SCIENTIFIC CONSULTATION – PROJECT PROPOSALS

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GLOBAL ANTIBIOTIC RESEARCH AND DEVELOPMENT (GARD) PARTNERSHIP

Developing new antibiotic treatments, promoting responsible use, and ensuring access for all

A joint WHO/DNDi initiative incubated by DNDi in support of the Global Action Plan for Antimicrobial Resistance

DNDi
Drugs for Neglected Diseases *initiative*

World Health Organization

Institut Pasteur

Contents

Platform: Antibiotic combinations

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Disease area:

Resistance rates are rapidly increasing globally. In low-resource countries, inexpensive first-line antibiotics have been the cornerstone of the therapy of infectious diseases. Now, high resistance rates cause frequent treatment failures and result in the increased need for second-line or even last-resort antibiotics. Access to some of these antibiotics or even future ones is often not affordable for those in need.

Though there is well-known geographical variation in resistance patterns, **critical resistance problem areas** can be summarised as follows:

– **Community-acquired infections caused by multidrug-resistant (MDR)** *Enterobacteriaceae*, which usually produce extended-spectrum beta-lactamases (ESBL), and are therefore resistant to aminopenicillins and cephalosporins and also frequently co-resistant to fluoroquinolones and other antibiotics. *Escherichia coli* and *Klebsiella pneumoniae* are recognised as important causes of hard-to-treat community-acquired urinary tract infections. Few oral treatment options remain in such cases, e.g. nitrofurantoin, fosfomycin trometamol, mecillinam.
    o Drug-resistant *Neisseria gonorrhoeae* may be treated with the old oral drug fosfomycin trometamol. Studies to support this treatment option are lacking.
– **Severe hospital-associated and, increasingly, community-acquired infections caused by carbapenem-resistant Gram-negative bacteria**.
    o The increasing prevalence of ESBL-producing *Enterobacteriaceae* has triggered an exponential empiric use of carbapenems, which in turn has created heavy selection pressure and an alarming increase in carbapenem resistance. In low-income countries, carbapenems may not be available. These bacteria are usually extensively drug resistant (XDR) and only susceptible to colistin and sometimes tigecycline, fosfomycin and/or an aminoglycoside. Indeed, alternative old drugs such as intravenous fosfomycin or temocillin to treat ESBL-producing bacteria are increasingly used where available. Combinations of antibiotics are used frequently, but have not been researched adequately thus far.

In the face of both increasing antimicrobial resistance and a dearth of novel antibiotics in the pipeline, it has become clear that we need new strategies. One of these must be to revisit old, still active antibiotics and **explore their potential for synergistic activity when used in combination**. Though numerous *in vitro* studies with antibiotic combinations are available, there is no evidence or consensus regarding methodology to predict clinical efficacy or finally, clinical evidence. Additionally, many old antibiotics have not been characterised in a structured process for drug assessment and regulatory approval according to current standards of science, as these standards and requirements have evolved over time (see proposal: Platform: Improving the usage of old antibiotics).

Disease burden:

MDR and XDR Gram-negative bacteria increasingly cause infections globally but are specifically prevalent in southern European countries, in some North American areas and in most Asian and African countries. Infections that are commonly caused by MDR bacteria include urinary tract infections, *N. gonorrhoeae* infections, skin and soft tissue infections, pneumonia, and hospital-associated infections.
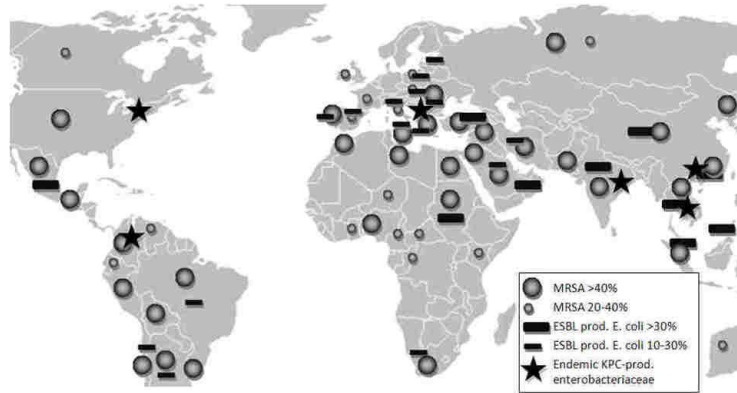
R&D gaps and needs:

Preliminary *in vitro* studies indicate improved activity of certain antibiotics when used in combination. Such combinations may improve bactericidal activity. Indeed, this synergy would in some cases allow for shorter antibiotic courses, which would translate to less exposure of "bystander organisms" to unnecessary antibiotics, and thus reduce the risk of emergence of resistance. Numerous *in vitro* studies as well as limited clinical observations are known and warrant the exploration of this strategy to respond to the resistance problem. Though used frequently, substantial knowledge gaps exist regarding optimal combinations, efficacy in infections caused by current resistant pathogens, optimal dosing regimens, and influence on emergence of resistance.

- Develop *in vitro, in vivo* and *in silico* methods to characterise the activity of combinations. Optimal and predicting methods have not been developed thus far.
- A thorough literature review and analysis of existing clinical data should be performed.
- Identifying knowledge gaps:
  The gap analysis provides the basis for the structured process for further studies of each prioritised antibiotic combination.
- Randomised controlled clinical study with the prioritised antibiotic combination to show its superiority to monotherapy
  - Develop a **master protocol template for randomised controlled clinical trials** for each selected antibiotic combination and invite clinical research groups from different geographic regions to follow this protocol. The main goal is to be able to pool the data in fields where patient recruitment is challenging.
- Open process
  All data should be open to the public.
- Communicating results
  - Organise dissemination and communication to all stakeholders.
  - Generate guidelines and summaries for the optimised use of each old antibiotic and integrate them into clear stewardship programs.

Project description:

We propose the implementation of an **open platform for the evaluation of combination antibiotic therapy**, as certain combinations are likely clinically synergistic. This synergy may translate into shorter overall antibiotic courses and thereby shorter exposure of "bystander" bacteria to antibiotics, with lower likelihood of resistance development. This platform will:

– Develop methodology to define predictive *in vitro* and *in vivo* studies
– Identify knowledge gaps
– Create a multi-expert consortium
– Develop a master protocol to study combinations
– Perform randomised controlled clinical studies of prioritised combinations
– Create an open data hub
– Define treatment and stewardship guidelines for the improved usage of old antibiotics

According to our current experience with colistin, a timeline up to 5 years is sufficient to perform important in vitro, in vivo and clinical studies.

Resources needed:

– Multi-expertise academic teams, including expertise in pharmacokinetics and –dynamics (PK/PD), *in vitro* and *in vivo* PK/PD expertise, PK/PD modelling, determination of breakpoints, clinical trials, evidence-based principles, meta-analysis, regulatory expertise, stakeholder communication.
– The financial resources depend on the number of antibiotics that are researched. According to current experience with the FP7-funded project AIDA, the costs for "redeveloping" one old antibiotic in a motivated academic European team is about 2,5-3 Million € (includes one randomised clinical trial)
– Potential partners involve experienced teams of the AIDA project, the ESCMID PK/PD study group (EPASG) and the members of the International Society of Anti-Infective Pharmacology (ISAP). Ursula Theuretzbacher is work package leader in AIDA, Founding President of the ESCMID PK/PD study group, President of ISAP, partner in the IMI project COMBACTE-MAGNET and work package leader in the IMI project DRIVE-AB with access to all needed experts.
– Access and conservation principles are currently researched in the IMI project DRIVE-AB.
– Leading key opinion leaders are partners in AIDA, EPASG, ISAP, DRIVE-AB, COMBACTE-MAGNET

Selected recent publications:

- Bergen PJ, Bulman ZP, Saju S, Bulitta JB, Landersdorfer C, Forrest A, Li J, Nation RL, Tsuji BT: **Polymyxin combinations: pharmacokinetics and pharmacodynamics for rationale use**. Pharmacotherapy. 2015 Jan;35(1):34-42.
- Ni W, Shao X, Di X, Cui J, Wang R, Liu Y.: **In vitro synergy of polymyxins with other antibiotics for Acinetobacter baumannii: a systematic review and meta-analysis.** Int J Antimicrob Agents. 2015 Jan;45(1):8-18.
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- Theuretzbacher U, Paul M: **Revival of old antibiotics: structuring the re-development process to optimize usage**. Clin Microbiol Infect. 2015 Oct;21(10):878-80.
- Theuretzbacher U, Van Bambeke F, Cantón R, Giske CG, Mouton JW, Nation RL, Paul M, Turnidge JD, Kahlmeter G: **Reviving old antibiotics**. J Antimicrob Chemother. 2015 Aug;70(8):2177-81.
- Muller AE, Theuretzbacher U, Mouton JW: **Use of old antibiotics now and in the future from a PK/PD perspective**. Clin Microbiol Infect. 2015 Oct;21(10):881-5.
- U. Theuretzbacher: **Global antibacterial resistance: The never-ending story**. J Global Antimicrob Resistance 2013, 1(2):63-69.

