its uptake (1), to activity of antibiotic-inactivating enzymes (2), to mutations that alter the activity of the enzyme activating the pro-drug, or to acquisition of new efflux pumps or mutations increasing expression of the chromosomally encoded ones.

antibiotic resistance would be very similar whatever the bacterial growth conditions. However, different works have shown that fitness costs are habitat-dependent [9], different mutations in the same gene can render different fitness costs [10] and fitness costs associated with acquisition of a resistance gene depend on the gene itself and cannot simply be attributed to energy required for replication, transcription or translation of the GME containing the resistance determinant [11].

While fitness costs are more specific than previously thought, a thorough understanding of the effect of acquiring resistance in bacterial physiology is required. For determining such effects, phenotype microarrays constitute a valuable tool. Throughout this review, we will discuss the different fields in which utilization of phenotype microarrays can be useful and will present examples in which this technology has already been used for deciphering the effects of acquiring antibiotic resistance in bacterial physiology.

2. Effects of acquisition of resistance to antimicrobials upon bacterial metabolism

Most antibiotic targets are macromolecules involved in fundamental bacterial processes such as cell division, DNA replication, transcription and translation. Mutations in targets such as DNA topoisomerases, RNA polymerases, elongation factors or ribosomal machinery (among others) confer resistance without inactivating these elements. Nevertheless, these mutations may modify their activity, hence altering bacterial replication, transcription and translation, reflected in changes in metabolism. Indeed, in some studies, a combination of transcriptomic and proteomic analysis has shown that antibiotic-resistant strains exhibit changes in levels of expression of proteins involved in energy metabolism, protein synthesis and envelope biogenesis, most frequently relating fitness costs of these resistance to the increase in the expression level of many enzymes involved in energy metabolism, including L-lactate dehydrogenase, glucose-6-phosphate isomerase, succinyl-CoA synthetase and phosphoglycerate kinase, among others [12–15]. This is the case for a combination of proteomic and transcriptomic studies comparing vancomycin-susceptible to vancomycin-intermediate *Staphylococcus aureus* strains, that showed a total of 155 differentially expressed proteins, most of them related to energy metabolism, cell envelope biosynthesis, protein turnover, amino acid transport and metabolism [15].

In another example, a study of rifampicin resistance mutations in *rpoB*, a gene that encodes the RNA polymerase β-subunit, has shown that the mutants display changes in their transcriptional profile at a genome-wide scale in such a way that *rpoB* mutations are considered to be global regulatory mutations that severely alter the transcriptional profiles of mutants [16]. Among the observed changes, the resistant mutants present increased expression of proteins involved in central metabolism (nucleotide and nucleoside, amino acid, carbohydrate, lipid and phospholipid biosynthesis), along with proteins belonging to other functional categories such as detoxification, signal transduction, protein synthesis and cell envelope [17,18]. Consistent with these findings, different works, some of them using phenotype microarrays, have shown that *rpoB* mutations have pleiotropic effects in carbon catabolism of *Escherichia coli* [19] and *Pseudomonas aeruginosa* [20], in the sporulation and metabolism in *Bacillus subtilis* [21] and in the metabolism of lipids in *Mycobacterium tuberculosis* [22].

Mutations conferring resistance to quinolones also may present differential fitness costs that impact bacterial metabolism. Indeed, mutations targeting genes encoding bacterial topoisomerases may alter DNA supercoiling, which can modify expression of genes coding proteins involved in bacterial metabolism and lead to different phenotypic changes in fluoroquinolone-resistant strains [23]. On the other hand, quinolone resistance mutations enabling increased expression of efflux pumps, capable of extruding quinolones, may have a dual effect, one due to energy required for functioning of the pump, another due to the substrate(s) extruded by the pump itself. For instance, high-level ciprofloxacin-resistant

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