Microbiota transplantation and/or CRISPR-Cas in the battle against antimicrobial resistance.

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In the last decades we have witnessed a dramatic increase in infections caused by multidrug-resistant bacteria (MDRB). Organizations such as the European Center for Disease Prevention and Control (ECDC) and the World Health Organization (WHO) consider these infections as an emerging global disease and a major public health problem. Although the development of new antibacterial drugs seems to have reached a dead-end, potential new therapeutic strategies can be pursued [1]. Recently, WHO has reported a list of antibiotic resistant bacteria to guide the investigation, discovery and development of new antibiotics, mentioning as priority # 1 (critical) those Gram-negative bacilli such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and 3rd generation cephalosporin and/or carbapenem resistant *Enterobacteriaceae* [2].

In recent years, measures are taken to control the emergence and spread of multi-resistant bacteria (Figure) as well as to encourage the pharmaceutical industry to design and develop new antibiotics or new therapeutic strategies [3].

In the nosocomial setting the control of the spread of MDRB is a crucial issue to prevent further infections. Patients colonized with MDRB can serve as reservoir for further dissemination of these bacteria and even be on risk to develop an infection caused by these bacteria. Although spontaneous loss of some of these MDRB can occur, selective digestive decontamination (SDD) with specific antibiotics has been used to decolonize the intestinal tract, eliminating MDRB. However, there is evidence both in favour and against this approach [4-8].

There are a couple of alternatives which may be useful to eliminate MDRB from the intestinal tract: 1. The use of “healthy” microbiota or faecal microbiota transplantation (FMT) to displace the MDRB, and 2. The use of the CRISPR-Cas system to specifically eliminate the gene(s) encoding resistant determinant(s).

The use of faecal microbiota transplantation in the decolonization of MDRB have been reported, showing an effect in some patients in whom the elimination of MDRB was successful and others have not [9-17] (See Table). In a recent clinical trial performed by Bilinski and colleagues [17] on the use of FMT in patients with hematological diseases colonized with MDRB, complete MDRB decolonization after FMT was reached in 15/20 (75%). Overall, although it seems that the use of FMT to eliminate MDRB from intestinal tract is a promising strategy, several aspects should be taken into account. How many times should FMT be performed? What will the impact of using FMT in healthy people just to eliminate MDRB be? Would it be as successful as in patients? What is the best way of administration? FMT may also have an impact on factors beyond intestinal homeostasis such as the function of the immune system or the metabolic and neuropsychological health of the recipients [18]. In the future it

will likely be possible to design synthetic antimicrobial susceptible microbiota to be used in the FMT.

The discovery of the clustered regularly interspaced short palindromic repeats and their associated proteins Cas (CRISPR-Cas system) is a cutting-edge technology that has different applications. One of this application is its use as a system to knock-out a specific bacterial gene, since CRISPR induces double stranded breaks. In this sense, CRISPR-Cas has been used to target specific genes for resistance located in plasmids. Knocking out these genes can re-establish the susceptibility of the bacteria to the antibiotic [19-22]. The major limitation is the delivery of the genetic construct to the bacteria. At present, it can be achieved using bacteriophages or plasmids transmissible by transduction or conjugation, respectively. Citorik et al. [19] used M13-derived phagemids encoding the genes of the CRISPR-Cas system. A phagemid is an engineered bacteriophage derived from a phage and a plasmid. This phagemid can be packaged into phage particles and used to deliver CRISR-Cas to the bacteria. The authors generated a genetic construct containing two spacers ("spacer" is the sequence in the CRISPR-Cas system which defines the genomic target to be modified) to target the $bla_{\text{NDM-1}}$ and $bla_{\text{SHV-18}}$ genes and observed a reduction of 2- to 3-$\log_{10}$ in viable *Escherichia coli* cells carrying plasmid containing the $bla_{\text{NDM-1}}$ or $bla_{\text{SHV-18}}$ genes but not in the wild type strain.