Platform: Improving the usage of old antibiotics (dosing regimens, indications)

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International Society for Anti-Infective Pharmacology (ISAP); ESCMID PK/PD study group (EPASG)

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Disease area:

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Though there is well-known geographical variation in resistance patterns, **critical resistance problem areas** can be summarised as follows:

— **Community-acquired infections caused by multidrug-resistant (MDR)** *Enterobacteriaceae*, which usually produce extended-spectrum beta-lactamases (ESBL), and are therefore resistant to aminopenicillins and cephalosporins and also frequently co-resistant to fluoroquinolones and other antibiotics. *Escherichia coli* and *Klebsiella pneumoniae* are recognised as important causes of hard-to-treat community-acquired urinary tract infections. Few oral treatment options remain in such cases, e.g. nitrofurantoin, fosfomycin trometamol, mecillinam.
  o Drug-resistant *Neisseria gonorrhoeae* may be treated with the old oral drug fosfomycin trometamol. Studies to support this treatment option are lacking.
— **Severe hospital-associated and, increasingly, community-acquired infections caused by carbapenem-resistant Gram-negative bacteria**.
  o The increasing prevalence of ESBL-producing *Enterobacteriaceae* has triggered an exponential empiric use of carbapenems, which in turn has created heavy selection pressure and an alarming increase in carbapenem resistance. In low-income countries, carbapenems may not be available. These bacteria are usually extensively drug resistant (XDR) and only susceptible to colistin and sometimes tigecycline, fosfomycin and/or an aminoglycoside. Indeed, alternative old drugs such as intravenous fosfomycin or temocillin to treat ESBL-producing bacteria are increasingly used where available, and combinations of antibiotics are used frequently (see proposal: Platform: Antibiotic combinations).

In the face of both increasing antimicrobial resistance and a dearth of novel antibiotics in the pipeline, it has become clear that we need new strategies. **One of these must be to revisit old, still active antibiotics to make sure that we are using them correctly and to their full potential**, as well as to determine if one or several of them can help alleviate the pressure on more recent agents. Some of the old antibiotics may be still active against MDR and XDR bacteria. Most of these old antibiotics have not been evaluated in a structured process for drug assessment and regulatory approval according to

current standards of science, as these standards and requirements have evolved over time. For most of these drugs, basic information is lacking. They are used according to potentially unreliable information collected 50-70 years ago. This situation is not acceptable as it risks suboptimal or inadequate treatment of patients and may accelerate emergence of resistance due to inadequate indications and dosing regimens.

Disease burden:

MDR and XDR Gram-negative bacteria increasingly cause infections globally but are specifically prevalent in southern European countries, in some North American areas and in most Asian and African countries. Infections that are commonly caused by MDR bacteria include urinary tract infections, *N. gonorrhoeae* infections, skin and soft tissue infections, pneumonia, and hospital-associated infections.
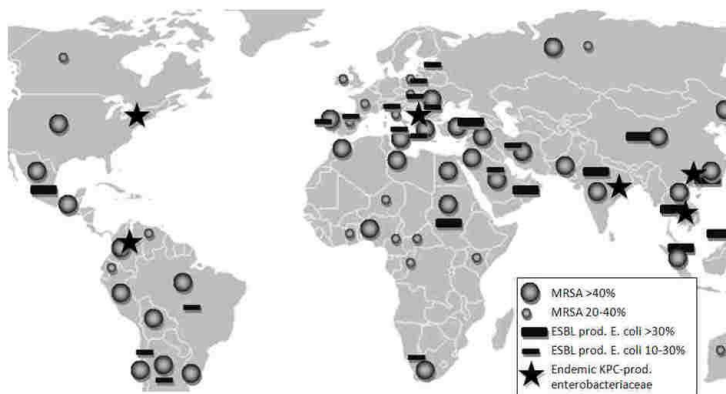
R&D gaps and needs:

Old and still useful antibiotics need to be "re-developed" to fill vital knowledge gaps regarding efficacy to current resistant pathogens, dosing regimens to improve activity, reduce risk of emergence of resistance, and minimise toxicity as well as suitable indications.

– Prioritisation
  A thorough literature review and analysis of usage data should provide the evidence for prioritisation. Based on this information, an expert stakeholder group should reach consensus regarding ranking of old antibiotics that are worthy of inclusion in such a project.
– Identifying knowledge gaps
  Structured reviews, meta-analyses and non-clinical studies are the basis for the gap analysis and prioritisation of required studies. The gap analysis provides the basis for the structured process for further studies of each prioritised antibiotic.
– "Re-development" process of old antibiotics
  o Perform all non-clinical studies in a collaborative consortium with a broad range of expertise
  o **Clinical trials**:
    Develop a **master protocol template for randomised controlled clinical trials** for each selected antibiotic and invite clinical research groups from different geographic regions to follow this protocol. The main goal is to be able to pool the data in fields where patient recruitment is challenging.
  o In contrast to a formal sequential development programme, the gap-filling strategy ("academic re-development") can perform many studies simultaneously and provide the opportunity for feedback and integration of non-clinical and clinical information in an extensive usage optimisation process.
– Open process
  o All data should be open to the public.

- Communicating results
  - Organise dissemination and communication to all stakeholders.
  - Inform and engage regulatory agencies and manufacturers as well as governments, policy makers and payers to ensure the availability of good-quality drugs and information to guide their use.
  - Generate guidelines and summaries for the optimised use of each old antibiotic and integrate them into clear stewardship programs.

Project description:

We propose the implementation of an **open platform for the reviving and "re-development" of old antibiotics**, which are (1) certainly not being used to their full potential today and, if optimised, (2) could alleviate the utilisation pressure on more recent and future antibiotics, and thus play a significant role in overall antibiotic conservation. This platform will:

- Prioritise old antibiotics
- Identify knowledge gaps
- Create a multi-expert consortium
- Significantly reduce the evidence gap with methodologically robust randomised clinical trials
- Create an open data hub
- Communicate with regulatory and national public health agencies
- Define treatment and stewardship guidelines for the improved usage of old antibiotics
- Ensure high-quality, controlled access to old antibiotics in low-income countries

According to our current experience with colistin, a timeline up to 5 years is sufficient to perform relevant *in vitro*, *in vivo* and clinical studies.

Resources needed:

- Multi-expertise academic teams, including expertise in pharmacokinetics and –dynamics (PK/PD), *in vitro* and *in vivo* PK/PD expertise, PK/PD modelling, determination of breakpoints, clinical trials, evidence-based principles, meta-analysis, regulatory expertise, stakeholder communication.
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- Tesh LD, Shaeer KM, Cho JC, Estrada SJ, Huang V, Bland CM, DiMondi VP, Potter AN, Hussein G, Bookstaver PB.: **Neisseria gonorrhoeae and fosfomycin: Past, present and future**. Int J Antimicrob Agents. 2015 Sep;46(3):290-6.
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- U. Theuretzbacher: **Global antibacterial resistance: The never-ending story**. J Global Antimicrob Resistance 2013, 1(2):63-69.

Oral Tebipenem for Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae

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Disease characteristics

Infections caused by Extended-Spectrum Beta-Lactamase (ESBL)-producing Enterobacteriaceae are very common in hospital-acquired infections and they have been increasing in community-acquired infections, especially urinary tract infections. Emergence of community-acquired infections due to ESBL-producing Enterobacteriaceae may be associated with a dramatic increase in prevalence of healthy carriers of ESBL-producing Enterobacteriaceae in their guts due to consumption of antibiotics and/or consumption of foods contaminated with ESBL-producing Enterobacteriaceae.

