« Stratégies innovantes de lutte contre la colonisation intestinale à bactéries multi-résistantes ».

Antoine Andremont
University Paris-Diderot Medical School (Paris France)
antoine.andremont@aphp.fr

DOI: Scientific advisor DaVolterra (LIR)
Three reasons why the impact of antibiotics on the microbiota is of utmost interest to clinicians.

1. It is the “epicenter” of resistance
2. It is the source of many infections
3. It « probably » can be manipulated
« New » natural history of bacterial infections

Contamination of the patient

Colonisation of commensal flora

Dissemination of pathogens

Dissemination of pathogens

Dissemination of pathogens

Sickness and disease

Iceberg’s tip

Human or animal populations and the environment

21/12/2015
« New » natural history of bacterial infections

Contamination of the patient

Colonisation of commensal flora

Dissemination of pathogens

Sickness and disease

Iceberg’s tip

Human or animal populations and the environment
But the medical vision of bacterial resistance is not enough!

A more holistic vision is needed.
Antibiotic Resistance (Credit F. Baquero)
This was hospital-born

Old types of genes
With new mutations

Hygiene programs in hospitals

This is community-born
(in part food-related)

New types of genes
« CTX-M »
Environmental sources

Incidence/100 admission

Paris (France) Bichat university hospital
Evolution of ESBL carriage rates in the community
Evolution of ESBL carriage rates in the community

Introduction

Generics 3GK

Gap between the « South » and the EU and the « South »
Three reasons for clinicians to « play » with the microbiota

1. It is the “epicenter” of resistance

2. It is the source of many infections

3. It « probably » can be manipulated
An historical paper demonstrating the gut was the source of Gram-negative bacteremia in severely neutropenic patients.
Since the concept has extended

✓ Intensive care unit patients
✓ Neonates
✓ Urinary tract infections
✓ Infections during pregnancy
✓ Infections post transrectal biopsies
✓ .....
But precise data are difficult to find...

1. It may be because colonisation « only » is not enough....

2. We suggest that **high densities** of bacteria are a very important factor.
The same occurs in pigs today with CTX-M genes.
Increase in ESBL fecal densities in women treated with antibiotics

FIG 2 Intestinal ESBL-RA in 63 women according to antibiotic exposure and to the concordance of urinary and fecal ESBL strains (concordance, ≥95% similarity between rep-PCR patterns [see Materials and Methods]) among the 31 women not exposed to antibiotics. The main horizontal bar represents the mean. Error bars represent 95% confidence intervals. a, determined by a two-tailed, unpaired t test.
574 travelers in intertropical zones

Global acquisition rate: 51% (n=293)
Rate of clearance of ESBL in returning travellers

![Graph showing clearance rates by visited area: Sub-Saharan Africa, Latin America, Asia]
Rates of clearance in returning travellers decreased with high abundance of colonising ESBL

Ruppe, et al. CID 2015
Are patients with high intestinal densities of ESBL at higher risk of (UTI) infections?
Fecal abundance of ESBL in women with *E. coli* UTI (ESBL vs non-ESBL)

<table>
<thead>
<tr>
<th></th>
<th>ESBL UTI</th>
<th>Non-ESBL UTI</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>7</td>
<td>24</td>
</tr>
</tbody>
</table>

Fecal abundance ESBL (% total enterobacteria)

P<0.05

Ruppe et al. AAC 2013
Three reasons for clinicians to « play » with the microbiota

1. It is the “epicenter” of resistance
2. It is the source of many infections
3. It « probably » can be manipulated
How can this interfere with their everyday practice in using antibiotics?

1. Reduce antibiotic usage
2. Modify antibiotic usage.
3. Decrease antibiotic concentrations in the colon during treatments
4. Use of « new » probiotics
5. Use of fecal transplant
A medical post with a permanent paramedic

~500 amerindians still living in a traditional manner

Trois-Sauts village
- South of French Guiana
- Restricted area

2°15'0.99"N, 52°52'58.99"W
**FIG 1** ESBL-E carriage rate in Wayampi volunteers (gray squares) and overall antibiotic exposure of the whole community (black diamonds) in 2001, 2006, and 2010. Linear regression ($y = 8.675x - 1.396; R^2 = 0.87$) and Pearson’s correlation ($P = 0.24$) were used to evaluate the evolution.
How can we do that?

