MICROBIALS 2018
**PROJECT PROPOSAL**

*Guidelines*
- When composing your proposal, please follow the order given below and answer all questions. We cannot accept proposals with incomplete information.
- Please use Times New Roman 11 in the entry field (shaded background).
- Limit your project proposal to 10 pages (excl. attachments).
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- Please upload one single PDF file (including the proposal, CV and all attachments) on the «Microbials» webportal.
- The PDF file is part of the full application and must be uploaded no later than 20 July 2018.
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**BASIC INFORMATION**

**01 LAST NAME, FIRST NAME** Egli, Adrian **DATE OF BIRTH** 27/09/1978

**02 PROJECT FULL TITLE** Developing microbials to fight extended beta-lactamase (ESBL)-producing Escherichia coli

**PROJECT SHORT TITLE** Displacing ESBL

**APPLICANT AND TEAM**

**03 MAIN APPLICANT** –Adrian Egli, PD, Dr. med., Dr. phil., Head of the Division of Clinical Microbiology at the University Hospital Basel; specialist in clinical microbiology and diagnostics, focusing on host-pathogen evolution.

**04 PROJECT TEAM** – *Short information on other persons involved in the project?*

We have formed an interdisciplinary team to develop innovative approaches to tackle the ESBL-producing E. coli problem. Dr. med. MSc. Esther Künzli is a specialist in Infectious Diseases and Travel medicine at the Swiss Tropical and Public Health Institute; she focusses on travel related transmission of multi-drug resistant bacteria and will recruit the India travelers for our clinical study. Dr. rer. nat. Wolf-Dietrich Hardt is a specialist in molecular infection biology and the evolution of virulence. He is professor of microbiology at ETH Zurich and will provide his world-leading expertise in mouse models for E. coli and Salmonella gut infections. Dr. Federica Sallusto is professor of immunology at the ETH Zurich and specialized in T- and B-cell immunology. Dr. Sebastian Bonhoeffer is a specialist on mathematical modelling of infectious disease dynamics. He has worked extensively on the evolution of resistance to antivirals and antibiotics. He is professsor of theoretical biology at the ETH Zurich where he leads a group working both theoretically and experimentally on microbial population biology.

**PROJECT DESCRIPTION**

**05 THEMATIC CONTEXT** – *What is the wider context of the project?*

Antibiotic resistance is a severe threat to contemporary medicine. We urgently need effective approaches to fight multi-drug resistant pathogenic bacteria. Here, we will focus on multidrug resistant E. coli strains, which express extended spectrum beta lactamases (ESBL E. coli). These notorious pathogens are on the rise world-wide and are classified priority 1 by the World Health Organization (WHO). So far, there are no effective means to prevent the spread of ESBL E. coli.

Our project develops a radically new approach to fight ESBL E. coli. We hypothesize that ESBL E. coli can be efficiently removed from patients by applying well-defined microbials. Our approach roots in the observation that >50% of the colonized people will spontaneously "loose" the colonizing ESBL E. coli strain within 6-18 months (1). Based on our previous work, we hypothesize that the ESBL E. coli is out-competed by other, ideally pan-sensitive strains (non-ESBL E. coli; Figure 1A, blue symbols). Ingestion of such competitive strains appears to be a random event that initiates the displacement of the ESBL E. coli strain. Such strains are natural microbials and could provide a powerful means to stop the spread ESBL E. coli. We want to verify this hypothesis in a clinical study of ESBL E. coli displacement in the patient's gut, isolate non-ESBL competitor E. coli strains and verify their capacity to decolonize ESBL E. coli patients. Such displacing microbials could be applied to ESBL colonized patients (but potentially also to other multi-drug resistant bacteria such as Carbapenemases or Vancomycin resistant Enterococcus feacium), which eliminates the waiting time for spontaneous exposure and swiftly stops colonization by ESBL E. coli. This would provide a powerful novel strategy for addressing the ESBL E. coli crisis.

## 06 STATE OF THE ART

Antibiotics have dramatically reduced the mortality burden inflicted by pathogenic bacteria. However, today these achievements are in jeopardy, as bacteria increasingly acquire antibiotic resistance. At the same time, virtually no new antibiotic classes are being discovered and developed for clinical use. Thus, there is concern that important bacterial

infections may become untreatable once again. In response to this threat, the G20 industrial countries, the World Health Organization (WHO), and numerous nations have initiated concerted efforts during the past five years. In 2017, the WHO has released a priority list to focus research and development into the most notorious antibiotic resistant pathogens. **ESBL *E. coli* is 1st priority**. Its incidence is rising and is currently at 13% of all *E. coli* isolates in Switzerland (www.anresis.ch), up to 40% in eastern European countries such as Bulgaria (ecdc.europa.eu), and up to 70-80% in other regions of the world (2). This underlines the urgent need for immediate action. However, **we currently lack proven, easy and fast solutions**. Therefore, we propose a radically new approach to fight back antibiotic resistance. Due to their extended spectrum beta lactamases (ESBL), these *E. coli* strains are resistant to third generation cephalosporins. This class of antibiotic drugs is commonly used as empiric treatment of severe Gram-negative infections. ESBL *E. coli* isolates often carry additional resistances rendering further highly effective antibiotics, such as cotrimoxazole or ciprofloxacin, useless (3, 4). While ESBL carriage itself is not associated with disease, the antibiotic resistances severely limit therapy options in the case of infections. Patients colonized with ESBL *E. coli* show a more severe clinical course in the case of infections (5-8). Infections with ESBL *E. coli* include urinary tract infection and pyelonephritis, which may progress to abscesses, sepsis and septic shock. Due to the lack of efficient empiric treatment, ESBL *E. coli* infections lead to higher morbidity and mortality than infections with non-ESBL *E. coli* (9, 10). In addition, the ESBL *E. coli* infections require antibiotics with even broader activity spectra (e.g. carbapenems or colistin), eventually selecting for more resistant strains. Thus, new approaches to combat the ESBL *E. coli* problem are urgently needed.

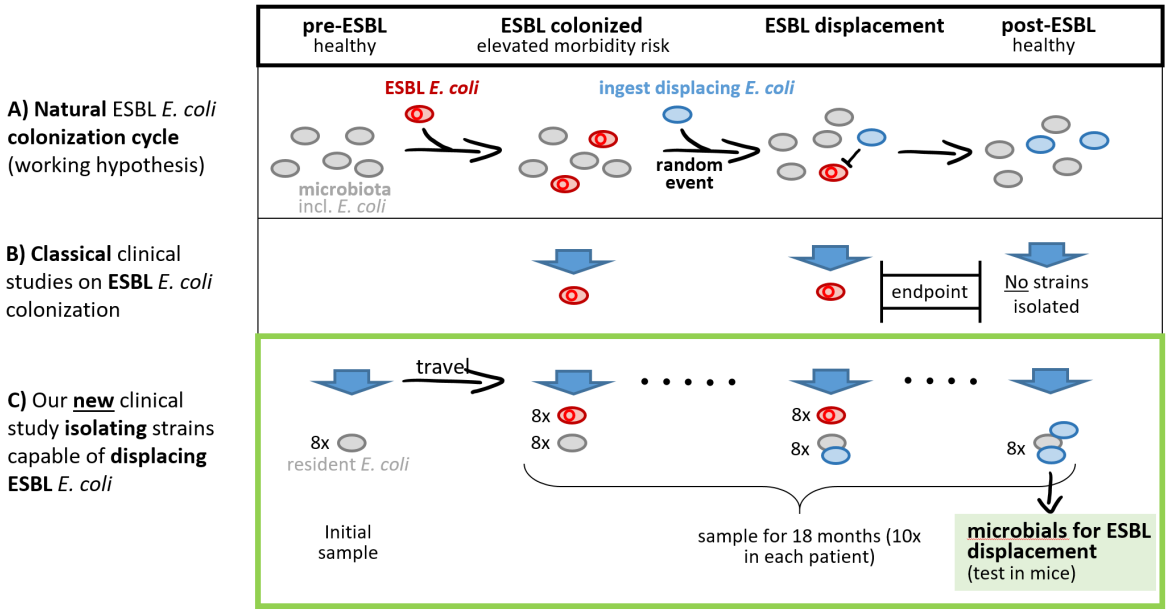


**Figure 1. *E. coli* strain displacement in the host's gut.** The normal human microbiota includes typically one or more *E. coli* strains. These are part of the normal microbiota. Most are pan-sensitive to antibiotics. **(A)** Individuals can be colonized with ESBL *E. coli* (red) upon exposure (random process, e.g. when travelling to East Asia). Without treatment, >50% of the colonized individuals return to an ESBL-free status within 6-18 month. We hypothesize that this is due to competitive displacement by benign, pan-sensitive *E. coli* strains (blue). **(B)** Typically, clinical studies monitor the ESBL *E. coli* strain itself, but fail to isolate pan-sensitive *E. coli* strains which may drive ESBL-displacement. **(C) Our new study design:** In our clinical study, we will systematically sample the entire *E. coli* population. We will employ genome sequencing and phenotypic resistance testing to characterize the ESBL *E. coli* strain of interest, to monitor gut resident (grey) and to identify (and collect) newly incoming non-ESBL *E. coli* strains (blue), which likely include strains capable of displacing the ESBL *E. coli* (red). In the second part of the project (**Figure 2**), we will use these *E. coli* strain collections to establish which non-ESBL, ideally pan-sensitive *E. coli* strains are quickly displacing ESBL *E. coli*. We propose that such strains are **ideal *microbials* to fight the global ESBL *E. coli* problem**.

