1 Title
Multimodal treatment of intestinal carriage of multi-drug resistant bacteria with probiotics, prebiotics and quorum-sensing inhibitors.

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3 Background

3.1 Evolving antimicrobial resistance
Antimicrobial resistance is one of the biggest public health challenges of our time. Indeed, resistance to critically important antimicrobials, especially carbapenems, 3rd and 4th generation cephalosporins, fluoroquinolones and antipseudomonal penicillins is high or even increasing in many settings. For instance among European isolates of Klebsiella pneumoniae, resistance to 3rd generation cephalosporins (3GC) increased and reached 56% in Italy and 47% in Portugal in 2017, while fluoroquinolone resistance reached 58% in Italy and 49% in Portugal [1]. Meanwhile, in Pseudomonas aeruginosa isolates, resistance to piperacillin-tazobactam increased in some countries (e.g., to 19% and 27% in Spain and Portugal, respectively), and a marked increase of carbapenem resistance was observed in Hungary (39% in 2017) [1]. High levels of antimicrobial resistance are also encountered in Palestine and surrounding countries. Indeed, a Palestinian recent study showed that 81% of Campylobacter isolates were resistant to ciprofloxacin [2]. Among E. coli isolated from urine in Paslestinian hospitals in 2011, 56%, 71% and 22% were resistant to ciprofloxacin, cefotaxime and imipenem, respectively [3]. multidrug resistant organisms (MDRO) were detected in 73% of Syrian patients with positive wound cultures in 2014-2016 [4].

3.2 The intestinal microbiota, a major reservoir of MDRO
Intestinal colonization by a MDRO may evolve from asymptomatic carriage to various infections - mainly urinary, digestive and bloodstream infections. Furthermore, digestive carriage of MDRO can lead to environmental contamination and transmission to healthy or diseased subjects. Hence, decreasing and even deleting the digestive carriage of MDRO is of major importance to limit the world-wide spread of antimicrobial resistance.
### 3.3 Bacterial species protective against MDRO carriage

Probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [5]. Using live bacteria to counter intestinal colonization by MDRO after antimicrobial therapy is becoming increasingly plausible with the improving knowledge of the structure and functions of the intestinal microbiota [6]. Lactic acid bacteria have been studied as potential probiotics for one century [7]. For instance, *Lactobacillus reuteri* colonizes the gastro-intestinal tract of humans and animals, and secretes various antimicrobial substances like reuterin, lactic acid, acetic acid, ethanol, and reutericyclin, that inhibit a wide range of pathogenic gram negative bacteria [7,8]. However, the antimicrobial activity of *Lactobacillus* show high variability across strains [9]. Furthermore, some authors have highlighted the need for combining two different bacterial species to reduce intestinal colonization of pathogenic Enterobacteriaceae [10,11].

The genus *Bacillus* comprises different species of soil bacteria that form endospores, that are commonly ingested with vegetables, leading to concentration of *Bacillus* spores in human faeces around $10^5$ colony-forming units (CFU) per gram [12]. Non pathogenic *Bacillus* species include *B. subtilis*, but also *B. clausii*, *B. pumilus* and *B. licheniformis* [12]. *Bacillus* produces compounds that confer an antimicrobial activity against various human pathogens [13]. Sporulation may confer an advantage to *Bacillus spp* over other potential probiotics, as it allows long term conservation in ambient conditions. Indeed, oral treatment with *Bacillus subtilis* has been shown to prevent various digestive infections in chicken, rabbit, fish and mouse, including infections due to *Escherichia coli* [13–17]. It also abrogated *S. aureus* asymptomatic colonization in a murine experimental model [18]. However, whether it decreases the asymptomatic intestinal colonization of other MDROs remains unknown.

### 3.4 Prebiotics to treat MDRO carriage

Prebiotics are substrates that are selectively utilized by host microorganisms conferring a health benefit [19]. As stated by an international consensus panel, "currently established prebiotics are carbohydrate-based, but other substances such as polyphenols and polyunsaturated fatty acids converted to respective conjugated fatty acids might fit the updated definition assuming convincing weight of evidence in the target host" [19]. For example, among carbohydrates, galacto-oligosaccharides and fructo-oligosaccharides selectively favors the in vitro growth of lactobacilli and bifidobacteria [20]. However, the efficacy of prebiotics to treat MDRO intestinal carriage in experimental models has been poorly documented. For instance, galacto-oligosaccharides had a limited effect on *Salmonella enteritidis* cecal colonization in day-old pullet chicks [21].

### 3.5 Modulation of quorum-sensing

Quorum sensing is a key process for the production of virulence determinants in pathogenic bacteria. *Bacillus*-induced abrogation of *S. aureus* intestinal colonization is mediated by the inhibition of *S. aureus* quorum sensing [18]. It has been recently shown that flavonoid molecules inhibit the quorum sensing of Gram negative bacteria [22]. In particular, quercetin inhibits expression of quorum sensing-associated genes, thereby downregulating the virulence attributes of *E. coli* O157:H7 both in vitro and in vivo [23]. However, it remains
unknown whether molecules that inhibit quorum-sensing in Enterobacteriaceae may decrease the asymptomatic intestinal carriage of MDROs.

4 Objectives
The aim of this PhD thesis is to identify multimodal preventive and curative treatments of intestinal carriage of MDROs in a murine experimental model. Multimodal treatments will include non pathogenic bacteria (so-called probiotics), in particular *Bacillus subtilis*, prebiotics and quorum sensing inhibitors. Targeted MDROs are ESBL producing *Escherichia coli* and *Klebsiella pneumoniae*, carbapenemase producing *Klebsiella pneumoniae*, Glycopeptide Resistant *Enterococcus faecium*.

5 Methods

5.1 Selection of MDROs and potential treatments of MDRO intestinal carriage

- **MDROs** that will be used in the intestinal colonization murine model will be selected from clinical isolates that have been studied in the MiHAR Lab.
- **Bacillus spp strains**: *Bacillus spp* strains will be screened from environmental samples or from faeces of mice treated with amoxicillin. They will be selected according to 2 criteria: (i), ability to persist in murine intestinal microbiota; (ii), in vitro activity against targeted MDROs (either growth inhibition or quorum sensing inhibition).
- **Prebiotics** will be selected through a systematic review of the literature, including experimental studies that assessed the efficacy of prebiotics on intestinal colonization and/or infection. This review will be the first paper of this PhD thesis.

5.2 Experimental assessment of the activity of selected treatments on MDRO intestinal carriage

- **adaptation of the murine model of asymptomatic intestinal colonization by ESBL-producing *E. coli***
  - Intestinal dysbiosis will be induced with amoxicillin according to a murine model currently used in the laboratory. The murine model will be adapted to allow a persistant carriage of *Bacillus spp*. *Bacillus* carriage will be assessed with culture and qPCR.
- **in vivo activity of *Bacillus spp* against intestinal colonization by ESBL-producing *E. coli***
  - The activity of each treatment, alone and in combination, will be assessed on the intestinal and faecal concentrations of cultivable ESBL-producing *E. coli*, by comparison with a control group.
5.3 Relationship between microbiome architecture and therapeutic efficacy

The microbiome structure will be assessed by amplification of the V4 region of bacterial 16S rRNA. Analyses will be conducted to assess whether treatments (Bacillus spp, prebiotics, flavonoids) alter the murine intestinal microbiome, and whether treatment efficacy is explained by the alteration of the intestinal microbionas. Furthermore, if the treatment efficacy shows inter-mouse variability, we will assess whether the individual pre-treatment microbiome predicts treatment's activity.

6 References


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