1. Reduce antibiotic usage
2. Modify antibiotic usage.
3. Decrease antibiotic concentrations in the colon during treatments
4. Use of probiotics
5. Use of fecal transplant
Antibiothérapie empirique et colonisation intestinale chez des nouveaux-nés de réanimation

Bacilli resistant to regimen of unit,
Bacilli sensitive to regimen of unit but resistant to regimen of the other unit

NICU A
- Ampicilline-Céfotaxime

NICU B
- Penicilline-tobramycine
- Penicilline-tobamycine

(from De Man P. et al., Lancet 2000, 355 : 973)
For vets: Modify the doses for metaphylaxia

Animals without symptoms have low inocula at most

✓ Thus small doses are enough for treatment
✓ This strongly decrease the impact on the microbiota

FIG 2 Impact of the different cefquinome dosage regimens on cefotaxime-resistant Enterobacteriaceae in the fecal flora of rats before, during, and after treatment. ◆, Patent-phase-adjusted dose (50 mg/kg of body weight); □, prepatent-phase-adjusted dose (5 mg/kg); ▲, control untreated group. Data are means ± standard deviations (SDs). The arrows indicate the days of antibiotic administration.
How can we do that?

1. Reduce antibiotic usage.
2. Modify antibiotic usage.
3. Decrease antibiotic concentrations in the colon during treatments.
4. Use of probiotics.
5. Use of fecal transplant.
The idea goes far back in the 80s

Use of β-Lactamase-Producing Anaerobes to Prevent Ceftriaxone from Degrading Intestinal Resistance to Colonization

Florence Léonard, Antoine Andremont, Bernard Leclercq, Roger Labia, and Cyrille Tancrède

From the Laboratoire d’Ecologie Microbiennne, Service de Réanimation Médico-Chirurgicale, Institut Gustave-Roussy, Villejuif, and Centre National de la Recherche Scientifique, Paris, France
Idée oubliée pendant plus de 10 ans, revisitée dans les années 90 :

1. Production d’enzymes purifiées recombinantes

2. Développement de systèmes de vectorisation au colon
Orally Administered Targeted Recombinant Beta-Lactamase Prevents Ampicillin-Induced Selective Pressure on the Gut Microbiota: a Novel Approach to Reducing Antimicrobial Resistance

Jaana Harmoinen, Silja Mentula, Matti Heikkilä, Michel van der Rest, Päivi J. Rajala-Schultz, Curtis J. Donskey, Rafael Frias, Pertti Koski, Nina Wickstrand, Hannele Jousimies-Somer, Elias Westermarck, and Kai Lindevall

**18 fistulated dogs randomized into 3 groups:**
1. Ampicillin IV 40mg/Kg IV + placebo
2. Ampicillin + P1A B-lactamase (penicillinase *Bacillus*) 8.2 mgX4
3. Placebo + placebo

**End points:**
1. Composition of the flora (DGGE)
2. Emergence of Ampi-R enterobacteria
Changes in similarity index and counts of amp-R coliforms in dogs

Similarity index  DGGE

Counts of amp-R coliforms

Tarkkanen AM et al. AAC 2009
36 healthy volunteers randomized into 3 groups:

1. Ampicillin IV 1gX4
2. P1A β-lactamase (penicillinase Bacillus) 8.2 mgX4
3. Both

End points:

1. Composition of the flora (DGGE)
2. Emergence of Ampi-R enterobacteria
Changes in similarity index and counts of amp-R coliforms in volunteers

Similarity index  DGGE

Counts of amp-R coliforms

Tarkkanen AM et al. AAC 2009
Oral Recombinant $\beta$-lactamase (Ipsat P1A) Prevents Ampicillin (AMP)-Induced Resistance in Gut Microflora in Patients with Respiratory Infections

<table>
<thead>
<tr>
<th>End-points</th>
<th>P A1 B-lactamase (n=54)</th>
<th>Placebo (n= 58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amp-R coliforms</td>
<td>Reduction 24.1% [CI 95% 39-9] p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>SIM % (DGGE)</td>
<td>Reduction 16.1% [CI 95% 9-24] p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Resolution of infection</td>
<td>No difference</td>
<td></td>
</tr>
<tr>
<td>Side effects</td>
<td>No information</td>
<td></td>
</tr>
</tbody>
</table>