To fight ESBL *E. coli*, we have formed a highly collaborative team with complementary world-class expertise in travel medicine, infectious diseases, clinical microbiology, immunology, pre-clinical gut colonization models, and mathematical modeling. Our project is rooted in the observation that ESBL *E. coli* carriage is typically transient (**Figure 1A**). Strikingly, colonized people tend to carry the ESBL *E. coli* strain for no more than 6-18 months, before they regain an ESBL-negative status, even without any clinical intervention (1). Thus, natural mechanisms must exist that terminate ESBL *E. coli* colonization. **We want to determine whether these natural mechanisms could be exploited for decolonization**.

The transient nature of ESBL *E. coli* colonization is particularly well-documented in travelers returning from East Asia and includes studies by our own team (Kuenzli, Jaeger et al. 2014). Before the journey, most Swiss travelers are ESBL-negative (ESBL colonization status of ≈2%). This is in line with a relatively low ESBL incidence in the young travelling population in Switzerland. During the travel, 70-80% become colonized with ESBL *E. coli* (11-15). These people can transmit the ESBL *E. coli* strains and are themselves at risk of increased morbidity and mortality during infections (see above). Multiple body sites can be colonized (16). Exposure time to colonized people is highly critical in terms of transmission at the hospital setting (17, 18). Thus, in communities and households, spread of ESBL *E. coli* by transmission can easily occur. Decolonization would be ideal to reduce both, the risk for the patient and the spread of ESBL *E. coli*. While colonization is clearly reversible, it has remained unexplored why the ESBL *E. coli* strains are lost. This is because of the design of the clinical studies that are classically used to monitor ESBL colonization most often did not include non-ESBL *E. coli* isolates, as on commonly used screening plates only ESBL E. coli grow – thereby the dynamic changes of ESBL and non-ESBL *E. coli* could not be explored. (**Figure 1B**). We expect, that the mechanisms that naturally terminate ESBL *E. coli* colonization could provide powerful means for cure and prevention. Our project aims to identify such mechanisms and exploit them for therapy (**Figure 1C**).

Based on our previous work on *Enterobacteriaceae* population dynamics in the mammalian gut (19, 20), we hypothesize that ESBL *E. coli* is eliminated by other bacterial strains that are ingested in a random fashion from the environment, e.g. via food or water (**Figure 1A**, blue). Due to their similar physiology, benign *E. coli* strains pan-sensitive to the commonly used antibiotics are likely candidates. This concept has not been explored yet for ESBL *E. coli* and might offer a powerful new decolonization approach.

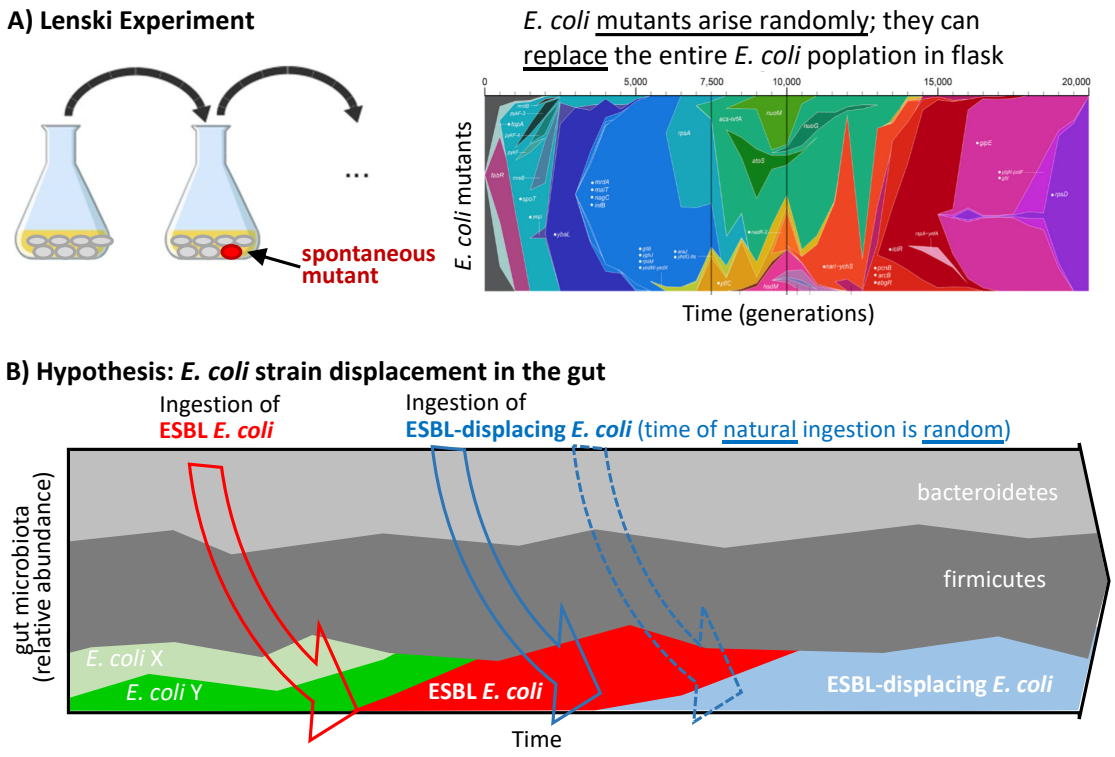


**Figure 2. *E. coli* strain displacement occurs in pure broth cultures and likely also in the gut. (A)** Lenski Experiment. Cultures of a well-defined *E. coli* clone were passaged for >60'000 generations. Sequence analysis of sampled clones revealed that mutants arise and quickly take over the entire flask-borne population (color changes in the right panel; adapted from (29, 30)). **(B)** The gut can be regarded as an open system, which is constantly exposed to newly incoming bacteria. Some newcomers, like ESBL *E. coli* (red) or pan-sensitive *E. coli* (blue) can successfully establish in this niche and even displace previous members (e.g. *E. coli* X (light green) and *E. coli* Y (dark green) by ESBL *E. coli* (red); or ESBL *E. coli* (red) by the ESBL-displacing *E. coli* (blue)). The graph focusses on the *E. coli* strains. Other phylotypes are represented in grey (e.g. bacteroidetes, firmicutes; hypothetical plot; not to scale).

It is well known that individual members of the gut microbiota can be replaced by others. While most "transiting" microbes are poorly adapted and incapable of colonizing the gut, some effectively colonize and can displace other strains (**Figure 2**). This is the principle for fecal transplantation therapy to resolve recurrent *Clostridium difficile* diarrhea (21). We have studied such strain replacements in mouse colonization models with *Salmonella* Typhimurium and closely related *E. coli* strains (19, 20, 22). Such strain displacement may be driven by numerous mechanisms, including nutrient competition, bacteriocins, Type VI secretion system-mediated killing or strain-specific bacteriophages (23-27). Based on this knowledge, we hypothesize that ESBL *E. coli* are under high competitive pressure in the gut of colonized hosts. The ESBL *E. coli* are likely in constant competition with other bacteria (i.e. other *E. coli* strains) that are constantly ingested from the environment. The famous long term evolution experiments by Richard Lenski on the evolution of isolated *E. coli* cultures have demonstrated that even spontaneously arising mutants, arising from an clonal *E. coli* strain, are sufficient to displace entire wild type *E. coli* populations (28)(**Figure 2A**). In the patient's gut the chance of displacement should be even higher, due to the abundant influx of new bacterial strains, including some pan-sensitive *E. coli* strains that can displace the ESBL *E. coli* (**Figure 2B**). Thus, ESBL *E. coli* should face a highly competitive environment in the patient's gut (**Figure 2B**, red symbols). This explains why ESBL *E. coli* are typically lost from the gut within 6-18 month and provides a huge untapped potential for decolonization therapy. We hypothesize that this displacement is driven by benign *E. coli* strains featuring two important characteristics: a) they are able to out-compete ESBL *E. coli* strains in the gut; b) they do not readily take up conjugative plasmids, such as those encoding ESBL enzymes; and c) they already harbor related plasmids that prevent the uptake of ESBL plasmids. Otherwise we would observe that one ESBL *E. coli* strain typically being replaced by another (which is not the case). Such **harmless, ideally pan-sensitive *E. coli* strains would be ideal microbials for decolonizing ESBL *E coli*-affected patients**. By applying such displacing *E. coli*, one could remove the "element of chance" (**Figure 2B**) and achieve immediate decolonization. The isolated benign, pan-sensitive *E. coli* strains could prove to be **powerful new "microbials"** to fight ESBL *E. coli*, a task of urgent worldwide importance.