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Boonyasiri A, Tangkoskul T, Seenama C, Saiyarin J, Tiengrim S, Thamlikitkul V. Prevalence of antibiotic resistant bacteria in healthy adults, foods, food animals, and the environment in selected areas in Thailand. Pathog Glob Health 2014;108:235-45.

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Suankratay C, Jutivorakool K, Jirajariyavej S. A prospective study of ceftriaxone treatment in acute pyelonephritis caused by extended-spectrum beta-lactamase-producing bacteria. J Med Assoc Thai 2008;91:1172-81.

Existing treatments and needs for new/additional tools
The patients with severe infections due to ESBL-producing Enterobacteriaceae need to be hospitalized and treated with parenteral carbapenems (ertapenem, imipenem, meropenem, doripenem). Although etapenem can be administered once a day and the patient can be treated on out-patient basis, it is still

parenteral drug and it has to be given at the hospital in most of developing countries. The existing oral antibiotics that are active against ESBL-producing *E.coli* include nitrofurantoin and fosfomycin trometanol. However, these antibiotics are usually effective for therapy of uncomplicated lower urinary tract infection (UTI), such as acute cystitis, but they are not indicated for therapy of upper UTI including acute pyelonephritis. Although amdinocillin (mecillinam) is also oral agent and is active against ESBL-producing *E.coli*, several studies observed that its efficacy for therapy of ESBL-producing *E.coli* infection is modest. Therefore, more effective oral antibiotics against infections due to ESBL-producing Enterobacteriaceae are needed.

One of the most interesting oral antibiotics with activity against ESBL-producing Enterobacteriaceae is tebipenem pivoxil. It is the first oral carbapenem antibiotic available for clinical use. Tebipenem shows a broad-spectrum activity against Gram-positive and Gram-negative bacteria including ESBL-producing Gram-negative bacteria. Tebipenam pivoxil is well absorbed from oral administration. It is quickly converted to tebipenem by carboxyesterase localized at the intestinal epithelial cells and then it is transferred into blood. The half life of tebipenem is one hour. Tebipenem pivoxil is mainly excreted by the kidney, 54% to 73% of a dose. There are several clinical trials of tebipenem for therapy of infections including otolaryngological infections and pneumonia. Tebipenem is only available in Japan as fine granules (Meiji Seika Kaisha, Ltd.) for treatment of otitis media, sinusitis and pneumonia in children.

In vitro study of tebipenem against ESBL-producing *E.coli* isolated from Thai patients revealed that MIC_{50} and MC_{90} of tebipenem against ESBL-producing *E. coli* were 0.06 mg/L with MIC range from ≤ 0.06 to 0.25 mg/L. In vivo study in healthy Thai subjects who received 300 mg of tebipenem pivoxil 3 times a day for 2 consecutive days showed very high inhibitory and bactericidal titers of their serum samples and urine samples against ESBL-producing *E.coli*. No subjects experienced side effects related to receiving tebipenem pivoxil.

Tebipenem was also found to be active against fluoroquinolone-resistant *Neisseria gonorrhoeae* and *Burkholderia pseudomallei* isolated from Thai patients.

References

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Thamlikitkul V, Lorchirachoonkul N, Tiengrim S. In vitro and in vivo activity of tebipenem against ESBL-producing E. coli. J Med Assoc Thai 2014;97:1259-68.

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Seenama C, Tiengrim S, Thamlikitkul V. In vitro activity of tebipenem against fluoroquinolone-resistant *Neisseria gonorrhoeae* (In published)

R&D gaps and needs

The following research and development of tebipenem pivoxil should be considered.
1. Formulate tebipenem pivoxil for use in adults
2. Conduct clinical studies on efficacy and safety of tebipenem pivoxil for
2.1. therapy of mild to moderate infections caused by ESBL-producing Enterobacteriaceae in out-patient setting
2.2. step down therapy for severe infections caused by ESBL-producing Enterobacteriaceae after receiving several days of parenteral antibiotics, especially carbapenems
2.3. therapy of infection due to multidrug-resistant *Neisseria gonorrhoeae*
2.4. step down therapy and maintenance therapy of melioidosis

One of the concerns on tebipenem pivoxil which is a very effective oral antibiotic if it is widely available is induction of carbapenem resistance in Gram-negative bacteria. Therefore, conservation measures for responsible use of tebipenem pivoxil are extremely important.

New Polymyxin Formulations

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Disease characteristics

Infections caused by extensively drug-resistant (XDR) or carbapenem-resistant Gram-negative bacteria, especially *Acinetobacter* spp., *Pseudomonas aeruginosa*, and Enterobacteriaceae have been increasing in many countries around the World over the past decades. The mortality and economic burdens of these XDR Gram negative bacterial infections are enormous.

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Existing treatments and needs for new/additional tools

Polymyxins, polymyxin B and polymyxin E (colistin), are polypeptide antibiotics that were developed in the 1940s, but fell into disfavor due to their high toxicity rates. These two antibiotics have been used for therapy of infections caused by XDR Gram-negative bacteria over the past decades due to their good activities against XDR Gram-negative bacteria, especially carbapenem-resistant (CR) *Acinetobacter* spp., CR *Pseudomonas aeruginosa*, and CR Enterobacteriaceae (CRE); and a lack of newer antibiotics that are more effective and safer than polymyxins. Polymyxin B is administered parenterally in its active form as polymyxin B sulphate, while polymyxin E (colistin) is administered parenterally as an inactive pro-drug, colistimethate sodium. Many meta-analyses of mosty observational studies on efficacy of polymyxins revealed that their efficacy was moderate. The mortality of the patients with XDR Gram-negative bacterial infections who received polymyxins was still high. The currently used dosing regimens of polymyxins for the patient with good renal function and impaired renal function are still complicated and their recommended doses are usually too low. Although combinations of polymyxins with other antibiotics were found to be synergistic in vitro, many reports on clinical studies of antibiotic combination of polymyxin with other antibiotics (such as carbapenems, rifampicin, fosfomycin, tigecycline) for therapy of XDR Gram-negative bacterial infections did not reveal any clear benefits on clinical outcomes over polymyxin monotherapy. Combination of parenteral antibiotics, including polymyxins, with inhaled colistin did not demonstrate better final clinical outcomes of the patients with respiratory tract infections caused by XDR Gram-negative bacteria than parenteral antibiotics alone. Many patients who received polymyxins developed acute kidney injury. Therefore, use of higher dose of

the currently available polymyxin formulations (polymyxin B sulphate and colistimethate sodium) to improve clinical outcomes of the patients infected with XDR Gram-negative bacteria should have more risk of developing polymyxin-associated adverse events than benefit.

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R&D gaps and needs

New antibiotics that may be more effective and safer than polymyxins for therapy of XDR Gram-negative bacterial infections, such as fluorocycline (eravacycline), siderophore cephalosporin (S-649266), are under clinical studies and they will not be available for clinical use over the next few years. Therefore, it may be worth developing new formulations of polymyxins in order to increase the dose of polymyxins that could lead to better clinical outcomes and to decrease a risk of developing polymyxin-associated nephrotoxicity.

Formulation of parenteral colistin sulphate which is an active form of polymyxin E should be further explored since the preliminary results of therapy in 15 patients with XDR Gram-negative bacterial infections with parenteral colistin sulphate showed promising efficacy and safety.

Formulation of other colistin salt (in addition to colistin sulphate) or polymyxin analogue that is active form of polymyxin E should also be searched.

Formulation of parenteral polymyxins that can be administered in higher doses to improve clinical outcomes with no higher risk of developing acute kiney injury should be considered. The preliminary data on in vitro and in vivo activity, efficacy and safety of many preparations of polymyxins, such as liposomal polymyxins in animals are available.

