Functional Characterization of the Antibiotic Resistance Reservoir in the Human Microflora

Morten O. A. Sommer,* † Gautam Dantas,†† George M. Church

To understand the process by which antibiotic resistance genes are acquired by human pathogens, we functionally characterized the resistance reservoir in the microbial flora of healthy individuals. Most of the resistance genes we identified using culture-independent sampling have not been previously identified and are evolutionarily distant from known resistance genes. By contrast, nearly half of the resistance genes we identified in cultured aerobic gut isolates (a small subset of the gut microbiome) are identical to resistance genes harbored by major pathogens. The immense diversity of resistance genes in the human microbiome could contribute to future emergence of antibiotic resistance in human pathogens.

Multiple antibiotic resistance in human pathogens has increased over the past decades and challenged our ability to treat bacterial infections (1, 2). For example, methicillin-resistant Staphylococcus aureus (MRSA) caused 18,964 mortalities in the United States in 2006 (3). The comparison with 14,627 AIDS-related mortalities that occurred in the same year (4) highlights the public health importance of just one multiresistant bacterial pathogen in an industrialized nation. Whole-genome sequencing of one multiresistant bacterial pathogen in an industrialized nation. Whole-genome sequencing of bacteria has revealed that many of the resistance genes harbored by these strains have not evolved within the sequenced strain but were acquired by lateral gene transfer events (5). Antibiotic resistance determinants encoded on mobilizable elements move between diverse bacteria to disseminate resistance genes into a variety of interacting microbial communities (6, 7). Consequently, there is an increasing interest in elucidating reservoirs of mobile antibiotic resistance genes that may be accessible to clinically relevant pathogens (8–10).

The human microbiome substantially impacts human health and plays beneficial roles in dietary processing and prevention of pathogen intrusion (11–15). The widespread use of antibiotics in human medicine and agriculture has likely induced substantial responsive changes in this community.

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29. R. N. Lipcius, Marine Science, Gloucester Point, VA, 2002). Oyster Reef Restoration Map Atlas


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References

Movies S1 to S6

Supporting Online Material

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Materials and Methods

Figs. S1 to S5

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Fig. 1. Distributions of (A) nucleotide identities and (B) amino acid identities for 93 resistance genes identified from DNA extracted directly from saliva and fecal samples to the most similar resistance gene from any organism (white bars) as well as the most similar resistance gene harbored by a pathogenic isolate (black bars) in GenBank (table S3) (24). Two resistance genes with no significant similarity to sequences in GenBank are not shown.
Bifidobacterium longum (26), as well as commensals with the capacity to become opportunistic pathogens such as Bacteroides fragilis and Bacteroides uniformis (table S3) (21). Interestingly, we also identified genes that encode proteins that are 100% identical to hypothetical proteins of unverified function in GenBank, for example, BACUNI_02013 from Bacteroides uniformis ATCC 8492, which we show encodes resistance to broad-spectrum beta-lactams such as amoxicillin and carbenicillin, and the third-generation oxyimino-cephalosporin cefdinir (table S3) (27). This highlights the utility of a functional selection approach to improve annotation of genomic and metagenomic sequencing data from the human microbiome project (28).

Most of the antibiotic resistance genes harbored by the human microflora were distantly related (60.7% at the nucleotide level and 54.9% at the amino acid level) to antibiotic resistance genes so far detected in pathogenic isolates (Fig. 1 and table S3) (24). In total, we identified 78 unique inserts with genes with low homology (<90% amino acid identity) to proteins in GenBank, encoding resistance to the 13 antibiotics profiled (tables S1 and S3). This may imply that the resistance genes of the human microbiome are inaccessible or infrequently exchanged with human pathogens; however, all the resistance genes we identified in this study were functional in E. coli, which suggests that if a barrier to gene transfer exists between the constituents of the human microbiome and pathogens, it must stem from processes other than functional compatibility.

Phylogenetic analysis of the inserts using PhylOpythia (29) indicates that they predominantly originate from the phyla Bacteroidetes and Firmicutes, which dominate the gut flora (14). However, the majority of the genes we discovered have low sequence identity to resistance genes previously identified in pathogens from these phyla (e.g., Staphylococcus aureus and Streptococcus pneumoniae), as well as from the numerous pathogens that are readily culturable facultative anaerobic bacteria from the phylum Proteobacteria. Although commensal Proteobacteria constitute less than 1% of the human gut microflora (14), they increase in abundance during antibiotic treatment at the expense of the normally abundant Bacteroidetes and Firmicutes (16, 17). As a consequence of their normal low abundance in healthy individuals, they are not well represented in unbiased metagenomic libraries.