## 07 PROJECT CONTENT

The project has two consecutive phases. **Firstly**, we will perform a prospective observational single-centre **clinical cohort study to monitor ESBL *E. coli* colonization dynamics in the human gut**. This study will enroll tourists travelling to India. This cohort has several important advantages over other cohorts (e.g. tertiary care patients): a) participants have very low ESBL colonization rates before the journey; b) they are otherwise healthy (which excludes many confounding artefacts); c) they can be sampled before the onset of ESBL *E. coli* colonization: this is particularly interesting to see, whether antibody responses contribute to ESBL displacement (discussed below; potential problems and pitfalls); d) they have a 70-80% chance of ESBL *E. coli* colonization during travelling. This maximizes the number of relevant cases to study during the 18 months monitoring phase (**Figure 3**). From the samples, we will generate a systematic strain collection of ESBL *E. coli* isolates and their corresponding pan-sensitive *E. coli* strains. We hypothesize that pan-sensitive *E. coli* strains capable of displacing the ESBL *E. coli* dominate the microbiota population as the ESBL strain is lost (see **Figure 2B**, blue strain), which will be tested in the **second phase** of the project. In the second phase, we will use a **mouse model for ESBL *E. coli* gut colonization**, which has recently been established in the Hardt lab (**Figure 4A**). These pre-clinical experiments will establish, whether pan-sensitive *E. coli* isolates from human participants can swiftly replace ESBL *E. coli* within the mammalian gut. Such natural strains would be the basis for future decolonization trials in ESBL *E. coli* positive humans.

### 7.A. Clinical study

Travelers to India will be identified and recruited by Dr. Esther Künzli via the travel clinic at the Swiss Tropical and Public Health Institute (Swiss TPH) in Basel, Switzerland. The study protocol has been prepared and will be send for approval by the local internal review board (Ethikkommission Nordwest – und Zentralschweiz, EKNZ). Inclusion criteria: age >18 years, travel to India, generally healthy with no need for regular antibiotic treatment. After written informed consent, patients will be instructed how to perform a rectal swab and how to ship the swabs directly to the laboratory for isolation of ESBL and non-EBSL producing *E. coli*. Before travel, we will enroll 40 patients. This will ensure that ≈30 are likely to return ESBL *E. coli* positive. Even if some participants might drop out along the study, we can still follow ESBL-displacement in at least 10 study participants (given a chance for ESBL *E. coli* clearance of ≥50% in 6-18 months). Participants will be reimbursed for travel costs and time investment.

A total of 40 participants will be sampled in our clinical study over 18 months (**Figure 3**). Duplicate rectal/stool swabs (ESwab, Copan) will be performed every two months. In our previous study 84% completed the study protocol. One swab will be used for isolating both a) ESBL *E. coli* strains (grow on ESBL selective screening agar, ChromID ESBL, bioMérieux); and b) pan-sensitive *E. coli* strains (grow on McConkey agar w/o antibiotics; replica-plating onto ESBL selective screening agar does not yield growth). We will store 8 ESBL and 8 pan-sensitive *E. coli* strains per time point per patient. All isolated strains will be stored at -80°C. The second swab will be stored to allow isolation of further *E. coli* clones, microbiota analysis of the isolation of other microbiota strains as (see below, problems and pitfalls). Every six months, we will invite the participants to Swiss TPH to obtain a blood sample (for anti-*E. coli* antibody analysis; see problems and pitfalls). A serum sample (5mL) for antibody measurement and peripheral blood mononuclear cells (PBMCs in 6 CPTs) for cell mediated immunity will be collected, aliquoted and stored at -80°C or liquid nitrogen, respectively. Serum and PBMCs will allow the analysis of anti-E. coli antibody responses, as needed by Dr. Federica Sallusto (see below, problems and pitfalls).

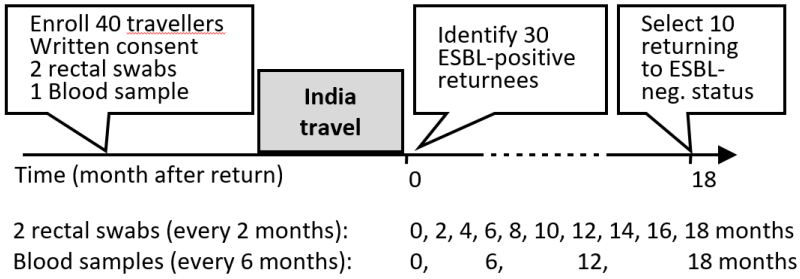


**Figure 3. Design of the clinical study.** The goal is to collect ESBL and pan-sensitive *E. coli* strains (8 per time point) and blood samples from 10 patients converting from ESBL E. *coli*-positive to -negative status.

Patients will receive, post travel, every 6 months a detailed questionnaire to document events such as additional travels, eventual antibiotic exposure, and changes in nutrition. The patient questionnaire will allow us to link particular external effects to changes observed in the *E. coli* composition.

The patient data will be pseudonymized and entered to a secured study related database. All medical and health care related data will be safely stored at the University Hospital Basel (A. Egli). The ESBL. *E. coli* and the pan-sensitive *E. coli* strains from 10 participants with evidence of ESBL-displacement will be subjected to detailed follow-up analysis at the Clinical Microbiology of the University Hospital Basel (for cost reasons limited to 8 ESBL and non-ESBL *E. coli* each per time point per patient).

i. Antibiotic resistance testing (routine clinical microbiology testing); minimal inhibitory concentrations determination for 20 commonly used antibiotics (31).
ii. Illumina sequencing (short read) of 8 ESBL *E. coli* clones per participant per time point (32-36). This will allow identification of the strain by core genome multi locus sequence typing (cgMLST) (37)) and detect background mutations that may arise.
iii. Illumina sequencing (short read) of 8 non-ESBL *E. coli* clones per participant per time point (32-36). This will establish the relationship between the most abundant non-ESBL *E. coli* strains present at each time point by cgMLST (37)).
iv. MinION sequencing (long read) of 1 representative ESBL *E. coli* clone per participant per time point (38). This will allow us to complete the chromosome sequence, identify the plasmids, determine the plasmid-localization of the detected resistances and pinpoint genetic rearrangements.
v. MinION sequencing (long read) of 1 representative non-ESBL *E. coli* clone per participant per time point (38). This will allow us to complete the chromosome sequence, verify the absence of unwanted virulence genes, identify the plasmids present in the strain and pinpoint genetic rearrangements.
vi. Plot of the *E. coli* population dynamics for each patient (as in **Figure 2**). Identify non-ESBL *E. coli* strains which appear during the demise of the ESBL *E. coli*. These non-ESBL *E. coli* are promising candidates for "ESBL displacement", ideally pan-sensitive to all tested antibiotics. This will be verified in the second phase of the project.

Potential problems and pitfalls:

a) Other bacterial species (not *E. coli*, such as *Klebsiella* spp. or *Enterococcus* spp.) displace the ESBL *E. coli* strains. In this case, we would turn to the second swab and analyze the patient's microbiota composition by plating (for isolating viable competitor strains) and by a 16S rRNA gene metagenomic sequencing approach (culture independent) to identify potential competitor strains. Independently of our study, this type of data is of high value to the community.

b) The immune response might help to eliminate the ESBL *E. coli* from the intestine (39). In this case, the ESBL competition experiments would be performed in naïve mice, to have no pre-existing adaptive immunity (see section 7B). We would analyze the patient's blood samples to quantify anti-*E. coli* antibody levels. If needed, we have suitable mouse vaccination protocols in place to test the antibody involvement in the mouse model: such as an attenuated vaccine from ESBL *E. coli*. If true, the patient's own ESBL isolates could serve as the basis for vaccine-driven ESBL elimination.

**7.B. Pre-clinical experiments to demonstrate E. coli driven displacement of ESBL *E. coli*.**

In the second phase, we will establish if non-ESBL *E. coli* strains (as isolated in section 7A) can efficiently displace ESBL *E. coli* from the mammalian gut. The Hardt lab has recently established a mouse model for *E. coli* gut colonization (20) which also works well for ESBL *E. coli* strains (**Figure 4A**). Using this mouse model, we will determine the colonization kinetics of the initial ESBL *E. coli* patient isolates (from 7.A.; ESBL *E. coli* isolated directly after the return from India) and quantitatively assess, whether non-ESBL *E. coli* (as isolated later from the same host) can efficiently displace ESBL *E. coli*.

Each ESBL *E. coli* isolate will be analyzed in two experimental groups (n=10 mice per group):

i. Undisturbed ESBL colonization kinetics: C57BL/6 mice (specific pathogen free; bred at the EPIC mouse facility of ETHZ) will be inoculated with the respective ESBL *E. coli* strain (5x10exp7 cfu, by gavage).

ii. Active displacement of ESBL *E. coli* by pan-sensitive *E. coli* (from 7.A.): C57BL/6 mice (n=10 mice per group; specific pathogen free; bred at the EPIC mouse facility of ETHZ) will be inoculated with the respective ESBL *E. coli* strain (5x10exp7 cfu, by gavage). After 7 days of ESBL *E. coli* colonization, we will orally inoculate with the non-resistant competitor *E. coli* strain of interest (from 7.A.).