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Novel treatment opportunities for acute melioidosis and other infections caused by intracellular pathogens

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1) Disease characteristics:
   Burkholderia pseudomallei (BP) is the causative agent for a severe and often deadly disease which is named melioidosis. BP is a Gram-negative, intracellular, aerobic rod which was classified until more recently as *Pseudomonas*. Melioidosis is an endemic disease of regions in southeast Asia (predominantly Thailand), northern Australia and expanding sporadic global distribution. The primary reservoir of BP is the soil and water. Transmission from the environment to humans most commonly occurs via open wounds and skin abrasions as well as via inhalation during severe weather conditions. Melioidosis can manifest a broad range of symptoms, with both acute and chronic manifestations. Acute disease can be severe, including fever, pneumonia, septic shock and death. Mortality rates of up to 50% are reported, depending to a large extent on diagnosis and onset of treatment. Co-morbidities such as diabetes, chronic renal failure or high alcohol consumption are strongly associated with severe infections and poor outcomes. Sub-acute or chronic manifestations are very common and include numerous sites and organs. Asymptomatic infections also occur and are able to relapse decades after the primary infection. (a.b)

2) Existing treatments and needs for new/additional tools:
   Current treatment modality of melioidosis is biphasic and very lengthy. Aim of treatment of the acute phase is to stop patients dying from overwhelming sepsis, while the aim of the second phase is eradication of any residual bacteria and minimising the risk of relapse.
   High-dose intravenous infusions of ceftazidime or meropenem (up to 6g or up to 3g /d, respectively) for 10-14 days are the treatments of choice for the acute phase, while treatment of the chronic phase usually takes up to 6 months by oral co-trimoxazole or co-amoxiclav. Parenteral high dose treatment of the acute phase, often in an ICU ward, severely limits treatment option by patients in rural regions and/or in developing countries with poor income profile. A low cost diagnostic as well as an oral treatment option would be highly preferable. In addition, effectiveness of the eradication needs improvement in terms of percentage eradication und of duration (and safety). (c,d)

3) Relevance in contributing to address AMR problem:
   2 characteristics of BP are relevant for a broader profile in the context of AMR:
   a) Gram-negative rod related to *Pseudomonas* which is classified as an "ESCAPE" organism by IDSA. Any new treatment of BP has a high probability of also succeeding against *Pseudomonas* et al.
   b) Exists as intracellular pathogen which are particularly difficult to address. Any new treatment of BP has a high probability of also succeeding against other intracellular pathogens, possibly both

facultative as well as obligate intracellular bacteria. As such *Coxiella, Legionella, Brucella* or Chlamydiae are of particular interest

4) Disease burden:
   a) Global epidemiology and populations affected
      A recent review summarises the global distribution of BP and burden of melioidosis. The disease is predicted to be ubiquitous throughout the tropics (southeast and south Asia, tropical Australia, western sub-Saharan Africa and South America) and few sporadic cases elsewhere, mostly by travellers. The review estimates an incidence of 150'000 cases annually of which 90'000 will die, 99% thereof in low- and middle-income countries. This incidence is approx. equivalent to measles and much higher than Dengue fever (e)

5) R&D gaps and needs:
   a) Lack of low cost diagnostic for rapid diagnosis and early start of treatment (see J Larsen BARDA presentation)
   b) Treatment limitations
      An effective oral treatment option for the acute phase of melioidosis seems to be highly desirable, both from an accessibility- as well as from a cost perspective for affected people in low-income countries.
   c) Resistance status
      BP shows high intrinsic resistance against numerous classes of established antibiotics, such as penicillin, early-generation cephalosporins, macrolides, rifamycin, colistin and aminoglycosides. Acquired resistance is relatively rare and chromosomally encoded. Most important is upregulation of efflux pumps, in particular BpeEF-OprC which should therefore be considered as resistance-target number 1 (f)
   d) Current R&D pipeline (key partners involved)
      Only a few BP organisms (10!!) are required to cause disease and as a result there is potential for use as a weaponized aerosol form both in a battlefield or terrorism situation. As a consequence, most of the current research work on new antibiotics against BP is funded by the US defence department in support of companies and institutions discovering and developing new BP treatments. Notably GSK, Basilea, Emergent Biosciences and Trius (now MSD). No breakthrough has been reported so far. Aim for those efforts is – as in other defence department funding projects – stockpiling of required doses as emergency preparedness in a bioterrorism attack

6) Project description:
   a) Outline R&D strategy
      Marketed or close-to-market drugs most probably exist which should be efficacious against BP – but which haven't been tested in melioidosis yet or which may need reformulation to become effective. The key issue in melioidosis appears to reside in the intracellular location of BP, because numerous antibiotics show activity against planktonic BP without activity in melioidosis. Therefore, one has to screen for antibiotics with the propensity to cross mammalian cell walls/membranes – or take measure to increase this propensity. Finally, combination of an

antibiotic with a host-directed drug may be a research route. In detail:

i) Screen available antibiotics for activity against intracellular pathogens. For example finafloxacin (Merlion) or JNJ-Q2 (Furiex) may be suitable candidates among quinolones. Test candidates in a murine or NHP BP aerosol model (g)

ii) Reformulate candidate drugs in a nanoparticulate drug delivery system to reach intracellular compartments – moxifloxacin or doxycycline as lead candidates? (h)

iii) Test host-directed antimicrobial drugs in the murine aerosol BP model, alone or in combination with suitable antibiotic candidates (i)

iv) Decide and execute on development strategy, depending on selection of candidate, its status and breadth of application (consider "animal rule" strategy)

b) Justify timelines (2 to 5 years project)
   i) i) to iii) to be done in parallel – 1.5 years
   ii) iv) between 2-6 years, depending on candidate and approach

c) Resources needed: type of expertise, rough estimate of financial and personal needs
   Virtual organisation – core group for supervision with antimicrobial and clinical expertise, all bench and clinical work outsourced

d) Potential partners (industry, academia, hospitals, etc.) to be involved/already contacted
   University of Chicago (host-directed antimicrobials)
   C. Ladavière (nanoparticulate drug delivery)
   University of Florida (Herbert Schweitzer and Hank Heine)
   BARDA/US-AMRI – NHP BP model and more
   Hospitals in Thailand

7) Access and conservation:
   Negotiate with industrial partners win-win scenarios, sharing rights on results and IP – have a scenario ready which makes results of R&D (see above) commercially and financially attractive to industrial partner (offer f.ex. rights to nosocomial pathogens, endocarditis, osteomyelitis…)

8) Key Scientific Opinion Leaders:
   Herbert Schweitzer – University of Florida
   Joe Larsen – BARDA
   Sharon Peacock – University of Cambridge UK
   David Dance – Oxford UK
   L. Czaplewski – Chemical Biology Ventures

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Addressing antibiotic treatment of neonatal & infant severe bacterial infections (SBI) in the context of high multidrug-resistant gram-negative bacteria (MDRGM)

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Disease area

1/4 of the 2.9 million neonatal deaths a year globally are attributable to infection. Whilst neonatal infection comprises 3% of DALYs globally, it attracts just £0.01 per DALY, the lowest of all infections. Neonates and young infants are at high risk of severe bacterial infections (SBIs) with significant associated morbidity and mortality. The overall possible SBI (pSBI) incidence in non-premature neonates in Latin America, Africa and South Asia is estimated to be 8% with a case-fatality risk of nearly 10%. pSBI accounts for nearly ¼ of deaths in this patient group in LMICs (Seale, TLID 2014). Recent modelling attributes 215,000 neonatal deaths a year due to resistant organisms alone in China, India, Pakistan, Nigeria and the Democratic Republic of Congo (Laxminarayan, Lancet 2016). Because SBI may present in a non-specific manner, initially most treatment is empiric (i.e. covering a range of target bacteria). Empiric treatment may provide inadequate antibiotic cover in settings where antimicrobial resistance is high. In HIC settings, guidelines defining the recommended empiric antibiotic regimens are highly variable (Spyridis, ADC 2016), perhaps in response to statements such as those made by the "Surviving Sepsis Campaign" that "[a]ntibiotic choices should be guided by local prevalence patterns of bacterial pathogens and susceptibility data" (Dellinger, ICM 2013). Data from Europe suggest that the broader-spectrum regimens used in some settings may not provide better cover when microbiological epidemiology is taken into account (Bielicki, JAC 2015). In LMICs, a standard regimen of an aminopenicillin plus gentamicin is recommended for neonatal SBI (WHO guideline in pSBI, 2015). Based on limited available surveillance data, a substantial proportion of neonatal and young infant SBI cases would not be adequately covered with this regimen (Downie, ADC 2012), particularly when considering Gram-negative bacteria (Le Doare, JPIDS 2015).