We isolated 572 bacterial strains on rich media under aerobic conditions from fecal samples from two healthy individuals (24). Phylogenetic profiling revealed that they belonged primarily to Proteobacteria, with a few Firmicutes and Actinobacteria (fig. S1). The isolates from individuals 1 and 2 were on average resistant to 9 and 5 out of 13 antibiotics, respectively (Fig. 2, C and D). Chloramphenicol and minocycline were the only antibiotics tested that were able to prevent the growth of more than 99% of the isolates (Fig. 2, A and B, and figs. S2 and S3).

Functional selections identified 115 unique inserts encoding transferable antibiotic resistance genes from the cultured aerobic gut microbiome isolates (Fig. 3 and table S4) (24). We found that 95% of these genes are over 90% identical at the nucleotide level to resistance genes in pathogenic isolates, and almost half of these genes were 100% identical (Fig. 3A), indicating an evolutionarily close relationship to the resistance genes harbored by clinical pathogens.

The group of resistance genes identical to those in pathogens belong to one class of tetracycline efflux pumps (TetA), two classes of aminoglycoside-modifying enzymes [AAC(3)-II and AAC(6)-II], and three classes of beta-lactam-inactivating enzymes (TEM, AmpC, and CTX-M) (Fig. 4). We identified a TEM-1 gene variant (Fig. 4 and Table 1) in cultured isolates from one gut microbiome on every sampling time (24) that has recently been reported in pathogenic strains of Bacteroides fragilis 21 and 26. Interestingly, we also identified CblA-1 and CblA-2, which previously were identified in pathogenic strains of Bacteroides uniformis (table S3) (27).}

Table 1. Unique beta-lactamase genes identified from gut and oral microbiomes from healthy humans. Gene ID refers to unique identifier in tables S3 and S4, and enzyme names use established nomenclature (24, 32). Percentage amino acid identity to the closest related gene in GenBank is calculated using the global alignment program clustalW (24, 33). NCBI, National Center for Biotechnology Information.

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<th>Beta-lactamase family</th>
<th>Enzyme name</th>
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<th>GenBank ID</th>
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### REPORTS

*E. coli, Salmonella enterica, Klebsiella pneumonia, Haemophilus parainfluenzae, Serratia marcescens, Pseudomonas aeruginosa,* and *Neisseria meningitidis* isolated around the globe (table S2). Nearly 80% of the depositions of this TEM-1 variant to GenBank have occurred between 2007 and 2008, which seems to indicate a relationship between the emergence of this resistance gene variant in the clinic and its occurrence in healthy humans.

The AmpC and CTX-M family of enzymes are extended-spectrum beta-lactamases that hydrolyze a wider variety of later generation beta-lactams (Table 1) (30). We identified the CTX-M-15 beta-lactamase (Fig. 4 and Table 1) in libraries from cultured gut microbiome isolates across multiple sampling days, as well as in our metagenomic libraries from the same microbiome.

Global sequence alignments of each of the 27 unique beta-lactamase sequences from our study identified 15 distinct sequence groups (Fig. 4). Of these groups, 5 were previously characterized (ChLA, CfcA, CTX-M, TEM, and AmpC) (31), whereas 10 constitute previously unknown beta-lactamase sequence families because they are between only 35% and 61% identical at the amino acid level to any gene products in GenBank (Fig. 4).

Interestingly, the known beta-lactamase genes we identified were from the cultured microbiome isolates, but the 10 previously unidentified gene families were identified solely by our culture-independent characterization (Fig. 4). In general, we found that the metagenomically derived resistance genes in our study were more distantly related to previously identified genes than those derived from aerobic gut isolates (Figs. 1 and 3 and figs. S5 and S6).

Of our 210 unique microbiome-derived inserts encoding antibiotic resistance, we found a subset of 29 that also contained genes similar (>96% nucleotide sequence identity) to previously characterized transposases (table S6). Of these, 14 transposases were identical to those previously identified on resistance genes from even closely related pathogenic isolates. Although this identity provides no information regarding the direction or mechanism of transfer, we can offer some speculation regarding the implications of our findings. First, the human microbiome may constitute a mobilizable reservoir of antibiotic resistance genes that are accessed by a pathogenic bacterium to acquire antibiotic resistance, although direct experimental proof of in vivo transfer of antibiotic resistance genes within the human microbiome remains to be shown. Second, despite selecting samples from untreated healthy humans, the aerobic cultured isolates may be dormant pathogens inhabiting the human microbiome. Third, by contrast with the cultured isolates, the resistance genes discovered by the culture-independent approach were distantly related to resistance genes from even closely related pathogenic isolates, which may reflect an unappreciated barrier to lateral gene transfer in vivo between the dominant commensals in healthy humans and disease-causing isolates. Fourth, this work exposes previous substantial undersampling of antibiotic resistance genes in the human microbiome.

