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In-depth resistome analysis by targeted metagenomics

Val F. Lanza[1,2,3,4], Fernando Baquero[1,2,3], José Luís Martínez[2,4], Ricardo Ramos-Ruíz[5], Bruno González-Zorn[6], Antoine Andremont[7], Antonio Sánchez-Valenzuela[1], Stanislav Dusko Ehrlich[8,9], Sean Kennedy[8,13], Etienne Ruppé[7,8], Willem van Schaik[10,14], Rob J. Willems[10], Fernando de la Cruz[11,12] and Teresa M. Coque[1,2,3*]

Abstract

Background: Antimicrobial resistance is a major global health challenge. Metagenomics allows analyzing the presence and dynamics of "resistomes" (the ensemble of genes encoding antimicrobial resistance in a given microbiome) in disparate microbial ecosystems. However, the low sensitivity and specificity of available metagenomic methods preclude the detection of minority populations (often present below their detection threshold) and/or the identification of allelic variants that differ in the resulting phenotype. Here, we describe a novel strategy that combines targeted metagenomics using last generation in-solution capture platforms, with novel bioinformatics tools to establish a standardized framework that allows both quantitative and qualitative analyses of resistomes.

Methods: We developed ResCap, a targeted sequence capture platform based on SeqCapEZ (NimbleGene) technology, which includes probes for 8667 canonical resistance genes (7963 antibiotic resistance genes and 704 genes conferring resistance to metals or biocides), and 2517 relaxase genes (plasmid markers) and 78,600 genes homologous to the previous identified targets (47,806 for antibiotics and 30,794 for biocides or metals). Its performance was compared with metagenomic shotgun sequencing (MSS) for 17 fecal samples (9 humans, 8 swine). ResCap significantly improves MSS to detect "gene abundance" (from 2.0 to 83.2%) and "gene diversity" (26 versus 14.9 genes unequivocally detected per sample per million of reads; the number of reads unequivocally mapped increasing up to 300-fold by using ResCap), which were calculated using novel bioinformatic tools. ResCap also facilitated the analysis of novel genes potentially involved in the resistance to antibiotics, metals, biocides, or any combination thereof.

Conclusions: ResCap, the first targeted sequence capture, specifically developed to analyze resistomes, greatly enhances the sensitivity and specificity of available metagenomic methods and offers the possibility to analyze genes related to the selection and transfer of antimicrobial resistance (biocides, heavy metals, plasmids). The model opens the possibility to study other complex microbial systems in which minority populations play a relevant role.

Keywords: Antimicrobial resistance, Resistome, Metagenomics, Differential abundance analysis, Targeted metagenomics

Background

Antimicrobial resistance is considered a major global health challenge that has been recently included in the agendas of major international bodies [1]. The adoption of measures to address the antibiotic resistance crisis [2] is impaired by the controversy over what resistance is and how and where it should be detected and analyzed [3–5].

Metagenomic methods are increasingly being used to analyze the ensemble of genes that may encode antibiotic resistance in various microbial ecosystems, which are defined as the *resistome* [6–17]. An important hurdle confronting current resistome analyses is low sensitivity in the detection of minority populations harboring resistance genes (often present at concentrations below the detection level of the methods used) [18] and/or low specificity in the identification of allelic variants that might confer different

* Correspondence: mariateresa.coque@salud.madrid.org; teresacoque@gmail.com
[1]Department of Microbiology, Ramón y Cajal University Hospital, Ramón y Cajal Health Research Institute (IRYCIS), Madrid, Spain
[2]Joint Unit of Antibiotic Resistance and Bacterial Virulence associated with the Spanish National Research Council (CSIC), Madrid, Spain
Full list of author information is available at the end of the article