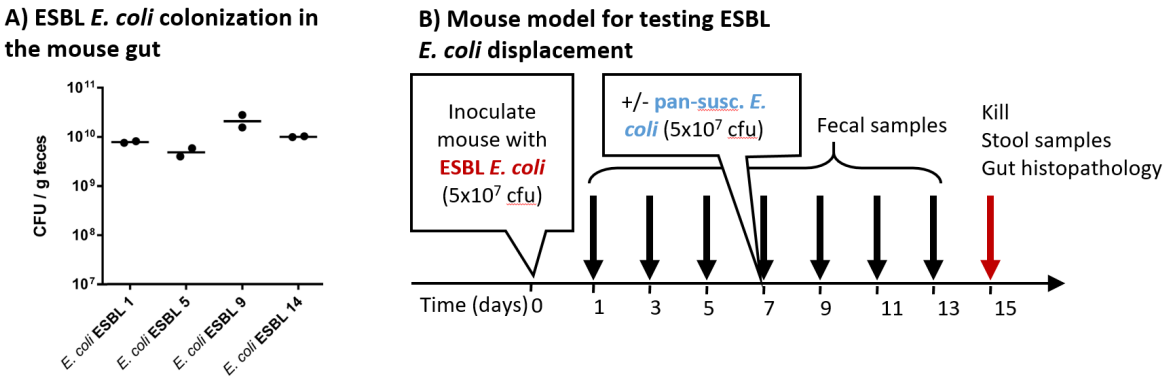


**Fig. 4 Mouse model for testing ESBL *E. coli* displacement**. **(A)** Pilot experiment for ESBL *E. coli* growth in the gut of C57BL/6 mice (fecal ESBL loads, 24h after colonization; Bakkeren, Egli, Hardt, unpubl.). **(B)** Experimental design for the mouse experiments from this proposal.

Gut luminal densities of the ESBL- and the non-resistant *E. coli* strains will be monitored by plating (i.e. using agar plates harboring appropriate antibiotics that differentiate between the ESBL and the non-resistant strains). We would focus on *E. coli* strains accelerating the displacement kinetics of ESBL strains. Histopathological analysis of mouse tissues will be an important step for verifying the benign nature of the pan-sensitive *E. coli* strains. To further ensure safety (to avoid the rise of new ESBL strains), we will perform *in vitro* and *in vivo* plasmid transfer experiments. This will aim to verify that displacing *E. coli* strains DO NOT pick up ESBL resistance plasmids. **Such *E. coli* strains would be a game changer allowing the swift decolonization of ESBL *E. coli* colonized patients**.

Potential problems and pitfalls:

a) *E. coli* mutants might arise during the experiments. These might affect the within host competition. Re-sequencing the whole genome of ESBL and non-resistant competitor *E. coli* strains will be performed to

determine if particular mutants of the acquisition/exchange of particular genetic elements might affect the strain displacement. If this were the case, we would re-construct such strains in a clean genetic background and perform competition experiments to verify the effect of the identified genetic changes.

b) The host's mucosal immune response may promote the displacement of the ESBL *E. coli* (see also 7.A). In this case, we would analyze the role of the mucosal secretory IgA response. We would generate attenuated vaccines of the respective ESBL *E. coli* strains, vaccinate the mice and establish if the mucosal IgA response accelerates ESBL displacement. Frozen blood samples from 7.A would serve as a clinical correlate.

c) Other bacterial species (not *E. coli*) are displacing the ESBL *E. coli* strains. Then, we would identify candidates displacing species from the second fecal swab samples stocked in 7.A. If bacteria cannot be cultured directly from the frozen samples, we would isolate similar strains from fresh human feces or retrieve similar strains from public repositories. Mouse experiments would then be performed to establish, if these non-*E. coli* strains can efficiently displace ESBL *E. coli* from the host's gut.

**7.C. Modelling of the replacement dynamics in humans and mice.**

Both the clinical study as well as the experiments in mice will generate temporal data of the replacement dynamics of ESBL producing and sensitive strains. To analyze these data and infer parameters we will develop models describing the competitive dynamics between strains of varying level of complexity for both the human and the mouse data. We will begin with models focusing just on the relative frequencies of ESBL producing and sensitive *E. coli* to infer relative growth rates of the two strains. Next, we will incorporate plasmid transmission between the two focal strain types, to determine whether such a model may better fit the data. Finally, we will incorporate the interaction with gut microbial community acting both as recipient or donor of the plasmid as well as direct competitors to the focal *E. coli* strains. These models will be relevant for those cases where replacement of the ESBL *E coli* is displaced by non-*E.coli* strains. These models can be used to help the comparison between the findings of the clinical study and the mouse experiments and they can be used for parameter.

Temporal analysis of the sequenced strains during replacement will also allow to assess to what extent the replacement dynamics is driven by immigration of strains from outside into the focal community versus the fixation of strains with adaptive mutations. Phylogenetic analysis will be used to distinguish patterns of evolution from patterns of immigration.

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37. Alikhan, N.F., et al., PLoS Genet, 2018. **14**(4): p. e1007261.
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## 08 PROJECT OBJECTIVES AND RESULTS

**WP1** (Month 1 - Month 24)**:**
*Objective1:* Describe the population dynamics (and possible evidence of clone evolution) of ESBL and non-ESBL *E. coli* in the human gut over time.
*Objective 2:* Perform whole genome sequencing and type the *E. coli* isolates. Identify clinical, microbiological and immunological factors associated with decolonization of ESBL E. coli.
*Objective 3:* Development of mathematical models for the replacement dynamics in humans.
*Deliverables:* Recruitment of 40 participants for pre-travel screening. Identify and follow up about 30 ESBL-positive returnees over up to 18 months. Build a biobank covering single ESBL (8 per participant per time point) and non ESBL *E. coli* isolates (8 per participant per time point); storage of more complex microbiome samples; collection of serum and immune cells for future immunological experiments.
*Milestones:* Ethical approval (before official project start), recruitment of 10 individuals with first sample (M3), Final recruitment of all samples (M18). Whole genome sequencing of first batch of isolates (M12). Finalized sequencing and sequence analysis (M24).

**WP2** (Month 25 - Month 36)**:**
*Objective 1*: Establish ESBL *E. coli* colonization dynamics in the mouse model (10 mice per ESBL strain; 10 ESBL strains). Establishment which pan-sensitive *E. coli* strains accelerate ESBL displacement and verify that they neither engage in plasmid transfer not elicit disease.
Objective 2: Adapt mathematical models to mouse model data.
*Objective 3:* Verify (or falsify) our main hypothesis, i.e. that exposure to certain pan-sensitive E. coli strains can efficiently terminate ESBL *E. coli* colonization.
*Objective 4:* Write up the data and publish our results.
*Deliverables:* Identity of pan-sensitive *E. coli* strains that can displace from the mammalian gut (M36). Strains could be the basis for future clinical studies to establish this decolonization approach in humans.

The knowledge will be published in scientific presentations and in relevant journals.

## 09 STAGES OF THE PROJECT

| | | |
|---|---|---|
| WP1 | Ethical approval | September 2018 |
| WP1 | Recruitment + initial sampling of 40 participants | Nov. '18 - Feb.'19 |
| WP1 | Identification of 30 ESBL-positive returnees | Until March 2019 |
| WP1 | Swab and blood sampling of the 30 participants; identification of 10 that regain ESBL-neg. status | Until October 2020 |
| WP1 | Biobank, Antibiograms, DNA sequencing | Until October 2020 |
| WP2 | Mouse experiments verifying that the pan-sensitive *E. coli* strains permit decolonization. | Oct. 2020-Oct. 2021 |
| WP2 | Publish the data | June-Oct. 2021 |

## 10 Quality Development

Sequencing is performed at an ISO accredited facility (ISO standard 17025) at the Division of Clinical Microbiology, University Hospital Basel, providing a high quality. All generated quantitative data will be assessed on a regular basis whether the objective has been achieved (for example ratio of ESBL and non ESBL *E. coli* bacteria over the course of colonization). The scientists (PhD student and bioinformaticians) involved will present their raw data at regular meetings e.g. lab meetings but also collaborative interdisciplinary group meetings. Regular organized meetings will provide continuous feedback on the study recruitment, microbiological and immunological progress within the project.

## 11 Innovation

Currently, there are no established and well documented procedures to decolonize ESBL *E. coli* carriers. Our project will establish a novel approach to this problem. Importantly, our approach will use benign *E. coli* strains from the gut microbiota of healthy patients. Our work will verify the benign nature of these strains and ensure that they do not accumulate resistance plasmids. Finally, our approach works without the need for antibiotics. Thus, the therapy using non-pathogenic microbes should be well tolerated.
Further points of innovation: Basis for later detailed analysis of ESBL and non-ESBL *E. coli* interactions in order to **identify novel ways for decolonization protocols**. Characteristics of non-ESBL sensitive *E. coli* as natural treatment. Testing of models based on a highly translational approach from the human situation.