We found many microbial DNA fragments encoding resistance genes that have never before been described, and our analysis suggests that we have just begun to scratch the surface of the im-

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**Fig. 2.** Antibiotic resistance profiles of cultured aerobic gut microbiome isolates. (A) Heat map displaying resistance profiles of 572 aerobic bacterial isolates obtained across three different sampling times from two human gut microbiomes. Linear color-scaled bars display 7436 growth measurements of aerobic cultured microbiome isolates after 24 hours at 37°C in Luria broth containing one of 13 antibiotics at concentrations between 20 and 100 μg/mL that prevent the growth of wild-type *E. coli* (table S1). White denotes no growth, and color intensity is proportional to growth in the presence of antibiotic, scaled to growth in the absence of antibiotic per individual isolate. (B) Percentage of aerobic gut isolates resistant to each of 13 antibiotics. Each data point represents the mean number of isolates resistant to each antibiotic, and error bars represent the standard deviation of this mean value from each of the three sampling times. Histograms depict the distribution of the number of different antibiotics that aerobic (C) gut microbiome 1 and (D) gut microbiome 2 isolates are resistant to.

**Fig. 3.** Distributions of (A) nucleotide identities and (B) amino acid identities for 114 resistance genes identified from cultured aerobic gut isolates to the most similar resistance gene from any organism (white bars) as well as the most similar resistance gene harbored by a pathogenic isolate (black bars) in GenBank (table S4) (24). One resistance gene with no significant similarity to sequences in GenBank is not shown.
mense diversity of antibiotic resistance machinery in the human microbiome. More than half of the inserts that were derived from metagenomic libraries and libraries from cultured gut aerobes were sequenced only once in our experiment (fig. S4), and we estimate that complete sequencing of these libraries would yield hundreds more resistance genes (24). Interestingly, when we compared the resistance genes derived from the microbiomes of the two different individuals, we found that over 65% of the resistance genes derived from cultured aerobes were highly similar (>90% nucleotide sequence identity) between the two individuals, whereas less than 10% of the metagenomically derived resistance genes were highly similar between the individuals (table S7) (24).

Many commensal bacterial species, which were once considered relatively harmless residents of the human microbiome, have recently emerged as multidrug-resistant disease-causing organisms (7). In the absence of in-depth characterization of the resistance reservoir of the human microbiome, the process by which antibiotic resistance emerges in human pathogens will remain unclear.

References and Notes
24. Materials and methods are available as supporting material on Science Online.
34. We acknowledge J. Davies, J. Jach, and M. Strong for helpful discussions regarding this manuscript; and the expert assistance of G. Jacoby and K. Bush for beta-lactamase enzyme family nomenclature, L. Kraal and G. Rockwell for sequence manipulations and similarity computations, and S. Caliri, A. Ellison, and T. Ellison for microbial culturing and DNA processing. We acknowledge National Genome Research Institute Centers of Excellence in Genomic Science, Personal Genome Project, Bill and Melinda Gates Foundation, Harvard Biophysics Program, Hartmann Foundation, and Det Kongelige Danske Videnkabernes Seelskab for funding. GenBank accession numbers GQ342978 to GQ343187 are listed in Table 1 and tables S3, S4, and S7. M.O.A.S. and G.M.C. advise many companies, listed in (24); none is working within fields related to the subject of the manuscript.

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Motile Cilia of Human Airway Epithelia Are Chemosensory

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Cilia are microscopic projections that extend from eukaryotic cells. There are two general types of cilia: primary cilia serve as sensory organelles, whereas motile cilia exert mechanical force. The motile cilia emerging from human airway epithelial cells propel harmful inhaled material out of the lung. We found that these cells express sensory bitter taste receptors, which localized on motile cilia. Bitter compounds increased the intracellular calcium ion concentration and stimulated ciliary beat frequency. Thus, airway epithelia contain a cell-autonomous system in which motile cilia both sense noxious substances entering airways and initiate a defensive mechanical mechanism to eliminate the offending compound. Hence, like primary cilia, classical motile cilia also contain sensors to detect the external environment.