JARVINEN A. et al. ICAAC 2008
Enzymatic approach : current status

• New developments in the US and in Europe suing cephalosporinases and carbapenemases
• However the spectrum is limited to beta-lactams.
• Therefore we chose an wider approach using a non specific adsorbent (The DaVolterra project)
The concept behind DAV 132
Pre-clinical efficacy of DAV 131

Chez les chiens traités par Lévofoxacin IV

Figure 2: Fecal concentrations of levofloxacin

Figure 3: Plasma concentrations of levofloxacin at D4

Chez les souris traitées par Cefotaxime IP

Figure 5: Efficacy of DAV131 in adsorbing the CTX residues in the colon

Figure 6: Efficacy of DAV131 in preventing CTX-induced establishment of K. pneumoniae PUG-2 strain

ICAAC 2012
Two phase 1 studies in human volunteers of the safety/efficacy of DAV132 to protect the microbiota during antibiotic treatments.

During 1-Day treatment with ampicillin
N=18

During 5-Days treatment with moxifloxacin
N=40
Figure 1: Free MOX fecal concentration (mean ± SD) over D1-D16 (LOQ 40 ng/g; MOX MIC for Enterobacteriaceae 0.5 μg/mL)

Figure 2: MOX plasma concentration (mean Log ± SD) over 24h on D5 (LOQ 10 ng/ml)

Figure 3: Normalized microbiome gene richness ratio (median, quartiles, 1.5 interquartile range, and outliers) from D1 to D37 in subjects treated with (a) MOX, (b) MOX+DAV132, (c) DAV132, and (d) negative control.
How can we do that?

1. Reduce antibiotic usage
2. Modify antibiotic usage.
3. Decrease antibiotic concentrations in the colon during treatments
4. Use of Fecal transplant or « new » probiotics
**E. coli** (Mutaflor) probiotic and MDR colonisation in elderly nursing home pts

<table>
<thead>
<tr>
<th>Site of colonisation</th>
<th>% positive after 5 wks Rx</th>
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<tbody>
<tr>
<td></td>
<td>Placebo N=36</td>
</tr>
<tr>
<td></td>
<td>Probiotic N=33</td>
</tr>
<tr>
<td>Feces</td>
<td>58</td>
</tr>
<tr>
<td></td>
<td>77</td>
</tr>
<tr>
<td>Urine</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>75</td>
</tr>
</tbody>
</table>

Tannock, GW et al. JMM 2011
Effect of probiotic on VRE colonisation in patients.

Multispecies probiotic
*Bifidus 4, Lactobacillus 5, Enterococcus 1*

\[\text{VRE colonisation} \]
Evolution of rectal carriage of ESBL before during and after colistin/tobramycin decontamination.
Evolution of rectal carriage of ESBL before during and after short colistin/tobramycin decontamination.

Can the addition of a probiotic-FT prevent the rebound of colonisation?
✓ 64 pts in 4 EU centers
✓ ESBL or CPE infections resolved but persistent carriage
✓ Decontamination plus FT vs No treatment
✓ End point: Lack of carriage 35-48 days after randomisation
✓ Starting T1-2016
Man 60y, HTA, end-stage renal diseases, Two allotransplantation (2000-3)
2006-12 X epidodes aof pyelonephritis ESBL E. Coli
2012 Graft failure, Peritoneal dialysis

Fecal transplantation in 2013 (protocole FECAL trial). No previous antibiotic
One single duodenal infusion
Restal culture negative for ESBL from Week 2
Follow-up 12 weeks. No symptom of infection.
A new type of probiotics coming from the americans?

Comparison of the « active » microbiota of 8 ESBL (CTX-M carriers to 24 non carriers controls
Heat maps and clustering based on taxon composition and abundance (Bray-Curtis distance) in the active microbiota (16S rRNA).

ESBL carriers blue
Non-carriers black.
Relative abundance of bacterial non-carriers biomarkers in active microbiota. Dashed line represents mean value.
A wrap-up

1. The impact on microbiota is clearly a key secondary effect in the dynamic of resistance and the occurrence of infections.

2. Its control, and possibly its manipulation, could lead shortly to significant progress in the management of infections caused by enteric bacteria.

3. These two facts may change atibiotic usage and stewardship in the future.
Merci beaucoup pour votre attention !