## 12 Impact

The project has the potential to identify the key factors regulating colonization with ESBL *E.coli*, knowledge, which might be transferred to other multi-drug-resistant pathogens e.g. carbapenems. It offers an **entirely new strategy to decolonize patients**. Whereas previous strategies used antibiotic treatment to reduce or remove ESBL E. coli isolates, this new approach would not be dependent on antibiotics. This would be extremely valuable in the face of the current antibiotic resistance crisis. We assume that such a treatment would significantly reduce the amount of broad-spectrum antibiotics needed in clinics as EBSL colonized patients could eventually be de-colonized.

## BUDGET

## 13 Project duration 11/2018 – 10/2021

## 14 Detailed project budget

In kind contributions from the Clinical Microbiology, University Hospital Basel focus on the laboratory assessment of the strains (laboratory work, bioinformatic analysis, and assay reagents). Laboratory technicians performing phenotypic resistance testing and Illumina sequencing of all isolates will be financed as in-kind contributions (laboratory work). Additionally, three bioinformaticians (Dr. Daniel Wüthrich, Dr. Ferdinando Bonfiglio, and Dr. Helena Seth-Smith) are available to train methods for the detailed genomic analysis.

## 15 Detailed project budget submitted to Gebert Rüf Stiftung

| **salaries** | **amount** | | **sum** |
|---|---|---|---|
| study nurse (enrollment, 400 samplings of stool + blood, data management) | 1/2 year salary | | 48000 |
| PhD student (mouse ESBL displacement experiments) | 1 | | 55000 |

| **consumables, materials** | **amount** | **quantity** | **sum** |
|---|---|---|---|
| Volunteer expense reembursement (after 10 samplings) | 500 | 40 | 20000 |
| sample processing + storage | 200 | 400 | 80000 |
| Illumina DNA Sequencing (8 ESBL strains at each time point) | 110 | 800 | 88000 |
| Illumina DNA Sequencing (8 non-ESBL strains at each time point) | 110 | 800 | 88000 |
| MinIon DNA sequencing (1 ESBL strain per time point) | 230 | 80 | 18400 |
| MinIon DNA sequencing (1 non-ESBL strain per time point) | 230 | 80 | 18400 |

| | | | |
|---|---|---|---|
| mice for ESBL displacement experiments<br>(10 per ESBL-non ESBL pair; controls = 10 inoculated with ESBL alone;<br>purchase + housing) | 50 | 400 | 20000 |
| Plates, reagents for mouse ESBL displacement analyses | | | 10000 |
| re-sequencing of re-isolates from mouse ESBL displacement experiments | 110 | 400 | 44000 |
| publication costs | 3000 | 1 | 3000 |

**Sum of all costs** **492800**

**PROJECT SETTING**

**16 HISTORY** – *Is the project already underway? If so, where?* Dr. Esther Künzli (Swiss TPH) and Dr. Adrian Egli (University Hospital Basel) will submit the application for ethical approval. This will ensure that approval is granted by the beginning of this project (October 2018).

**17 IP PROTECTION** – *Are patents available or planned? Do any third parties have claims over or interest in the targeted results?*
Patents might arise if interesting ESBL displacing E. coli strains can be identified. These would be patented in collaboration with Unitectra, Switzerland (https://www.unitectra.ch).

**18 SUBMISSION** – *Has the project been submitted elsewhere? Has it been rejected or awarded? If so, where?* No.

**19 CO-FINANCING** – *Has the project been partially financed elsewhere? If so, where and to what extent?* No. The preparation of the ethical application for the clinical study is currently performed from own resources. The Hardt lab holds an animal license to perform the mouse work described in 7B.

**20 GRANTS** – *Short list of other grants relevant for the topic and difference of the submitted project from these grants.* Dr. Adrian Egli, Prof. Wolf-Dietrich Hardt and Prof. Sebastian Bonhoeffer work in a NRP72 funded consortium addressing the spread of ESBL plasmids ("Towards quantification of the contribution of plasmids to the spread of antibiotic resistance"). This project has no direct scientific connection. The project tries to fundamentally understand what the driving factors are in plasmid transmission. In contrast, the current project proposal for the Gerbert Rüf foundation aims to identify non-pathogenic E. coli isolates which could be used as a treatment to de-colonize patients.

Dr. Adrian Egli is PI of another NRP72 funded project aiming to build a molecular surveillance database for multi-drug resistant bacteria ("Development of a Swiss surveillance database for molecular epidemiology of multi-drug resistant pathogens"). All sequenced isolates from the current proposal will be shared via this database.

**21 REFERENCES** – *Provide three references with addresses: From your university, another Swiss university or an international contact:* Prof. Dan I. Andersson, Uppsala Antibiotic Center, Uppsala Sweden

PD Dr. Urs Karrer, Infectious Diseases, Kantonsspital Winterthur, Winterthur, Switzerland

Prof. Dr. Ingo B. Autenrieth, Uni Tübingen, Germany

**PLACE:** Basel, **DATE** 18/07/2018, **SIGNATURE (ELECTRONIC)**:

**ATTACHMENTS**

- 1 page CV of main applicant.
- List of top publications from the last two years.
- Poss. 1 page CV's of persons involved in the project.

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Basel, July 17, 2018

**Letter of endorsement for the application of Dr. Adrian Egli**

Dear members of the scientific board

With great pleasure I support the research proposal by Dr. Adrian Egli and his collaborative partners.

The project proposal addresses a highly urgent need in medicine - reducing the transmission of multi-drug resistant bacteria, in particular ESBL-producing *E. coli*. Multi-drug resistant bacteria are continuously increasing around the globe and drive the usage of broad-spectrum antibiotics for empiric antibiotic therapy. In his proposal Dr. Egli and interdisciplinary group of collaborators from the ETH Zurich (Wolf-Dietrich Hardt and Sebastian Bonhoeffer) and the Swiss Tropical and Public Health (STPH) Institute (Esther Künzil) offer a very novel and highly innovative approach to understand bacterial colonization with ESBL *E. coli* and use this knowledge to decolonize patients.

Travel returners from India show an 80% colonization rate with ESBL *E. coli*. Interestingly, a high proportion of them lose the ESBL *E. coli* strains over the following two years. Dr. Egli's hypothesis is that non-ESBL *E. coli* will replace ESBL *E. coli* over time. This hypothesis will be explored using whole genome sequencing of *E. coli* isolates from patients over time and findings will be further studied in detail using mouse models. Their knowledge should allow to define a completely novel decolonization procedure in order to reduce the burden of ESBL carriage in patients. Thereby antibiotic consumption of broad-spectrum agents could be significantly reduced.

Dr. Egli is a research group leader at the Department of Biomedicine at the University Hospital Basel. I am highly confident that he and his collaborating partner will execute this important project successfully and generate novel and important knowledge to provide a new way of decolonizing patients from multi-drug resistant bacteria.

I fully support this application and very much hope that you will be able to fund the project.

Yours sincerely,

Prof. Radek Skoda
Chair of the Department of Biomedicine

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## Academic Positions & Education

15- Head of Division, Clinical Microbiology, University Hospital Basel, Switzerland.
14- Research Group Leader, "Applied Microbiology Research" Laboratory, Department of Biomedicine, University of Basel, Switzerland.
12-15 Fellow in Clinical Microbiology (FAMH), University Hospital Basel, Switzerland.
10-11 Clinical Fellowship "Transplant Infectious Disease", University of Alberta, Canada, as well as Post-doctoral fellowship, Li Ka Shing Institute for Virology, University of Alberta, Canada.
08-09 Resident Internal Medicine, University Hospital Basel, Switzerland. Focusing on Haematology and Infectious Diseases.
06-08 PhD thesis (summa cum laude), „Cellular and humoral immunity against Polyomavirus BK and human Cytomegalovirus in kidney transplanted patients", University of Basel, Switzerland.
05 Resident Internal Medicine, Spital Dornach, Switzerland).
04 MD thesis „Suicide and depression: suicides in the family and social environment", University of Basel, Switzerland.
98-04 Medicine at the University Basel, Switzerland.

## Current Teaching and Supervision

17- *PhD thesis*: Aline Cuenod, MSc, Department Biomedicine, University of Basel, Switzerland, Project: "Pathogen evolution: focus on resistance"
13-18 *Master thesis*: Yvonne Hollenstein, Julia Hartmann, Lukas Kaufmann, University of Basel, Switzerland, *MD thesis*: Yvonne Hollenstein, University of Basel, Switzerland.
14- *PhD thesis*: Mohamedyaseen Syedbasha, M.Sc., Department Biomedicine, University of Basel, Switzerland, Project: „Interferon lambda blocking molecules".
15- *PhD thesis*: Janina Linnik, M.Sc., Department Biomedicine, University of Basel, Switzerland, Project: „Computational Modelling of IFN lambda in vaccination".
15- *Undergraduate teaching* at the University of Basel, Switzerland.
14- *Postgraduate teaching*, immunomeetings at the University of Basel, Switzerland.