Multidrug-resistant Gram-negative bacteria (MDRGNB) SBI is associated with a very high mortality amongst neonates and young infants. Several reviews have identified lower- and middle-income countries (LMICs), such as India or Nigeria, as having high MDRGNB prevalence, but outbreaks and endemicity of MDRGNB have also been described for neonatal units in high-income countries (HIC). Together with an increasing hospital birth rate in LMICs, this may explain why meropenem was amongst the antibiotics accounting for 90% of prescriptions for hospitalized neonates in Africa, Asia and Latin America (Versporten, JAC 2015).

R&D gaps and needs

Increased access to the standard recommended antibiotic regimen of amoxicillin and gentamicin administered as a simplified regimen in the outpatient setting may follow the AFRINEST and SATT trials (Wall, PIDJ 2013; Baqul, Lancet 2015; Tshefu, Lancet 2015). However, AFRINEST and SATT have not as yet reported detailed microbiological data, meaning conclusions about treatment failure rate cannot be drawn. A strategy regarding the monitoring and treatment of this subset of patients must be developed. The unjustified use of third-generation cephalosporins and rapid escalation to carbapenems in community and hospital based management of neonatal and infant pSBI is likely to exacerbate the challenges of antimicrobial resistance and to raise issues around access to antibiotic treatment for infants with pSBI, when the only route of delivery is parenteral. The potential to optimise the standard regimen of ampicillin and gentamicin through more detailed pharmacological investigation is very important to the

management of neonatal and infant pSBI in LMIC settings. The potential impact of more widespread use of co-amoxiclav in neonates and infants with pSBI on resistance prevalence is unclear.

A review of older agents and their availability throughout HICs also pinpointed colistin, fosfomycin, temocillin and tobramycin as key options to be considered in the context of MDRGNB infections (Pulcini, CID 2012). Among these fosfomycin is an important potential agent to consider, as dosing recommendations for intravenous use in neonates exist, the agent can be administered intravenously, intramuscularly and orally, the side-effect profile is favourable (Raz, CMI 2012), in vitro susceptibility of MDRGNB is high (Falagas, Lancet ID 2010) and there may be a synergistic effect when fosfomycin is combined with other antibiotic agents (Kastoris, EJCP 2010). This proposal aims to both optimise existing antibiotic treatment options for global neonatal sepsis and develop a pharmacometric, microbiological and clinical trial network for rapidly and efficiently re-entering older antimicrobials into routine clinical practice.

Two stream research programme proposal:

1. Pharmacology/Pharmaceutical work stream: A systematic literature review is needed to collate what is known about the potentially anti-MDGRN synergistic effects of the possible combinations of amoxicillin (+/-clavulanate) gentamicin/amikacin, co-trimoxazole and other routinely used antibiotics combined with fosfomycin. The review should be complemented by grey literature, e.g. summary of medical product characteristics from pharmaceutical companies, and should identify any gaps in PK data that would have to be addressed within a clinical trial. Formulation issues must be addressed to facilitate access to antibiotic treatment for pSBI including consideration of IM and oral administration.

Standard regimen: (i) Evaluation of expected failure rate in target populations using improved surveillance data; (ii) evaluation of desired target total daily dose, taking into account efficacy and toxicity; (iii) definition of appropriate weight bands; (iv) development of appropriate formulations and/or single doses/concentrations to deliver optimal dosing regimens whilst avoiding development of resistance. The potential of significantly higher doses to overcome different molecular resistance mechanisms and the potential added risks/benefit of co-amoxiclav use will be investigated.

Novel combinations: We will use existing clinical surveillance networks such as neonIN (www.neonin.org.uk) and GARPEC (www.garpec.org) (both coordinated by SGUL) to identify strains of Enterobacteriacae (*E. coli* and *Klebsiella pneumoniae)* endemic in the target population with defined resistance mechanisms (e.g. KPC, VIM, OXA, NDM, CTX-M)) and *Acinetobacter baumannii* (both wild-type and defined mutants). We will study the following fosfomycin-based combinations: (1) fosfomycin + amikacin; (2) fosfomycin + ciprofloxacin; (3) fosfomycin + aztreonam and (4) meropenem + fosfomycin. We will study these combinations using shaking flasks, which will enable the temporal changes in total bacterial density and the resistant subpopulations to be estimated. The interactions will also be ranked in terms of their ability to suppress the emergence of drug resistance by subculturing to antimicrobial-containing plates. The leading 3-4 regimens that result in killing and prevent the emergence of drug resistance for key pathogens/ resistance mechanisms will be taken forwards for further study in the Hollow Fibre Infection Model (HFIM) (see Stage 2). Experimental models will be conducted for up to 14 days to provide a study duration that is clinically relevant and will provide a robust test of combination chemotherapy (shorter experimental periods may not provide time for the emergence of drug resistance). Human-like pharmacokinetics will be simulated in the HFIM. Drug concentrations in the HFIM will be measured using liquid chromatography/ mass spectrometry. We will describe the relationship between drug exposure (i.e. PK) and the antimicrobial effect (bacterial killing and the emergence of drug resistance) using mathematical models. The final solutions to these mathematical models will be used to define drug exposure targets that result in near maximal antimicrobial effect and minimise the emergence of drug resistance.

Proposed collaborators for this stream:

- Professor William Hope, University of Liverpool, Professor John Van den Anker, Director of Pediatric Clinical Pharmacology at **Children's National Medical Center in Washington**, Dr Joe Standing, Principal Research Associate, Institute of Child Health, UC, Professor Herman Goossens, Professor of Microbiology at the University of Antwerp, Professor Samir Saha, Professor of Microbiology and Executive Director of The Child Health Research Foundation at the Bangladesh Institute of Child Health, Dhaka Shishu Hospital, Dhaka, Bangladesh

2. Clinical trial stream: The clinical studies will build on the recent global SPRING initiative (Strengthening Publications Reporting Infections in Newborns Globally), which included most of the key individuals, funders and expert groups conducting global neonatal clinical sepsis research. This group have focussed on the need to increase quality and efficiency in global neonatal clinical trial reporting and design. An initial global consensus meeting will be held to facilitate agreement between relevant stakeholders on the definition of pSBI and optimal design for neonatal sepsis clinical trials in the context of current regulatory requirements for licensing of existing and older antimicrobial therapies. With regards to a definition of pSBI, minimal basic criteria will be agreed upon, to which additional criteria can be specified depending upon clinical setting. This will include reaching a consensus on appropriate p23harmacodynamic measures of sepsis resolution in neonates. This consensus meeting would consider if the most relevant and important antimicrobials for re-development, taking into account their availability internationally, the resistance landscape and the characteristics of the drugs in a global and geographically diverse context, have been selected. This consensus on neonatal sepsis monitoring, markers of treatment failure and clinical outcomes will be validated and used to evaluate the success/failure rate of current neonatal sepsis regimes in target populations. Existing cohort data will be identified and pooled where possible to aid study design, feasibility and power calculations. The aim of this component of the project is to build a collaborative platform of global neonatal sepsis clinical trials, through which a programme of potential optimal treatment regimens could be studied rapidly and efficiently. Pending the pre-clinical results, the initial proposed study is a targeted phase 1/2a PK and safety study of fosfomycin in combination with one of the above antibiotics in neonates. The clinical trial will incorporate risk stratification of patients, including the utilization of diagnostic methods with appropriate technology for that resource setting.

Proposed collaborators for this stream:
- Joy E Lawn, London School of Hygiene and Tropical Medicine and the SPRING Consensus group – listed in accompanying paper.

Access and conservation
The programme of research is built on the premise that currently a large number of neonates and infants cannot access appropriate antibiotic treatment for MDRGN pSBI. Building on the success of project partners in developing and providing child-friendly antiretroviral formulations through generic manufacturers (e.g. CIPLA), we would aim to engage a range of companies to address global antibiotic accessibility and roll-out. Furthermore, the focus on practical dosing regimens and development of risk stratification approaches will facilitate integration into IMCI and rollout by key stakeholders, such as the World Health Organization and UNICEF. Close collaboration with the planned expansion in microbiological surveillance and laboratory capacity building through collaboration with the new WHO surveillance network GLASS and the Fleming Fund will be a core part of ensuring that the proposed programme is embedded in the evolving platform of global alliances for tackling antimicrobial resistance.

