Project proposal form– 2018 entry

Project title:	**Predicting the fate of resistance genes, plasmids and hosts in mixed communities**
Project code: FOR CENTA USE ONLY	
Host institution:	**University of Birmingham**
Theme:	**Anthropogenic Impact; Evolution & Ecosystems (fits 2 CENTA themes)**
Key words:	**Individual-based modelling, Plasmid transfer, Fitness cost, Antimicrobial resistance**

Supervisory team (including institution & email address):

Dr Jan-Ulrich Kreft (University of Birmingham, j.kreft@bham.ac.uk)

Prof Elizabeth Wellington (University of Warwick, E.M.H.Wellington@warwick.ac.uk)

Project Highlights:

- Effect of biodiversity on fate of resistance plasmids: are there preferential transfer paths? How would that affect risk analysis?
- Can we predict persistence of resistance based on transfer rates and fitness costs? Or do we need to include co-evolution of plasmids and hosts?
- Is inhibition of plasmid transfer the most effective way to reduce resistance?

Overview:

The widespread use of antibiotics in human and veterinary medicine and as growth promoters in agriculture has not only selected for resistance genes but also for plasmids carrying resistance genes. As a result, plasmids as vectors of resistance genes have become prevalent, enhancing the rate of aquisition and dissemination of any new resistance genes as soon as new antimicrobials are introduced. Likewise, the simultaneous or alternating presence of several antimicrobials has selected for insertion sequence elements (IS) and other mobilizers of genes as they facilitate the accumulation of multiple resistances on single plasmids. Together, this has increased the potential for pathogens to aquire new resistances and new combinations of resistances. Multidrug resistant pathogens have brought us back to the pre-antibiotic era.

Hotspots of resistance genes in the environment are wastewater treatment plants and animal manures and slurrys on farms, which are being spread on arable land. Runoff from these fields and effluent from wasterwater treatment enter rivers and river sediments, also untreated sewage is discharged into rivers through storm overflow drains.

The group of Prof Wellington has isolated numerous *Escherichia coli* strains from river sediments. They carry a range of different types (IncF, IncA/C, IncQ) of plasmids with various resistance genes, eg the extended spectrum beta-lactamase $bla_{\text{CTX-M-15}}$. Most of these strains are commensals but more than 10% carry virulence genes and resistance plasmids and are well known pathogens such as ST131, a common cause of urinary tract infections in hospitals, clearly linking human pathogens to environmental reservoirs.

We will use simple mass-action and more powerfull individual-based models to understand and predict the fate of resistance plasmids in mixed communities of several E. coli strains. The models will be based on plasmid transfer rates and fitness costs measured by a MIBTP PhD student in the Wellington lab (Victoria Clark) who has been investigating the growth of these strains and plasmid dynamics in chemostats and is co-supervised by Dr Kreft. She will be two years ahead of this joint modelling project, which will enable the data to feed into the modelling and the modelling to feed into the experiments.

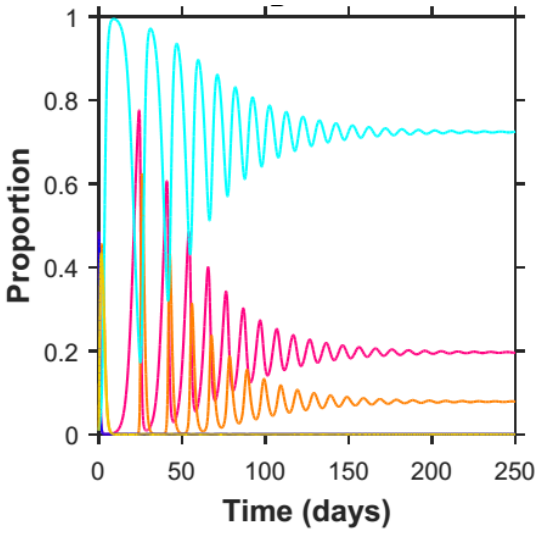

Figure 1: *A simple mass-action model of plasmid dynamics in chemostats shows damped oscillations and that Narrow Host Range (NHR) plasmids (cyan) benefit from the presence of Broad Host Range (BHR) plasmids (orange). The only plasmid free hosts not becoming extinct can carry the BHR plasmid (magenta). From Zhang et al., in preparation.*

Methodology:

The modelling will be based on our investigation of the competition of Narrow Host Range (NHR) and Broad Host Range (BHR) plasmids both with mass-action and individual-based models. We find that BHR plasmids have an advantage in multi-species communities despite their higher fitness costs but that they help the persistence of NHR plasmids by infecting the strains competing with the NHR plasmid carrying strains (Figure 1).

Individual-based models have a number of advantages, most importantly for this study they can encompass the presence of an indiviual plasmid in an individual host cell facilitating the modelling of co-evolution of plasmid and host, as well as transfer to another particular host.

The data will mostly come from chemostat experiments, where cells can be grown at lower growth rates relevant to river sediments and the gut. Most of the data will come from Victoria Clark at Warwick but the DR will also carry out some experiments in collaboration with other students in the Kreft lab.