## Professional Activities

*Grants*: reviewer for ESCMID scientific grants
*Conferences*: Poster Judge Swiss Society of Microbiology (2016/2017), Abstract for ECCMID (2017/2018), as well as organization of the following three conferences (2015/2017): *Full-day, international symposia*: Translation of Next Generation Sequencing into routine application for Clinical Microbiology. Basel, Switzerland, Main organizer. *1st ESCMID capacity forming workshop* (3 days with 18 European faculty members) on MALDI-TOF based diagnostics, Basel, Switzerland, Main organizer with a total of 80 participants and *MALDI-TOF MS based typing: capacity forming workshop,* Basel, Switzerland, Main organizer with a total of 25 participants.
*Journals*: regular of sporadic reviewing for the American Journal of Transplantation, BMC Infectious Diseases, Clinical Trans-plantation, Journal of Clinical Virology, Journal for Leucocyte Biology, Journal of Medical Virology, Journal for Microbiological Methods, Plos one, Swiss Medical Weekly, Transplantation, Transplant Infectious Diseases

**ADRIAN EGLI – PUBLICATIONS 2016 - 2018**

Transmission of ESBL-producing Enterobacteriaceae and their mobile genetic elements-identification of sources by whole genome sequencing: study protocol for an observational study in Switzerland. Stadler T, Meinel D, Aguilar-Bultet L, Huisman JS, Schindler R, **Egli A**, Seth-Smith HMB, Eichenberger L, Brodmann P, Hübner P, Bagutti C, Tschudin-Sutter S. BMJ Open. 2018 Feb 17;8(2):e021823. doi: 10.1136/bmjopen-2018-021823.

Gut microbiota modulate T cell trafficking into human colorectal cancer. Cremonesi E, Governa V, Garzon JFG, Mele V, Amicarella F, Muraro MG, Trella E, Galati-Fournier V, Oertli D, Däster SR, Droeser RA, Weixler B, Bolli M, Rosso R, Nitsche U, Khanna N, **Egli A**, Keck S, Slotta-Huspenina J, Terracciano LM, Zajac P, Spagnoli GC, Eppenberger-Castori S, Janssen KP, Borsig L, Iezzi G. Gut. 2018 Feb 6. pii: gutjnl-2016-313498. doi: 10.1136/gutjnl-2016-313498. [Epub ahead of print]

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Increasing prevalence of infectious diseases in asylum seekers at a tertiary care hospital in Switzerland. Bloch-Infanger C, Bättig V, Kremo J, Widmer AF, **Egli A**, Bingisser R, Battegay M, Erb S. PLoS One. 2017 Jun 15;12(6):e0179537. doi: 10.1371/journal.pone.0179537. eCollection 2017.

ESCMID postgraduate education course: applications of MALDI-TOF mass spectrometry in clinical microbiology. Greub G, Moran-Gilad J, Rossen J, **Egli A**; ESCMID Study Group for Genomic and Molecular Diagnostics (ESGMD). Microbes Infect. 2017 Sep - Oct;19(9-10):433-442. doi: 10.1016/j.micinf.2017.06.004. Epub 2017 Jun 30.

Respiratory Syncytial Virus Infection Control Challenges with a Novel Polymerase Chain Reaction Assay in a Tertiary Medical Center. Sendi P, **Egli A**, Dangel M, Frei R, Tschudin-Sutter S, Widmer AF. Infect Control Hosp Epidemiol. 2017 Nov;38(11):1291-1297. doi: 10.1017/ice.2017.213. Epub 2017 Oct 23.

Evaluation of the rapid biochemical β-CARBA™ test for detection of carbapenemase-producing Gram-negative bacteria. Hinić V, Reist J, **Egli A**. J Microbiol Methods. 2018 Jan;144:44-46. doi: 10.1016/j.mimet.2017.10.008. Epub 2017 Oct 19.

Associations among Antibiotic and Phage Resistance Phenotypes in Natural and Clinical Escherichia coli Isolates. Allen RC, Pfrunder-Cardozo KR, Meinel D, **Egli A**, Hall AR. MBio. 2017 Oct 31;8(5). pii: e01341-17. doi: 10.1128/mBio.01341-17.

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Concern regarding the alleged spread of the hypervirulent lymphogranuloma venereum chlamydia trachomatis strain in Europe. Seth-Smith HM, Galán JC, Goldenberger D, Lewis DA, Peuchant O, Bébéar C, de Barbeyrac B, Bénard A, Carter I, Kok J, Bruisten SM, Versteeg B, Morré SA, Thomson NR, **Egli A**, de Vries HJ. Euro Surveill. 2017;22(15):pii=30511. DOI: http://dx.doi.org/10.2807/1560-7917.ES.2017.22.15.30511

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A Cross-Sectional Study of Colonization Rates with Methicillin-Resistant Staphylococcus aureus (MRSA) and Extended-Spectrum Beta-Lactamase (ESBL) and Carbapenemase-Producing Enterobacteriaceae in Four Swiss Refugee Centres. Piso RJ, Käch R, Pop R, Zillig D, Schibli U, Bassetti S, Meinel DM, **Egli A**. PLoS One. 2017 Jan 13;12(1):e0170251. doi: 10.1371/journal.pone.0170251.

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Association of daptomycin use with resistance development in Enterococcus faecium bacteraemia-a 7-year individual and population-based analysis. **Egli A**, Schmid H, Kuenzli E, Widmer AF, Battegay M, Plagge H, Frei R, Achermann R, Weisser M. Clin Microbiol Infect. 2016 Oct 13. pii: S1198-743X(16)30462-1. doi: 10.1016/j.cmi.2016.10.003. [Epub ahead of print]

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Prevention of hepatitis C virus infection using a broad cross-neutralizing monoclonal antibody (AR4A) and epigallocatechin gallate. O'Shea D, Law J, **Egli A**, Douglas D, Lund G, Forester S, Lambert J, Law M, Burton DR, Tyrrell DL, Houghton M, Humar A, Kneteman N.
Liver Transpl. 2016 Mar;22(3):324-32. doi: 10.1002/lt.24344. Epub 2016 Jan 29.

Complexity of Host Micro-RNA Response to Cytomegalovirus Reactivation After Organ Transplantation. **Egli A**, Lisboa LF, O'Shea D, Asberg A, Mueller T, Emery V, Kumar D, Humar A. Am J Transplant. 2016 Feb;16(2):650-60. doi: 10.1111/ajt.13464. Epub 2015 Oct 13.

## CV: Prof. Dr. rer. nat. Wolf-Dietrich Hardt (July 2018)

**Affiliation:** ETH Zürich, Institute of Microbiology, D-BIOL, Office HCI G417, Vladimir-Prelog-Weg 4, 8093 Zürich, Switzerland; Tel: 0041-44-632 5143; email: hardt@micro.biol.ethz.ch

**Short Biography**

'87-'92 Biochemistry at Freie Universität Berlin, Germany; Diploma thesis with PD Dr. Roland Hartmann and Prof. Dr. Volker A Erdmann, Freie Universität Berlin, Germany.

'92-'95 Graduate student with PD Dr. Roland Hartmann and Prof. Dr. Volker A Erdmann, Freie Universität Berlin, Germany; PhD topic: Enzyme kinetics and mutagenesis of RibonucleaseP.

'95-'97 Postdoc with Prof. Jorge E. Galan, State University of New York, Stony Brook, NY, USA; Research area: *Salmonella* Typhimurium molecular and cellular infection biology.

'98-'01 Group leader, Max von Pettenkofer-Institut für Hygiene und Mikrobiologie, Ludwig-Maximilians Universität München, Germany; Research area: Virulence factors of *Salmonella* Typhimurium; Evolution of *Salmonella* as a pathogen.

'01-'10 Associate Prof. of Microbiology, Institute of Microbiology, D-BIOL, ETH-Zürich; Research area: Molecular basis of *Salmonella* diarrhea; Mouse models for gut infection biology; Evolution of *Salmonella* as a pathogen; Type III secretion.

'10-… Full Prof. of Microbiology, Institute of Microbiology, D-BIOL, ETH-Zürich.

**Qualification, motivation and contribution to the project**

The Hardt lab studies the molecular basis of infection, focussing on *Salmonella* diarrhea. Recently, we developed interest in gut colonization by *E. coli* strains. We combine pre-clinical approaches from molecular biology, genetics, cell biology, immunology and evolution biology to decipher the complex network of interactions between *Salmonella* Typhimurium, *E. coli* strains, the microbiota and the host's gut. In the past, we have developed mouse models that have proven instrumental for identifying the virulence factors of the pathogen, mucosal immune responses, how inflammation boosts pathogen growth in the gut lumen and how this boosts evolution in *Salmonella* spp. and the transfer of antibiotic resistance plasmids between *Salmonella* spp. and *E. coli* strains. We have extended this pre-clinical work to study how the microbiota, microbiota-derived vitamins and the adaptive immune response of the host can interfere with enteropathogen infection and antibiotic resistance plasmid transfer. Now, we need to obtain data from the human patient for benchmarking our mouse models and identifying the most relevant parameters that determine the disease progression in the human gut. The Gebert Rüf project consortium will be an ideal test ground for our hypotheses. We are excited to help deciphering the basis of ESBL *E. coli* gut colonization, develop de-colonization strategies and to test them in our mouse infection models. This pre-clinical work will allow us to verify the competitive potential of the isolated competitive *E. coli* strains and could be the basis for future work on the underlying molecular mechanisms, the specialty of my lab. In either way, this mouse work will provide exciting data verifying our ESBL *E. coli* competition hypothesis. We are happy to provide the basis for any future clinical decolonization trials.

