Antibiotic Resistance Genes: From The Farm To The Human Gut

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The threat of drug resistance

- An estimated 25,000 people die every year in Europe from antibiotic-resistant bacteria.
- The US CDC: at least 23,000 deaths a year in the USA were caused by antibiotic resistance.
- WHO: About 170,000 deaths in 2012 were caused by multi-drug resistant tuberculosis.

Nearly 1000 β-lactamases that inactivate these antibiotics have been identified, a ten-times increase since before 1990.

**“Golden era”: 1940s to 1960s**

- In April, the WHO declared that the problem “threatens the achievements of modern medicine. A post-antibiotic era — in which common infections and minor injuries can kill — is a very real possibility for the 21st century.”

*The Lancet Infectious Diseases. 2014*

*Nature. 2012*
The mechanism of drug resistance

- Impermeable membrane
- Efflux pumps
- Resistance mutations
- Inactivation enzyme

- Horizontal gene transfer (HGT): conjugation, transformation, transduction
- Genome mutation
The circles represent potential anthropogenic antimicrobial selection pressure. Some pathways are well described, while others are plausible but lack substantive evidence. Not all plausible pathways are depicted.
The human gut microbiota is a reservoir of antibiotic resistant bacteria.

It is like a bazaar of genes where bacteria trade their antibiotic resistance genes (ARG): taking, giving, and exchanging.
Antibiotic Resistance Gene (ARG) Datasets

- the Antibiotic Resistance Genes Database (ARDB): a core set of 7,825 antibiotic resistance genes, which belong to 380 gene types and corresponding to 249 antibiotics.
First Analysis on Human Gut Microbiota

- The 4.1 million human gut gene catalog created BGI (from 85 Danish, 39 Spanish and 38 Chinese gut microbiota).
Different countries have different AR gene types

- 1,093 ARGs in the human gut, belonging to 149 AR gene types

More than 50% individuals: China 70 types; Denmark 45 types; Spain 49 types.
Abundance of the resistance genes in China is the highest

- To compare the abundances of antibiotic resistance genes in the Chinese, Danish and Spanish populations, we computed the relative enrichment of each of the genes based on sequencing coverage by using the original Illumina GA short reads.

Comparison of relative abundance of total AR genes in each individual. The diamond boxes on the right denote the interquartile range (IQR) between the first and third quartiles (25th and 75th percentiles, respectively) and the line and the square inside denote the median and mean, respectively. China: n=38; Denmark: n=85; Spain: n=39
Resistance gene abundance and SNPs are regionally different.

Hit map of relative abundance of each genotype in each individual and hierarchical clustering. The sample names of Chinese, Danish and Spanish individuals were colored by red, green and blue, respectively.
Prevailing resistance gene types in different countries

The high abundance of TcR genes was further confirmed through functional screening of resistant clones from the fosmid libraries of three healthy Chinese individuals (200,000 clones). Among 100 drug-resistant fosmid clones, 74 clones were resistant to tetracycline, 14 to gentamicin and 12 to amoxicillin.

Hu et al. Nature Communications 2013
The Abundance of AR genes in human gut and the agricultural use of Antibiotics

Huet et al. Gut Microbes 2014
Diversity of Antibiotic Resistance Genes in Different Age Groups

124 samples: from 3 to 53 years old; male: 63 samples, female: 61 samples

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age range</th>
<th>Sample No.</th>
<th>Male/female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-school-aged children (CH)</td>
<td>3-5</td>
<td>23</td>
<td>9/14</td>
</tr>
<tr>
<td>School-aged children (SC)</td>
<td>10-11</td>
<td>25</td>
<td>11/13</td>
</tr>
<tr>
<td>High school students (HSS)</td>
<td>15-17</td>
<td>26</td>
<td>9/17</td>
</tr>
<tr>
<td>Adults (AD)</td>
<td>26-55</td>
<td>50</td>
<td>24/26</td>
</tr>
</tbody>
</table>
The antibiotic resistance gene-type diversity gradually increased with age.

Spearman correlation coefficient between the age of each individual and the number AR gene types was 0.824 ($p<0.001$)
How Bacteria are Trading their AR genes
-Horizontal Gene Transfer

What drives the Horizontal Gene Transfer?
The ecology or the phylogeny?
Recent HGT is enriched in the human microbiome across all phylogenetic distances

Ecology is the dominant force shaping recent HGT in the human microbiome

Human and non-human
Different locations

Bacterial phylogeny is more related with AR gene horizontal transfer

A set of 1,680 mobile AR genes (carried by mobile genetic element), with a total of 2,134 representative sequences.