Training and skills:

CENTA students are required to complete 45 days training throughout their PhD including a 10 day placement. In the first year, students will be trained as a single cohort on environmental science, research methods and core skills. Throughout the PhD, training will progress from core skills sets to masterclasses specific to the student's projects and themes.
The Doctoral Researcher (DR) will acquire a broad set of mathematical modelling and statistical data analysis skills within an interdisciplinary project. The DR will learn to communicate and collaborate with an experimental DR, where the modelling will require certain data and generate predictions to be tested in the lab. The modelling will also guide experimental effort by identifying the most important parameters and processes.

Moreover, the DR will have the opportunity for public outreach activities to inform the AMR debate with the results of our project. Programming (Java, Matlab, Python, R), project management and communication skills will also be gained.

Partners and collaboration (including CASE):

The Kreft group has >15 years' experience in individual-based and other kinds of mathematical modelling. Current research includes modelling plasmid dynamics (Zhang et al., in preparation) and investigating the fate of resistance on a dairy farm (Kreft is Co-I on the NERC AMR-in-the-real-world grant "EVAL-FARMS").

The Wellington lab has isolated a host of E. coli strains with various plasmids and resistance genes from river sediments. These genome sequenced strains are currently being investigated in chemostats by the joint PhD student Victoria Clark.

There is strong potential for conversion to CASE that will be pursued before project start.

Possible timeline:

Year 1: Learn modelling of plasmid dynamics by expanding the existing model with co-evolution of plasmid and host reducing the fitness cost of the plasmid over time. Learn the possibilities and limitations of the experimental work so that the modelling reflects what can be measured. Use data already available to parameterize the model and learn how to do this. Perform sensitivity analysis to identify most important parameters and processes feeding into the lab work.

Year 2: Use and further develop the models to investigate the effect of increasing diversity on the persistence of plasmids in the absence of selection. Investigate the effect of growth rate (which can be controlled in the chemostats) on plasmid dynamics. Select some of the model predictions for experimental testing, either by Victoria in Warwick or the Kreft lab with help from PhD student Eleni Christidi.

Year 3: Investigate the effect of co-evolution and antibiotic selection on the spread and persistence of plasmids. Investigate potential mitigation mechanisms such as reducing antibiotic concentrations during sewage treatment, inhibiting horizontal transfer of plasmids or using CRISPR or other mechanisms to get rid of plasmids, avoiding co-selection by mixtures of antibiotics and/or heavy metals. Inform risk analysis – how likely is it that pathogens aquire resistance genes in the environment? Is it more or less likely at lower growth rates?

Further reading:

Merkey BV, Lardon LA, Seoane JM, Kreft J-U, Smets BF. 2011. Growth dependence of conjugation explains limited plasmid invasion in biofilms: an individual-based modelling study. Environ Microbiol 13:2435–2452.

Kreft J-U. 2014. Mathematical modelling of plasmid dynamics. *In:* Bell E et al. (eds.), Molecular Life Sciences: An Encyclopedic Reference. Springer-Verlag, Berlin Heidelberg.

Hellweger FL, Clegg RJ, Clark JR, Plugge CM, Kreft J-U. 2016. Advancing microbial sciences by individual-based modelling. Nat Rev Micro 14:461–471.

Amos GCA, Hawkey PM, Gaze WH, Wellington EM. 2014. Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment. J Antimicrob Chemother 69:1785–1791.

Amos GCA, Zhang L, Hawkey PM, Gaze WH, Wellington EM. 2014. Functional metagenomic analysis reveals rivers are a reservoir for diverse antibiotic resistance genes. Vet Microbiol 171:441–447.

Lehmann K, Bell T, Bowes MJ, Amos GCA, Gaze WH, Wellington EMH, Singer AC. 2016. Trace levels of sewage effluent are sufficient to increase class 1 integron prevalence in freshwater biofilms without changing the core community. Water Res 106:163–170.

Further details:

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Training proposal form

For guidance on training please see below – please note that a CENTA training session must be a minimum 5hrs

Session title:

Agent-based modelling: a hands-on masterclass

Brief description of content: (sufficient to allow us to identify duplication or possibilities for combination)

The course will last two full days to allow the DRs to learn to program a simple model of the spread of disease in NetLogo. An introduction into agent- or individual-based modelling (lectures, day 1, am) will be followed by a demonstration of designing and coding a core model of disease dynamics (workshop, day 1, pm). On the second day, participants can extend the core model in various ways to address specific research questions under guidance and help (workshop, day 2 am + pm). At the end the participants will leave with a working if simple model of disease outbreaks.

Who will deliver the training (including institution/s):

Jan-Ulrich Kreft, University of Birmingham

Which part of the programme does it fit? *Please circle or underline*

- **Which year:** either year
- **Which training group:** Theme Science
- **If Theme Science, which theme:** Anthropogenic Impact and Evolution & Ecosystems

NB: I am happy to discuss where this best fits.

Where will it be delivered? *Please circle or underline*

Birmingham

Are there any limits on class size?

Maximum about 10 (potentially more with the help of demonstrators)

Any specific equipment or software needed?

NetLogo