**List of 5 publications relevant for the project:**

h-factor 53; >10'000 citations; 157 original publications and reviews

1. Diard, M., Garcia, V., Maier, L., Remus-Emsermann, M.N.P., Regoes, R.R.*, Ackermann, M.* and **W.D. Hardt*** (2013) Stabilization of cooperative virulence by the expression of an avirulent phenotype. **Nature**, 494(7437):353-6.

2. Stecher, B., Maier, L. and **W. D. Hardt*** (2013) 'Blooming' in the gut: how dysbiosis might contribute to pathogen evolution. **Nat Rev Microbiol.** (4):277-84.

3. Miki, T.*, Goto, R., Fujimoto, M., Okada, N. and **W.D. Hardt** (2017) The Bactericidal Lectin RegIIIβ Prolongs Gut Colonization and Enteropathy in the Streptomycin Mouse Model for Salmonella Diarrhea. **Cell Host&Microbe** 21(2):195-207.

4. Diard, M., Bakkeren, E., Cornuault, J.K., Moor, K., Hausmann, A., Sellin, M.E., Loverdo, C., Aertsen, A., Ackermann, M., De Paepe,M., Slack, E. and **W.D. Hardt*** (2017) Inflammation boosts bacteriophage transfer between Salmonella. **Science** 355(6330):1211-1215.

5. Moor, K., Diard, M., Sellin, M.E., Felmy, B., …, Regoes, R.R., Loverdo, C., Stocker, R., Brumley, D.R.*, **Hardt, W.D.*** and Emma Slack* (2017) High-avidity IgA protects the intestine by enchaining growing bacteria. **Nature**, doi: 10.1038/nature22058.

# CV: Sebastian Bonhoeffer

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## Nationalities

German (by birth) and Swiss (by naturalization in 2015)

## Degrees

| | |
|---|---|
| 88 | Classical Music (cello), Music Academy Basel, Switzerland (with Prof. Heinrich Schiff) |
| 92 | Diploma in physics, University of Vienna, Austria (with Prof. Peter Schuster) |
| 95 | D. phil in Zoology, University of Oxford (with Prof Martin Nowak and Prof. Robert May) |

## Academic positions

| | |
|---|---|
| 95-96 | Florey Research Fellow at Lady Margaret Hall, Oxford |
| 95-98 | Wellcome Research Assistant at the Wellcome Centre for the Epidemiology of Infectious Disease, Oxford |
| 97-98 | Senior Research Fellow, Wolfson College, Oxford |
| 97 | Visiting scientist at the Aaron Diamond AIDS Research Center, Rockefeller University, New York (with Prof David Ho) |
| 98 | Senior Assistant of Prof. P. Schmid-Hempel, ETH Zurich |
| 99-01 | Junior Group Leader at the Friedrich Miescher Institut, Basel |
| 01-05 | SNF Research Professor, ETH Zurich |
| 05- | Full Professor, ETH Zurich |

## Membership of scientific bodies and journals

| | |
|---|---|
| 05- | PLoS Computational Biology (currently as Deputy Editor in Chief) |
| 08- | Epidemics (Editorial Board) |
| 02-10 | Journal of Theoretical Biology (Editorial Board) |
| 01-08 | Proceedings of the Royal Society Series B (Editorial Board) |
| 10-18 | Member of the Research Council of the Swiss National Science Foundation |
| 14- | Elected Member of European Molecular Biology Organization |

## Recent grants

| | |
|---|---|
| 11-16 | European Research Council Advanced Researcher Grant |
| 17-22 | Co-Investigator (1 out of 10): Simons Foundation |
| 18-20 | Principal Investigator: Swiss National Science Foundation Project Grant |

## Research fields

Evolution and dynamics of infectious diseases, in particular within an infected host; Evolutionary dynamics of drug resistance (antiviral, antibiotics, antifungal, antimalarial, cancer); Mathematical modelling; Phylogenetic analysis; Network models of disease epidemiology; Host-parasite coevolution; Evolution of recombination; Evolution on complex fitness landscapes; Evolution of heterotrophic energy metabolism; Evolution of plasmids; Experimental microbial evolution;

# Curriculum vitae: Dr. med. Esther Künzli, MSc

**Affiliation:** Swiss Tropical and Public Health Institute, Socinstrasse 57, 4051 Basel, Switzerland and Epidemiology, Biostatistics and Prevention Institute, Hirschengraben 84, 8001 Zürich; Tel: 0041 61 284 82 55; Email: esther.kuenzli@swisstph.ch

**Short Biography:**

| | |
|---|---|
| 1999 – 2005 | Study of Medicine, University of Basel |
| 2010 | FMH Exam Internal Medicine |
| 2010 – 2011 | Master in Control of Infectious Diseases at the London School of Hygiene and Tropical Medicine |
| 2014 | FMH Exam Infectious Diseases |
| 2015 – dato | Scientific Collaborator, Epidemiology, Biostatistics and Prevention Institute, University of Zurich |
| 2017 – dato | Scientific Collaborator / Consultant, Swiss Tropical and Public Health Institute Basel |

**Qualification, motivation and contribution to the project:**

In the past years, I have coordinated several national and international studies on the worldwide spread of antimicrobial resistance with a special focus on multidrug resistant Enterobacteriaceae at the Swiss TPH Basel and the EBPI Zürich.

Seeing the increasing threat of multidrug resistant bacteria, we are excited to participate in the proposed project providing a basis for potential decolonizing options.

**List of the 5 most relevant publications for this project:**

Kuenzli E, Jaeger VK, Frei R, Neumayr A, DeCrom S, Haller S, Blum J, Widmer AF, Furrer H, Battegay M, Endimiani A, Hatz C. High colonization rates of extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli in Swiss travellers to South Asia – a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. BMC Infect Dis 14 (1): 528 (2014).

Bernasconi OJ, Kuenzli E, Pires J, Tinguely R, Carattoli, A, Hatz C, Perreten V, Endimiani A. Travelers can import Colistin-resistant Enterobacteriaceae including those possessing the plasmid-mediated mcr-1 gene. Antimicrob Agents Chemother 2016 June 13. pii: AAC.00731-16.

Pires J, Kuenzli E, Kasraian S, Tinguely R, Furrer H, Hilty M, Hatz C, Endimiani A. Polyclonal intestinal colonization with extended-spectrum cephalosporin-resistant Enterobacteriaceae upon traveling to India. Front. Microbiol 2016 July 12. http://dx.doi.org/10.3389/fmicb.2016.01069.

Kuenzli E, Juergensen D, Kling K, Jaeger VK, DeCrom S, Steffen R, Widmer AF, Battegay M, Hatz C, Neumayr A. Previous exposure in a high-risk area for travellers' diarrhoea within the past year is associated with a significant protective effect for travellers' diarrhoea. A prospective observational cohort study in travellers to South Asia. Journal of Travel Medicine: 24(5). DOI: 10.1093/jtm/tax056

Bernasconi OJ, Donà V, Pires J, Kuenzli E, Hatz C, Luzzaro F, Perreten V, Endimiani A. Deciphering the complete deletion of the mgrb locus in an unusal colistin-resistant Klebsiella pneumoniae colonizing the gut of traveler returning from India. Int J Antimicrob Agents 2017. DOI: 10.1016/j.ijantimicag

**Fragebogen vor der Reise**

**Probandennummer**:

**Beruf** ……

**Ausbildung**

□ Obligatorische Schulzeit Anzahl Jahre: ……

□ Anlehre

□ Lehre

□ Fachhochschule

□ Universität

**Länder, die innerhalb der letzten 12 Monate bereist wurden**

Land: …….. Dauer: ……..

Land: …….. Dauer: ……..

Land: …….. Dauer: ……..

Land: …….. Dauer: ……..

Land: …….. Dauer: ……..

**Hatten Sie während einer der Reisen in den letzten 12 Monaten Durchfall** (bitte geben Sie auch jeweils das zu dem Zeitpunkt bereiste Land an)?

□ Ja □ Nein

**Waren Sie in den letzten 12 Monaten im Ausland im Spital?**

□ Nein

□ Ja Land: ……

Dauer: ……..

Anzahl Tage: ……..