New formulations of Amoxicillin/clavulanic acid and challenges of heat stability

Organization: Médecins sans frontières (MSF)

Disease area: Pediatric infectious diseases

Within MSF field programs, co-amoxiclav is prescribed in two distinct clinical situations:

1. In recurrent common infections such as otitis media, sinusitis or community-acquired pneumonia treated in outpatient department (mostly by general practitioners)
2. In severe conditions such as infections of soft tissues (cellulitis, fasciitis, noma), of bones and articulations given as oral therapy following initial intravenous therapy

Existing treatments and needs for new/additional tools

Co-amoxiclav exists in different formulations and dosages. Theses dosages have different ratios of amoxicillin/clavulanic acid. The 4/1 ratio formulation is listed as a WHO Essential Drug and in most of the national lists in developing countries.

The 4/1 ratio formulation contains a too high dosage of clavulanic acid and insufficient amoxicillin for paediatric age groups. The clavulanic acid dose can lead to severe diarrhoea, a side effect that further endangers the vulnerable patient. The ideal amoxicillin/clavulanic acid ratio would be 14/1 although a 7-8/1 ratio is acceptable. A 14/1 ratio formulation is available in high income countries but is too expensive to be used widely in low-income contexts.

At present, the 4/1 ratio formulation is used within MSF for mainly for patients with resistant forms of tuberculosis.

Among 500 children admitted between May and August 2013 and between May and August 2014 in a district hospital in Koutiala, Mali, co-amoxiclav was prescribed in 20% of children who received an antibiotic. When an IV antibiotic was prescribed, co-amoxiclav was then given as relay per os treatment in 25% of cases. Co-amoxiclav was the drug with the 2nd highest daily defined dose for 100 patient-days (ceftriaxone being the first).

With respect to per os, MSF uses mostly the 7/1 and 8/1 ratio (75% of total consumption volume). However, this proportion falls to 56% when it comes on reconstituted solutions. This is partly explained by the fact that MSF works within public hospitals and follows national protocols based on WHO recommendations and available formulations.

Aside from the ratio, there is a need for a formulation as dispersible tablets and not as solid tablets.

Regarding severe infections of soft tissues and bones or articulations, a suspension of co-amoxiclav is available with a 7-8/1 ratio. However, the dosage of clavulanic acid is not stable after reconstitution. The degradation of the clavulanic acid in a reconstituted suspension (ideally kept at all times between 2 to 8°C) was reported by a manufacturer (Sandoz); a 13% loss over 7 days was observed. MSF makes the hypothesis that even larger loses may occur at higher temperatures. Heat stable formulations are therefore required for use in many low-income settings.

Overall, MSF field teams face three major constraints:

- Need for an appropriate ratio of amoxicillin/clavulanic acid
- Need for a heat stable syrup or dispersible tablets
- Too high price of co-amoxiclav with a 14/1 ratio

Relevance in contributing to address AMR problem

1 – Regarding ENT and respiratory infections, the inappropriately low dose of amoxicillin means patients may receive an under-dosage even when the prescription is properly followed. The clavulanic acid also has the potential to decrease adherence to treatment when a child presents with diarrhoea induced by co-amoxiclav. In both cases under-dosing and under-adherence leads to incomplete treatment and promotes drug resistance.

2 – Regarding severe infections of soft tissues and bones or joints, the instability of the clavulanic acid can lead to under-dosing.

3 – In general, appropriate dosage and formulation increase adherence thereby contributing to the limitation of the emergence of resistance.

Disease burden:

Children represent approximately 20% of the general population in developing countries and account for a high proportion of the disease burden requiring medical attention where infectious diseases remain a leading cause of childhood mortality. Pneumonia is the leading cause of death among children under 5 worldwide. The situation is particularly critical among malnourished or HIV infected children.

In 2014, around 2 million children under 5 were treated in MSF programs with an estimated 222 000 inpatient admissions. Severely malnourished children and neonates represented a substantial proportion of admissions. Upper and lower respiratory tract infections were the largest cause of consultation in children under 5; LRTI was the first cause of death among under-5s worldwide and one of the leading causes in MSF in-patient departments.

Among patients treated by MSF, approximately 150 000 children and 160 000 adults were treated with co-amoxiclav in 2014.

R&D gaps and needs:

- Treatment limitations

Inappropriate ratio of amoxicillin/clavulanic acid for a large patient group. Currently available formulations are only in suspension or non-breakable tablet form both of which complicate dosing and acceptance. Suspension is unstable with probably significant loss of clavulanic acid at high temperature over time leading to an unknown and improper dosing.

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Zoliflodacin for the management of uncomplicated gonorrhoea

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Disease area: Uncomplicated gonorrhoea

1. **Description of disease**:
Uncomplicated gonorrhea is a sexually transmitted infectious disease caused by *Neisseria gonorrhoeae*. *N. gonorrhoeae* most commonly infects the lower genital tract (i.e. urethra/cervix; urogenital gonorrhea), rectum, and pharynx; these infections are classified as uncomplicated. Infections of the upper genital tract and of non-/extra-genital sites also occur and are classified as complicated. Untreated, uncomplicated gonococcal infection may lead to severe secondary sequelae, such as pelvic inflammatory disease, ectopic pregnancy, and infertility. Briefly, the major syndromes include:

- **Uncomplicated gonorrhoea**: Ninety percent of men with urethral infection have symptomatic mucopurulent penile discharge and dysuria. In contrast, women may be asymptomatic or exhibit non-specific symptoms such as odorless mucopurulent vaginal discharge and vaginal bleeding.
- **Disseminated gonococcal infection (DGI), including septic arthritis**: DGI, a rare complication, frequently results in skin lesions, asymmetric polyarthralgia, tenosynovitis, or oligoarticular septic arthritis; occasionally perihepatitis and rarely endocarditis or meningitis can occur.
- **Gonococcal ophthalmia neonatorum**: Gonococcal conjunctivitis, acquired during perinatal exposure, is an acute illness that may result, if untreated, in blindness.

2. **Treatment**:
Combination therapy with a single dose intramuscular ceftriaxone and a single dose of oral azithromycin is the generally recommended treatment for uncomplicated gonorrhoea; combination therapy has been adopted because of the theoretical benefit that two antimicrobials with different mechanisms of action can potentially delay emergence/spread of resistance and because of the need to treat undiagnosed concurrent *Chlamydia trachomatis* infection. The generally recommended doses varies by geography; e.g., the US regimen is 250 mg ceftriaxone/1 g azithromycin while the EU regimen is 500 mg ceftriaxone/2 g azithromycin. Treatment of complicated gonorrhoea requires at least 7-days of parenteral therapy (e.g., ceftriaxone, ceftizoxime, or cefotaxime).

3. **Antimicrobial resistance**:
Development of *N. gonorrhoeae* resistance to available therapies is of major concern; e.g., in 2013 the US CDC ranked drug-resistant *N. gonorrhoeae* as an "Urgent Threat" (CDC 2015). Examples of drug classes that are no longer recommended as monotherapy due to resistance include sulfanilamides, penicillin, tetracyclines, and fluoroquinolones. Most recently, resistance to macrolides and to extended spectrum cephalosporins and subsequent clinical failures have been reported. Because the extended spectrum cephalosporins are the only current first-line option for treatment of gonococcal infection, resistance and treatment failures with these agents is of particular concern. The threat of widespread ceftriaxone resistance and untreatable gonococcal infection is real.

Disease burden including antimicrobial resistance:

WHO estimated that in 2008 there were approximately 106 million new cases of gonorrhoea in adults aged 15-49 (WHO 2012). Furthermore, this represented a 21.0% increase since 2005 in the global incidence of gonorrhoea. The global epidemiology by WHO Region (WHO 2012), summarized below, suggests that gonorrhoea disproportionately impacts low/middle-income countries.

WHO Region	Incidence (per 1,000)		Incidence (Millions of cases)
	Male	Female	
African	60.3	49.7	21.1
Americas	27.6	18.5	11.0
South-East Asia	37.0	16.2	25.4
European	7.0	8.3	3.4
Eastern Mediterranean	11.6	8.1	3.1
Western Pacific	49.9	34.9	42.0

Derived from WHO 2012

The WHO GASP (Gonococcal Antimicrobial Surveillance Programme) network is a worldwide laboratory network that monitors for resistance of *N. gonorrhoeae* to antimicrobial agents (WHO 2013). High rates of fluoroquinolone resistance are reported in all but a "handful of countries". There are growing reports of decreased susceptibility to ceftriaxone and cefixime. Furthermore reports of failure to treat pharyngeal gonorrhoea with ceftriaxone have been verified in several countries. Data on antimicrobial resistance in resource constrained settings, however, are scarce. WHO assumes therefore that "the treatment failures … represent only the tip of a silent epidemic of antimicrobial resistance".