Similarly, Yosef et al. [21] engineered a temperate λ prophage to carry the CRISPR-Cas system encoding spacers that target the $bla_{\text{NDM-1}}$ and $bla_{\text{CTX-M-15}}$ genes. The authors also found that lysogenic phage carrying CRISPR confer lytic phage resistance. An advantage is that the CRISPR-Cas system allows multiplexing against different targets, enabling simultaneous targeting of various resistance genes. Would this approach be efficacious in removing resistant genes from MDR bacteria found in the intestinal microbiota? The main limitation is to have a collection of appropriate temperate phages designed against multiple resistance genes and that resistance gene carried by the bacteria should be known. At present this is feasible. Phages seem to be well-tolerated when orally administered. Oral phage therapy for targeting bacteria located in the intestinal tract has been successfully used. In order to use the CRISPR-Cas approach *in vivo,* the stomach must first be passed since deactivation of bacteriophages by acid may occur. Further investigation is needed to determine how this can be achieved in order for the phages to reach to the intestinal tract and still be active as well as establish the optimal doses to be used. One of the main advantages of this approach is that the susceptibility to antibiotics can be restored without compromising the normal microbiota of the individual.

The development of either of these two approaches to an extent to which they can be efficiently and safely used to eliminate MDRB from the intestinal tract or any other location

would be a revolution in the battle against the threat of the antimicrobial resistance. They could be applied to patients carrying MDRB in different settings to prevent subsequent infections by these MDRB and dissemination of the MDR strain as well as to people returning from a trip to a country with a higher prevalence of MDRB and who have a high probability of carrying these bacteria in the intestinal tract and transferring the bacteria to relatives living in the same house [23]. In addition, they could be applied to animals since they have shown to play an important role as reservoirs of MDRBs. In conclusion, I am very optimistic about the use of these two approaches to combat antimicrobial resistance. In an ideal situation, it would be possible to use either "natural" or synthetic FMT to restore the disturbed microbiota, whereas CRISPR-Cas could be used to specifically sensitize resistant bacteria.

Whatever the future may hold, it will certainly be interesting to watch and participate in the advancement of these two approaches in containing antimicrobial resistance.

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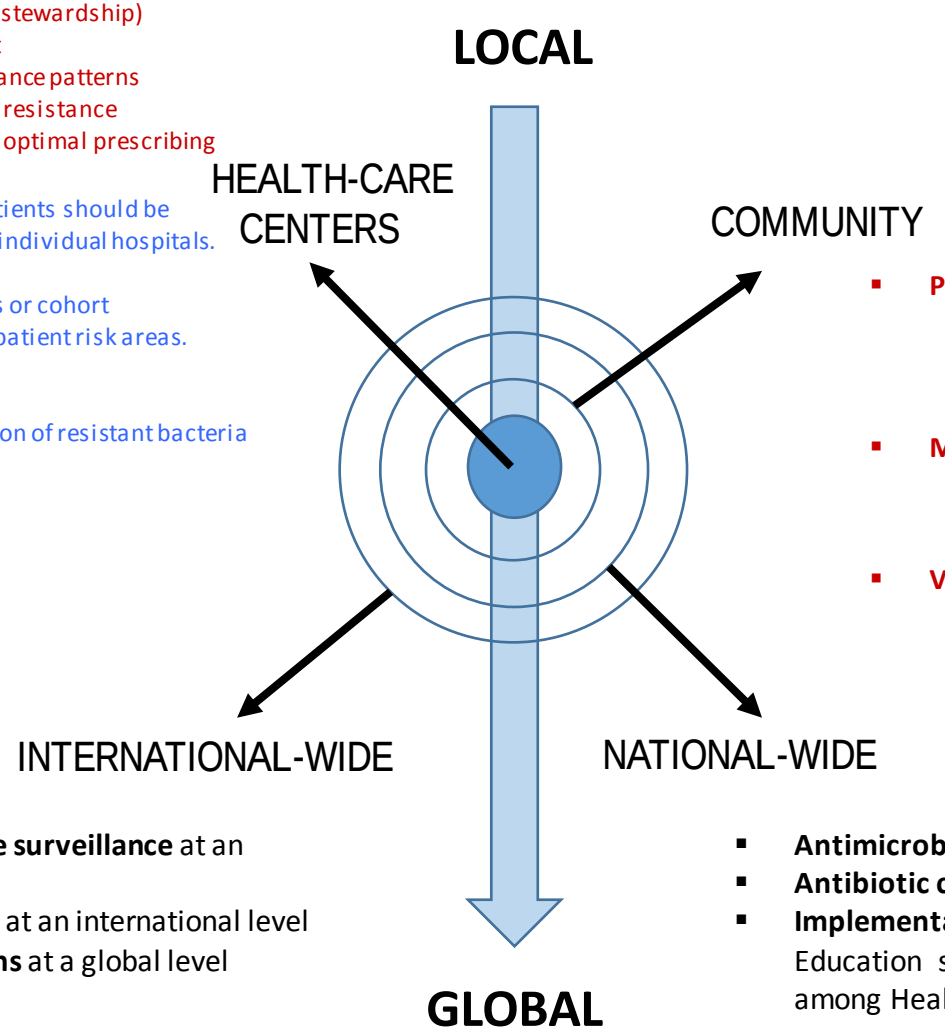
How to diminish the emergence and spread of antimicrobial resistance bacteria?