□ Nein

**Waren Sie während den letzten 12 Monaten in der Schweiz im Spital?**

□ Nein

□ Ja Wie oft: ……

Anzahl Tage: ……

Grund: ……

**Haben Sie in den letzten 12 Monaten Antibiotika eingenommen?**

□ Nein

□ Ja Was: ……

Wie oft: ……

Anzahl Tage: ……

**Leiden Sie unter einer der untenstehenden Erkrankungen** (Mehrfachnennungen möglich)**?**

□ Diabetes □ Bluthochdruck □ Reizdarmsyndrom

□ Nahrungsmittelallergien □ Chronisch-entzündliche Darmerkrankung

**Haben Sie eines der untenstehenden Symptome an mindestens 3 Tagen pro Monate** (Mehrfachnennungen möglich)?

□ Bauchschmerzen □ Durchfall □ Verstopfung

□ schleimiger Stuhlgang □ Blähungen

**Sind Sie Vegetarier?**

□ Nein □ Ja

**Haben Sie in Ihrem Alltag regelmässigen Tierkontakt (streicheln, füttern, ausmisten)** (Mehrfachnennungen möglich)**?**

□ Nein

□ Ja □ Katzen

□ Hunde

□ Kühe

□ Pferde

□ Geflügel

□ Andere ……

Häufigkeit: □ täglich

□ 1-3x/Woche □ 4-6x/Woche

□ < 1x/Woche

**Fragebogen für Reisende nach der Reise (**in der Woche nach der Reise auszufüllen**)**

**Probandennummer**: ☐

**Datum, an dem der Fragebogen ausgefüllt wurde: …….**

**Frage 1:**

**Haben Sie ausser Indien noch weitere Länder bereist?**

□ Ja □ Nein

Länder:_____

**Frage 2:**

**Was war der *Hauptzweck* Ihrer Reise?**

| | | |
|---|---|---|
| **Tourist** | □ Ja | □ Nein |
| **Beruflich** | □ Ja | □ Nein |

**Frage 3:**

**Welche Art von Unterkunft haben Sie *am häufigsten* benutzt (**bitte nur eine Möglichkeit auswählen**)?**

| | | |
|---|---|---|
| **Hotels (**mittlere bis obere Kategorie**)** | □ Ja | □ Nein |
| **Einfache Hotels/Guest Houses** | □ Ja | □ Nein |
| **Privatunterkünfte bei Fremden** | □ Ja | □ Nein |
| **Privatunterkünfte bei Familie / Freunden** | □ Ja | □ Nein |
| **Anderes** | □ Ja, nämlich …… | |
| | □ Nein | |

**Frage 4:**

**Wo haben Sie *hauptsächlich* gegessen (**bitte nur eine Möglichkeit auswählen**)?**

| | | |
|---|---|---|
| **Restaurant/Hotel** | □ Ja | □ Nein |
| **Strassenstand/Take away** | □ Ja | □ Nein |
| **Privat (**selbst gekocht/Familie/Freunde**)** | □ Ja | □ Nein |

**Frage 5:**

**Haben Sie täglich Alkohol getrunken?**

□ Ja □ Nein

Falls ja: täglich mehr als einen halben Liter Bier oder ein Glas Wein oder ein Glas Schnaps?

□ Ja □ Nein

**Frage 6:**

**Welche der folgenden Speisen/Getränke haben Sie während der Reise zu sich genommen (**Mehrfachnennungen möglich**)?**

| | | |
|---|---|---|
| **Leitungswasser** | □ Ja | □ Nein |
| **Eiswürfel** | □ Ja | □ Nein |
| **Milchprodukte** | □ Ja | □ Nein |
| **Eiscrème** | □ Ja | □ Nein |
| **rohes Fleisch** | □ Ja | □ Nein |
| **Hamburger** | □ Ja | □ Nein |
| **anderes Fleisch** | □ Ja | □ Nein |
| **rohe Austern** | □ Ja | □ Nein |
| **Hummer/Shrimps** | □ Ja | □ Nein |
| **Salat** | □ Ja | □ Nein |
| **Mayonnaise-haltige Saucen** | □ Ja | □ Nein |
| **Essen von Strassenständen** | □ Ja | □ Nein |
| **ungeschälte/ungewaschene Früchte** | □ Ja | □ Nein |
| **Patisserie/Mousses** | □ Ja | □ Nein |

**Frage 7:**

**Haben Sie während dieser Reise unter einer oder mehreren der unten aufgeführten Erkrankungen gelitten (**bitte geben Sie jeweils das zu dem Zeitpunkt bereiste Land an**)?**

**Durchfallerkrankung**

□ Ja Land: ……

□ Nein

**Übelkeit/Erbrechen**

□ Ja Land: ……

□ Nein

**Fieber (> 37.5°C)**

□ Ja Land: ……

□ Nein

**Anderes**

□ Ja Was: ……

Land: ……

□ Nein

**Die folgenden Fragen müssen nur beantwortet werden, falls Sie während der Reise Durchfall hatten:**

*7.1 Traten zusätzlich zum Durchfall eines oder mehrere der folgenden Symptome auf?*

| | | |
|---|---|---|
| Übelkeit/Erbrechen | □ Ja | □ Nein |
| Bauchkrämpfen | □ Ja | □ Nein |
| Fieber | □ Ja | □ Nein |
| Blut im Stuhl | □ Ja | □ Nein |
| Gewichtsverlust | □ Ja | □ Nein |

*7.2 Waren Sie wegen des Durchfalls während der Reise bei einem Arzt/in einem Spital?*

□ Ja □ Nein

*7.3 Wurde eine Stuhluntersuchung durchgeführt?*

□ Ja Resultat: ……

□ Nein

□ weiss nicht

*7.4 Haben Sie ein Antibiotikum erhalten?*

□ Ja
- □ Ciprofloxacin
- □ Azithromycin
- □ Metronidazol
- □ Name des Antibiotikums unbekannt
- □ anderes: ……

□ Nein

□ weiss nicht

*7.5 Hatten Sie während der Reise mehr als eine Durchfallepisode **(**mit mind. 3 Tagen ohne Symptome dazwischen**)?***

□ Ja Anzahl Episoden .....

□ Nein

*7.6 Wie schwer war der Durchfall **(**bei mehreren Episoden die schlimmste Episode angeben**)**?*

Leicht (es hat meine Reisepläne nicht beeinträchtigt)

□ Ja □ Nein

Mittel (ich konnte gewisse Sachen nicht machen)

□ Ja □ Nein

Schwer (ich musste im Bett bleiben und/oder bin zum Arzt gegangen)

□ Ja □ Nein

*7.7 Hatten andere Personen, die mitgereist sind, ähnliche Symptome?*

□ Ja □ Nein □ weiss nicht

**Frage 8:**

**Haben sie wegen einer der oben genannten Erkrankung während der Reise Medikamente eingenommen (**bitte geben Sie jeweils den Namen oder Wirkstoff des Medikamentes an**)?**

□ Ja (Mehrfachnennungen möglich)

Antibiotika

□ Ja, nämlich ……

□ Name/Wirkstoff nicht bekannt

□ Nein

Malaria-Medikament

□ Ja, nämlich ……

□ Name/Wirkstoff nicht bekannt

□ Nein

Medikamente gegen Durchfall

□ Ja, nämlich ……

□ Name/Wirkstoff nicht bekannt

□ Nein

Schmerzmittel

□ Ja, nämlich ……

□ Name/Wirkstoff nicht bekannt

□ Nein

Andere

□ Ja, nämlich ……

□ Name/Wirkstoff nicht bekannt

□ Nein

□ Nein

**Frage 10:**

**Waren Sie während der *aktuellen* Reise im Spital?**

□ Ja

Land: ……

Dauer (Tage): ……

Grund: ……

□ Nein

**Frage 11:**

**Nehmen Sie regelmässig Säureblocker für den Magen (Nexium®, Pantozol®) ein und habe Sie dies auch währen der Reise getan?**

□ Ja

□ täglich

□ mehrmals pro Woche

□ weniger als 1x/Woche

□ Nein

**Fragebogen zur Nachverfolgung von Reisenden nach der Reise**

**Probandennummer**:

**Datum, an dem der Fragebogen ausgefüllt wurde**: ……

**Frage 1:**

**Waren Sie in den letzten 6 Monaten im Ausland in den Ferien?**

□ Ja

Land: ……..

Dauer (Tage): ……

Land: .....

Dauer (Tage): .....

Land: .....

Dauer (Tage): .....

□ Nein

**Frage 2:**

**Waren Sie in den letzten 6 Monaten im Ausland im Spital?**

□ Ja

Land: ……

Dauer (Tage): ……

Grund: ……

□ Nein

**Frage 3:**

**Waren Sie in den letzten 6 Monaten in der Schweiz im Spital?**

□ Ja

Dauer (Tage): ……

Grund: ……

□ Nein

**Frage 4:**

**Haben Sie während den letzten 6 Monaten eines der untenstehenden Symptome an mindestens 3 Tagen jeden Monate gehabt (**Mehrfachnennungen möglich**)?**

| | | |
|---|---|---|
| **Bauchschmerzen** | □ Ja | □ Nein |
| **Durchfall** | □ Ja | □ Nein |
| **Verstopfung** | □ Ja | □ Nein |
| **schleimiger Stuhlgang** | □ Ja | □ Nein |
| **Blähungen** | □ Ja | □ Nein |

**Frage 5:**

**Haben Sie in den letzten 6 Monaten Antibiotika eingenommen?**

□ Ja

Name des Antibiotikums: …….

□ Name unbekannt

Dauer (Tage): ……

□ Dauer unbekannt

Grund: ……

□ Grund unbekannt

□ Nein

**Frage 6:**

**Nehmen Sie regelmässig Säureblocker für den Magen (Nexium®, Pantozol®)?**

□ Ja

□ täglich

□ mehrmals pro Woche

□ weniger als 1x/Woche

□ Nein