A total of 23,425 published genomes (2,768 complete + 20,657 draft) until the end of the year 2014;

(These genomes belong to 6,335 species where 36 species have more than 50 genomes presented, and 5,362 species have only one genome)
Mobile AR genes are enriched in Proteobacteria

Figure 1

<table>
<thead>
<tr>
<th>Class</th>
<th>Species</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>Klebsiella pneumoniae</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>Acinetobacter baumannii</td>
<td>105</td>
</tr>
<tr>
<td></td>
<td>Escherichia coli</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>Enterobacter cloacae</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Klebsiella oxytoca</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Salmonella enterica</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>Staphylococcus aureus</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Enterococcus faecium</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Enterococcus faecalis</td>
<td>31</td>
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<tr>
<td></td>
<td>Shigella flexneri</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Vibrio cholerae</td>
<td>24</td>
</tr>
<tr>
<td></td>
<td>Morganella morganii</td>
<td>22</td>
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<tr>
<td></td>
<td>Staphylococcus epidermidis</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Bacteroides fragilis</td>
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<tr>
<td></td>
<td>Streptococcus suis</td>
<td>21</td>
</tr>
</tbody>
</table>

$P=2.9 \times 10^{-4}$; Mann-Whitney $U$-test
The genome of *Klebsiella pneumoniae* is still acquiring more AR genes

Figure S1 Rarefaction curve plots the observed number of antibiotic resistance gene against the number of genome in each species.
Figure S2

A

Count

8000

6000

4000

2000

0

ARG cluster size (gene number)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18

Frequency (%) 100

80

60

40

20

0

Flanking int/rec/trans

1000

6000

4000

2000

0

B

Frequency (%) 100

50

25

0

int/rec and trans

trans

int/rec

no int/rec and/or trans

ARG cluster size (gene number)

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18
The AR genes shared by different phyla

39 AR genes are present in at least 2 phyla

<table>
<thead>
<tr>
<th>Phylum</th>
<th>AR Genes Present</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proteobacteria</td>
<td>369/399</td>
<td>92.5%</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>57/86</td>
<td>66.3%</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>32/46</td>
<td>69.6%</td>
</tr>
<tr>
<td>Actinobacteria</td>
<td>15/40</td>
<td>37.5%</td>
</tr>
</tbody>
</table>
The frequency of mobile ARGs in different phylogenetic level and ecology
There is almost no HGT between Gram(-) and (+) bacteria.
Human and animal gut microbiomes
Metagenome Database used in this study

The 10 million human gut gene catalog from MetaHIT, and metagenomic database from different environments from NCBI

The pig, chicken and cattle gut metagenomic sequencing data

A set of 1,680 mobile AR genes (carried by mobile genetic element), with a total of 2,134 representative sequences.
Mobile Antibiotic Resistance Genes in Human and Animal Guts

- are surprisingly, more related to the ones from chicken gut, followed by pig gut; much less related to the cattle gut;
- are much less related to different parts of the human body except the mouth;

<table>
<thead>
<tr>
<th></th>
<th>Count</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gut_IGC&amp;Gut_chicken</td>
<td>36</td>
</tr>
<tr>
<td>Gut_IGC&amp;Gut_pig</td>
<td>20</td>
</tr>
<tr>
<td>Gut_IGC&amp;Buccal mucosa</td>
<td>18</td>
</tr>
<tr>
<td>Gut_IGC&amp;Supragingival plaque</td>
<td>17</td>
</tr>
<tr>
<td>Gut_chicken&amp;Gut_pig</td>
<td>16</td>
</tr>
</tbody>
</table>
when we separate the metagenomic data from China, Europe and America, the results are the same
Figure 4
Conclusions:

- The human gut antibiotic resistance gene abundance profiling indicates that they are more related to antibiotic use in animal;
- The human gut antibiotic resistance gene type analysis from different age groups showed the diversity of antibiotic resistance genes are age related (accumulated);
- Metagenome sequencing and analysis for farm animals showed evidences that mobile antibiotic resistance genes are more related to chicken gut, meaning that the antibiotic resistance genes in humans may come from chickens.
Acknowledgements

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