R&D gaps and needs:

The totality of data indicate that gonorrhoea is widely prevalent, disproportionately impacts low/middle-income countries, and is becoming more difficult to treat due to antimicrobial resistance. There is an urgent need to identify new agents to treat gonorrhoea. Three new agents are in clinical development: ETX0914 (zoliflodacin, Phase 2), solithromycin (Phase 3), and GSK2140944 (Phase 2). **ETX0914, the focus of this current proposal**, is a first-in-class oral antimicrobial agent with no pre-existing clinical resistance because of a novel mechanism of action—inhibition of bacterial DNA type II topoisomerase by a mechanism distinct from other DNA topoisomerase inhibitors (Basarab 2015; Unemo 2015).

Project description:

ETX0914 is currently in Phase 2 clinical development; the Phase 2 study (https://clinicaltrials.gov/ct2/show/NCT02257918?term=etx0914&rank=1) has completed enrolment. Assuming a successful outcome, ETX0914 will be ready to enter Phase 3 in 2017.

Entasis Therapeutics is seeking a partner to support the Phase 3 clinical development program. It is anticipated that licensure of ETX0914 in the US and in the EU for treatment of uncomplicated gonorrhoea will require a single Phase 3 study of approximately 600 patients in addition to the Phase 2 study. Preliminary assessment does not predict any significant impact on ETX0914 exposure by body weight, by race, or by age. As such, the results from the Phase 2 and Phase 3 program should be directly relevant to low/middle-income areas which have the greatest burden of disease. Key supportive data on ETX0914 and a summary timeline are in the attached slides.

The Phase 3 study will take approximately 18-24 months from first-patient enrolled to final clinical study report.

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Enteric Fever

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Disease area: Typhoid fever (enteric fever): blood-borne infection with *Salmonella enterica* serovars Typhi (accounting for about 75% of enteric fevers) and Paratyphi (accounting for the remaining 25%). [General reference is Parry & Basnyat, *Oxford Textbook of Medicine*]. Transmission is mainly through contaminated food and water, hallmarks of low socio-economic status; typhoid fever is a disease of poverty.

Typhoid fever has been largely neglected in global health, and case detection and treatment has taken a back seat to a push for increased vaccine use. A broader focus suggests that typhoid has the potential to be eradicated. It is a disease exclusively of humans with no known animal reservoirs, which has largely disappeared from high-income countries as a consequence of improved living conditions. Whole genome analysis suggests that *S.* Typhi and Paratyphi have become so specialized to humans that they could be vulnerable to an eradication strategy that includes case detection and treating clinical cases and typhoid carriers, in combination with vaccination and improved WASH access [Parkhill et al, 2002]. Even short of an eradication strategy, however, attention to the problems of diagnosis and treatment are urgently needed.

The first-line treatments for typhoid fever in many endemic areas are fluoroquinolones, third-generation cephalosporins and azithromycin. Antibiotics that were effective in the past (ampicillin, chloramphenicol, co-trimoxazole) have been lost to resistance over the past three decades, although in some parts of the world they remain active. In a recent randomised controlled trial from Nepal, a high failure rate of the fourth generation fluoroquinolone gatifloxacin was found for culture-confirmed cases of *S.* Typhi (Arjyal et al., 2016). Many of these failures were in patients infected with the H58 clade of antibiotic-resistant typhoid, which has spread from the Indian subcontinent to southeast Asia and most recently, to Africa [Wong V et al, 2015]. Also of interest, in this Nepal trial, the clinical responses to gatifloxacin and ceftriaxone were dramatically different between the culture-positive and culture-negative patients.

NOTE: A related area is invasive non-typhoidal *Salmonella* (NTS), which is highly prevalent in some parts of Africa and has similar problems in case detection and treatment. However, the diseases are significantly different and are not addressed in this proposal.

Disease burden:

The typhoid fever burden falls almost entirely on LMICs, with an estimated 20.6 million (17.5–24.2) cases and 223,000 (131,000–344,000) deaths each year (Mogasale et al. 2014). South Asia (the Indian subcontinent), with the highest death toll; Central and SE Asia; Indonesia and sub-Saharan Africa are the most endemic areas, with annual incidence rates of 100-1600/100,000 (OTM). Children and young

adults are most affected, suggesting some acquired immunity. The additional burden of paratyphoid fever has been difficult to estimate, although high rates have been reported in Asia, particularly China (Arndt et al., 2014).

An interest in clarifying the worldwide burden of typhoid fever has been sparked by greater interest in the use of typhoid vaccines, in advance of the impending availability of a Vi conjugate vaccine. To fill this information gap, the Gates Foundation has funded two large studies through the International Vaccine Institute (IVI): one focusing on Africa, that has recently been completed (soon to be published) and a similar study that is under way in Asia. A new follow-on study in Africa is focusing on the burden of severe typhoid and typhoid deaths.

These studies should provide a strong base for planning research on diagnosis and treatment. However, more studies like the recent Nepal study are needed to understand the status of antibiotic resistance in typhoid. Studies in Africa and Asia are essential.

R&D gaps and needs

TREATMENT

Optimizing treatment is crucial for individual patient care, but also as a control mechanism to complement vaccination. Resistance to the previously effective drugs (ampicillin, chloramphenicol, co-trimoxazole) means that in many endemic areas the treatment of typhoid relies on cephalosporins, fluoroquinolones, and azithromycin. Unfortunately, additional resistance to fluoroquinolones is now common in many parts of Asia. Resistance rates vary significantly around the world, but in some areas very limited choices are now available (Wain et al, 2015). The evidence base for global recommendations on the use of existing, and potentially new, antibiotics for typhoid is limited by the fact that most regimens have not been systematically tested in adequately powered clinical trials. One site and group of investigators—the Oxford Clinical Research Unit in Kathmandu and collaborators—is responsible for all recent large treatment trials, including the most recent (Arjyal et al., 2016). No similar trials have taken place in Africa. Few studies have measured the pharmacokinetics/pharmacodynamics (PK/PD) of antibiotics used for typhoid, or the efficacy of modern antibiotics in combinations (Crump et al, 2015). A crucial difference with other Gram-negative bacteraemias is the predominantly intracellular location of the pathogen and that infection can be followed by convalescent and chronic faecal carriage. Intestinal carriers are the source of infection for others in the community. Although most currently used antibiotic regimens appear to be reasonably effective, the optimum dose and duration and their effect on the eradication of carriage and prevention of resistance emergence has been little studied. The steady, step-wise emergence of resistance over the last 20 to 30 years raises the issue of whether higher dosages and/or combinations might slow the evolution and spread of resistant organisms.

The following areas of inquiry could lead to better treatment and should be pursued:

- Adequately powered trials of existing antibiotic regimens with a particular focus, in addition to efficacy, on the prevention of resistance emergence in *S.*Typhi and *S.* Paratyphi A, and also the normal bacterial flora and the eradication of intestinal carriage.
- Trials of combinations of antibiotics (concentrating on the cephalosporins, fluoroquinolones, and azithromycin initially, but eventually including other agents) for treatment efficacy and for prevention of the emergence of resistant organisms and eradication of carriage.

- These studies should include PK/PD components, as dosage is a concern, including studies of the intracellular penetration of the antibiotics. It is likely that current treatment regimens are often underdosing patients and that long-term underdosing with fluoroquinolones, in particular, has been driving resistance. The importance of this type of information is illustrated by the revision of fluoroquinolone laboratory breakpoints for *Salmonella* and the introduction of tentative breakpoints for azithromycin based on clinical trial data (Crump et al., 2015)
  - An in-vitro hollow fibre modelling component could add to the value of these studies. Little has been done in this area, and it is worth some effort to determine the value of the information produced (Booker et al., 2005).
- Trials of new antibiotics active against Gram-negative infections should be tried against typhoid as they become available so that reserve options are available if pan-resistance should emerge to the existing antibiotics.
- Trials of screening and treatment of carriage as part of a global control strategy. The last trial of treatment of typhoid carriers was conducted in the 1980s.
- Evaluation of novel strategies – for example, linking existing antibiotics to nanoparticles to improve intracellular penetration of the drug.