- **Antimicrobial stewardship**
  - ✓ Leadership commitment and appointing AS team
  - ✓ Rapid and affordable diagnostic (Diagnostic stewardship)
  - ✓ Systematic evaluation of on-going treatment
  - ✓ Monitoring antibiotic prescribing and resistance patterns
  - ✓ Reporting information on antibiotic use and resistance
  - ✓ Education of clinicians about resistance and optimal prescribing
- **Control**
  - ✓ Active screening of contacts of MDR-GNB patients should be decided on the background of prevalence in individual hospitals.
  - ✓ Screening of patients upon admission
  - ✓ Barrier isolation and isolation in single rooms or cohort isolation are implemented on the basis of inpatient risk areas.
  - ✓ Hand hygiene
  - ✓ Environmental cleaning
  - ✓ Decolonization of the MDRB or or sensitization of resistant bacteria



- **General Population**
  - ✓ Adhere strictly to therapeutic schemes
  - ✓ Respect proper treatment duration
  - ✓ Avoid self-medication with antibiotics
  - ✓ Proper cooking and handling of food
  - ✓ Hand-washing, when:
    - ✓ Before eating
    - ✓ Before and after touching a sick person
    - ✓ After using the bathroom
    - ✓ After touching an animal or handle animal waste
    - ✓ After handling rubbish
    - ✓ After being in places frequented by many people (example: public transportation)
- **Pharmacists**
  - ✓ Reject to sale without prescription
  - ✓ Inform patients about when are antibiotics needed, how to take them correctly and the consequences of a misuse.
- **Medical doctors**
  - ✓ Practice safe prescription of antibiotics
  - ✓ Use of point-of-care tools to ensure when are antibiotics needed
- **Veterinarians**
  - ✓ Reduce the use of antibiotics in livestock
  - ✓ Avoid using antibiotics as prophylaxis
  - ✓ Provide veterinarians with latest information regarding antimicrobial resistance
  - ✓ Sensitization of resistant bacteria

- **Antimicrobial resistance surveillance** at an international level
- **Antibiotic consumption** at an international level
- **Defining integrated plans** at a global level

- **Antimicrobial resistance surveillance** at a national level
- **Antibiotic consumption** at a national level
- **Implementation of action plan** in each country in terms of Education strategies among General Population as well as among Health specialists and Veterinarians

Table. Use of FMT for patients with intestinal tract colonization with resistant bacteria.

Design of the study	N° Patients	Bacteria targeted	Intervention used	% of success	Length of follow up	Ref.
Case report	1	ESBL-producing *E.coli*	FMT by naso-duodenal tube	ESBL-*E.coli* negative in stool after 2 week	1,2,4 and 12 weeks	9
Case report	1	Several MDROs	FMT by colonoscopy	Success	25 month	10
Case report	1	VRE	FMT by naso-duodenal tube	Relative abundance of VRE (84%) before FMT (24%) after 3 week (0.2%) after 7 month	1, 3 weeks and 7 months	11
Case report	1	OXA-48 producing *Klebsiella pneumoniae*	FMT by naso-duodenal tube	Success	7 and 14 days	12
Case report	1	VIM-1 producing *Klebsiella pneumoniae*	Colonoscopy	Success	1 and 6 weeks and 6 months	13
Case report	1	ESBL-producing *E.coli*	FMT by naso-duodenal tube	No success	1 week to 3 month	14
Prospective single-centre study*	11	VRE	FMT via enema	72.7%	7, 30, and 60 days and 6 months	15
Pilot prospective multicentre study	8	CRE and VRE	FMT by naso-duodenal tube	25% (1st month) 37.5% (3rd month)	1 and 3 month	16
Prospective single-centre study	20	Several MDROs	FMT by naso-duodenal tube	75% (1 month) 93% (6 month)	1 and 6 months	17

***** Stool VRE clearance in a post hoc analysis of the Phase 2 PUNCH CD study assessing a microbiota-based drug for recurrent *Clostridium difficile* infection.