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Metagenomics and antibiotics

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Abstract

Most of the bacterial species that form part of the biosphere have never been cultivated. In this situation, a comprehensive study of bacterial communities requires the utilization of non-culture-based methods, which have been named metagenomics. In this paper we review the use of different metagenomic techniques for understanding the effect of antibiotics on microbial communities, to synthesize new antimicrobial compounds and to analyse the distribution of antibiotic resistance genes in different ecosystems. These techniques include functional metagenomics, which serves to find new antibiotics or new antibiotic resistance genes, and descriptive metagenomics, which serves to analyse changes in the composition of the microbiota and to track the presence and abundance of already known antibiotic resistance genes in different ecosystems.

Keywords: Antibiotic resistance, metagenomics, microbiota, mobilome, resistome
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Introduction

Antibiotics are among the most successful therapeutic compounds developed by humans. However, one issue that is not so frequently taken into consideration is that antibiotics are also pollutants that may produce important effects on the structure of natural bacterial populations [1]. Most studies on antibiotic resistance refer to the mechanisms of emergence and dissemination of resistance determinants in bacterial pathogens [2]. Nevertheless, antibiotics may alter as well the overall microbial populations in natural ecosystems, being particularly relevant for the microorganisms (pathogens and commensals) that colonize ecosystems in close contact with humans. These habitats are the ones presenting the highest probabilities of suffering contamination by antibiotics together with gene transfer elements containing antibiotic resistance determinants [3–5]. Learning the impact that the release of antibiotics and resistance determinants may have on natural microbial populations is a relevant topic for a comprehensive understanding of the population dynamics of antibiotic resistance [4]. For this goal, the utilization of non-culture-based techniques (metagenomics) is required for describing more accurately the changes in bacterial populations confronted with antibiotic contamination.

Antibiotic resistance is a relevant health problem with consequences, not just for the treatment of infections, but also for the utilization of medical and surgical techniques that, like cancer chemotherapy or transplantation, involve immunosuppression of the patient. In the current situation, with few new antibiotics in the discovery pipeline, functional metagenomics can be useful for searching for new antimicrobials without the need for culturing the producer organisms. In this short review, we summarize recent progress on the utilization of metagenomics tools for tracking resistance elements, deciphering the effect of antibiotics on microbial communities and searching for new antimicrobials useful for treating infections by human pathogens.

Functional metagenomics

Functional genomics is being used for searching for specific activities without the need for culturing the organisms harbouring the pathways involved. This approach was used earlier and two antibiotic compounds (turbomycin A and B), with a wide spectrum of activity against Gram-positive and Gram-negative bacteria, were described [6]. More recently, a project based on the analysis of so-called disease-suppressive soils [7] further demonstrated the feasibility of metagenomic approaches for searching novel antimicrobials. This project was based on the original idea from Waksman and Woodruff [8] indicating that soils must have compounds capable of inhibiting pathogens because these bacteria are rarely found in natural, non-clinical ecosystems. Using this theoretical approach, libraries were constructed using DNA from soils in which pathogens were absent. Two types of screening were performed, one by direct searching of clones capable of inhibiting growth of target pathogens, and another looking for polyketide synthases because polyketides constitute a relevant family of drugs. By using both approaches, novel activities and novel synthases were found indicating that functional metagenomic approaches constitute an appealing alternative in the search for new antimicrobials [7].

Descriptive metagenomics

Metagenomics is currently being used for describing the structure of microbial populations. Since most microorganisms have not been cultured, metagenomics is a more comprehensive methodology than culture-based techniques. The description of microbiota can be addressed by two complementary approaches: analysis of the distribution of microbial species or analysis of the distribution of genes. Whereas the first approach serves for understanding broadly the population dynamics of the microorganisms present in a given ecosystem, the second is useful for understanding the global metabolism of the microbiota present in a given ecosystem. The analysis of ribosomal RNA has been the method of choice for describing the structure of microbiota. Originally, the method was based on study of the most abundant species using denaturing gradient gel electrophoresis and sequencing. However, the availability of high-throughput methodologies allows a more precise description of the species present in a given environment. The most useful technologies for in-depth analysis of microbiota are based on the use of 16S RNA microarrays [9,10] and straightforward analysis using recent massive sequencing methods. Massive sequencing has served as well to study correlations between the microbiota of people with different diets, diseases and ages and for tracking what can be considered as the core human metagenome [11,12]. Nevertheless, and although antibiotics can dramatically impact environmental and human microbiota and there exist useful tools for tracking these changes, the number of papers referenced in PubMed and that can be found using the query [(metagenomic*) AND (antibiotic* OR antimicrobial*)] is still very low, although these types of studies are increasing (Fig. 1). Among the papers on this topic, it has been described that the use of antibiotics challenges gut microbiota and that these changes correlate with changes in the host response as well [13], thus highlighting that antibiotics are not only good therapeutic drugs but that they can produce relevant secondary effects in our microbiota, which are not just selection of resistance. Indeed, treatment with sub-inhibitory concentrations of antibiotics produces a relevant shift in the structure of gut microbiota; however, it does not correlate with enrichment in antibiotic resistance determinants [14]. It is important to know whether antibiotic-induced changes in the microbiota are stable or the system is recovered once antibiotic treatment ceases. One recently published paper suggests that human microbiota presents a large degree of homeostasis, because it suffers dramatic changes in the presence of antibiotics but the system is recovered just 1 week after antibiotic removal [15]. An important aspect, not taken into consideration in these papers that analyse the overall population structure based upon 16S RNA, is whether this recovery also occurs at the fine grain level. Studies on this topic would address two issues: clonal expansion and gene

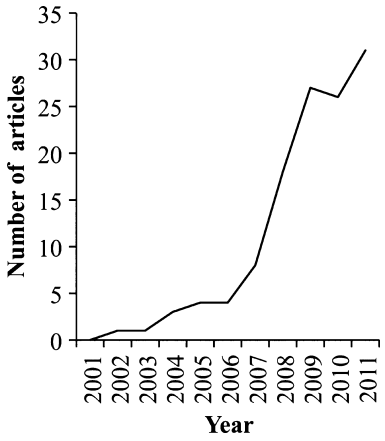

FIG. 1. Number of papers on antibiotics and metagenomics that can be found on PubMed. The figure shows the distribution along time of papers recorded in PubMed using the query [(metagenomic*) AND (antibiotic* OR antimicrobial*)]. The search was made on 17 October 2011.