DIAGNOSIS

Typhoid fever symptoms are similar to those of a range of febrile illnesses. Only bacterial blood culture is definitive, but multiple cultures may be needed as culture is often unsuccessful, in part because of low numbers of organisms present in blood and prior antibiotic therapy. Blood culture is expensive for the areas where typhoid is most common. Some attempts have been made to improve diagnostics, but no cheap, simple point-of-care test for typhoid has been developed. This leaves an enormous gap for treating patients, but also for understanding the burden. It is clear from the few studies that have been done that many cases treated presumptively may not be typhoid. The use of malaria rapid tests has probably exacerbated the situation, as people who were inappropriately treated presumptively for malaria now are treated for an inappropriate presumptive diagnosis of typhoid. In Africa, the ideal test would be a multiplex diagnositic for malaria, typhoid, "other bacteria," and possible some viruses. However, a single spot test for typhoid would also be very useful.

There are already many rapid tests on the market, mainly antibody tests, many of which have not been properly evaluated or evaluated in poor quality studies. The main RDTs that have been evaluated are the Typhidot-M® test; the TUBEX™ test; and the LifeAssay Test-it Typhoid ICT. They use a variety of formats for detecting IgM antibodies against *S.* Typhi antigens (LPS; 50KDa OPMP). They all lack sufficient sensitivity and specificity to be recommended for routine use (Crump et al, 2015). A priority is to conduct a systematic evaluation of these tests (similar to the malaria RDT evaluation conducted by FIND). A biobank of patient samples could be assembled for this purpose.

A number of current efforts for better typhoid diagnostics are under way. These include (with lead contacts):

- Molecular tests using PCR and real-time PCR directly on blood, so far with mixed results, at least partly because of low numbers of organisms in blood, the same problem that limits blood culture.

- Real-time PCR kit after short-term blood culture (~1 day), currently in trials.
- Circulating lymphocytes producing typhoid-specific IgA antibodies (TP test).
- Better antibody tests using antigens generated from the genome sequences, potentially aiming to use a combination of antigens.
- Metabolites expressed in typhoid patients, with some promising results from patients in Nepal and Bangladesh; at proof-of-principle stage.
- LAMP (simple PCR done at one temperature in one tube); early stage.

Describe the current R&D pipeline (and list key partners involved) and demonstrate gaps

None other than the R&D pipeline for the treatment of Gram-negative bacterial sepsis

Project description

Evaluate PK & PD of existing antibiotics alone and in combination in patients with typhoid fever and in an in vitro (hollow fibre) model

Candidate regimens of single antibiotics or combinations to be taken forward into randomised controlled trials in Africa and Asia. The RCT to assess efficacy but also the potential to prevent resistance using whole genome sequencing

The clinical trials will be used to collect a well-characterised biobank of relevant samples for evaluation of existing and new diagnostic tests

Systematic evaluation of marketed typhoid rapid tests

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Novel antibiotics for H. pylori infection

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Disease area: Gastric ulcers and gastric cancer du to Helicobacter pylori infection of the stomach

- Existing treatments and needs for new/additional tools

Existing treatments involved a tri-therapy associating two antibiotics among amoxicillin, metronidazole, chlarithromycin, fluoroquinolone and tetracycline with a proton pump inhibitor. In the absence of resistance, treatments are efficient in eradicating 80-85% of *Helicobacter pylori* infections.

- Relevance in contributing to address AMR problem

Resistance to first and second line treatments are increasing with primary resistance to chlarithromycin and fluoroquinolones being now above 20%, and metronidazole being around 40-50% leading to a high level of treatment failure.

Disease burden:

- Global epidemiology (if possible give specific information on low-/middle-income countries and high-income countries)

H. pylori infections affect around 50% of the human population with a very high burden in developing countries reaching around 80-90% while it is steadily decreasing in developed countries, the lowest incidence being Australia with less than 20% of the population.

- Populations affected (geographical, socio-economic)

In developing countries such in Asia and South America, the incidence is so high that there is no social-economic bias. In developed countries, the H. pylori infection is becoming more and more a problem affecting low income populations and recently immigrated populations from developing countries.

R&D gaps and needs:

- Treatment limitations

The resistance to treatment is increasing steadily particularly in developing countries. There is no available vaccination and no new therapeutic options.

- Resistance status

Monotherapy is not efficient against *H. pylori* infection. Eradication efficiency only reaches high levels (80-90%) with the use of tripletherapy. However, due to the particular niche of *H. pylori*, the number of efficient drugs is limited to amoxicillin, chlarithromycin, fluorquinolones, metronidazole and tetracycline. Unfortunately, resistance to three of these drugs is above 20% of the clinical strains (chlarithromycin, fluoroquinolones and metronidazole). Tetracycline is authorized for human use only in very few countries. Hence, only amoxicillin has remained active against *H. pylori* but has the disadvantage to be poorly efficient in eradicating the infection as monotherapy (around 20%).

- Describe the current R&D pipeline (and list key partners involved) and demonstrate gaps

We have be studying the peptidoglycan cell wall machinery of *H. pylori* to find new targets and develop new inhibitors of peptidoglycan assembly to use in combination with amoxicillin and proton pump inhibitors. Assembly of this essential macromolecule requires the coordination of multiple proteins that function in protein complexes. Beta-lactams such as amoxicillin only target the enzymatic activity of one of such activities, the transpeptidase activity of penicillin-binding proteins (PBPs). Our goal is to inhibit simultaneously different protein targets involved in peptidoglycan assembly reducing the ability of bacteria to raise resistance mechanisms. We are currently collaborating on the structural aspects with the team of Andrea Dessem of the IBS, Grenoble. We are negotiating a R&D project with DeBioPharma to screen new molecules and improve existing hits through medicinal chemistry.

Project description:

- Outline R&D strategy

Our current strategy is to develop small molecules targeting the assembly of the protein complexes involved in peptidoglycan synthesis. We have shown in the past that the assembly of the complexes themselves is essential for function. We have identified an essential core complex between PBP2 and the morphogenic protein MreC which association as a complex is essential for bacterial viability. We have screened for small molecules using a FRET-based assay that inhibit the protein complex association. We have also solved the crystal structure of the PBP2-MreC complex. We want to use this knowledge to further develop our hits and identify additional inhibitors with additional chemical libraries screens to develop novel antibiotics that inhibit peptidoglycan assembly. These molecules can be used by themselves or in association with other peptidoglycan synthesis inhibitors such as beta-lactams or glycopeptides.

- Justify timelines (2 to 5 years project)

Our project is based on extensive preliminary data (existing crystal structure, a first screen that has led to two promising natural products as inhibitors of PBP2-MreC assembly) and is aimed at

1) testing these molecules and novel ones for their inhibitory effect on bacterial growth first on H. pylori and then of relevant bacteria of the ESKAPE group
2) screen for additional chemical libraries using of FRET-based assay
3) improve existing hits using modelling-docking bioinformatics analysis and medicinal chemistry

In order to accomplish these three goals we have defined a timely of three years.

- Resources needed: type of expertise, rough estimate of financial and personal needs

We require the financial resources to perform additional chemical libraries screens and to test the hits and leads on relevant bacterial and in vivo infection models (180 k€)

We require a three-year post-doctoral fellow to accomplish task 1 and 2 (180 k€)

- Potential partners (industry, academia, hospitals, etc.) to be involved/already contacted

We already have advanced discussions with DebioPharma to develop the medicinal chemistry of our hits. We will continue our collaboration with Andrea Dessen on structural aspects of the protein complexes. We will screen new chemical libraries using the Institut Pasteur screening facility and French national chemical library collection.

Access and conservation:

- Ideally, provide suggestions on how the conservation and access of the new product can be addressed/tested.

Key Scientific Opinion Leaders:

- List Key Scientific Opinion Leaders supporting the project, or that potentially will support the project.

Waldemar Vollmer (University of Newcastle), Terry Roemer (MSD), Patrick Muzzin (DebioPharma), Karen Bush (University of Indiana), Gerry Wright (McMaster University)

Meeting contacts:

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