Universiteit Utrecht

Faculty of Veterinary Medicine

in collaboration with

CENTRAL VETERINARY INSTITUTE WAGENINGEN UR

Is loss of antimicrobial resistance possible?

ESBL dynamics in broiler chickens in absence of antibiotics

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Background

ESBL: resistance on plasmids (in *E. coli*)

Antimicrobial resistance paradigm: plasmid causes fitness disadvantage, so resistant bacteria will be lost in absence of antibiotics

However:

- Plasmids can be horizontally transmitted by conjugation
- *In vitro* we did not observe fitness disadvantage due to plasmid carriage

But maybe:

- Fitness effect was too small to observe *in vitro*
- Less efficient conjugation in real life due to lower concentration of *E coli* in the gut

Aim of *in vivo* experiment

Is extrapolation from *in vitro* to *in vivo* valid?

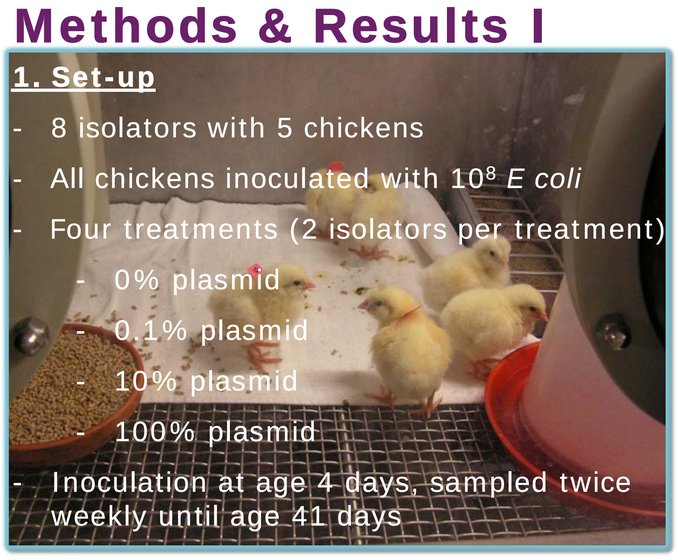
1. Is conjugation rate the same?
2. Do plasmids cause fitness disadvantage?

What is the consequence of the more complex *in vivo* environment?

3. How does the inoculum strain compete with resident *E coli*?
4. Are *E.coli* and ESBL dynamics dependent across chickens in one group?

Methods & Results I

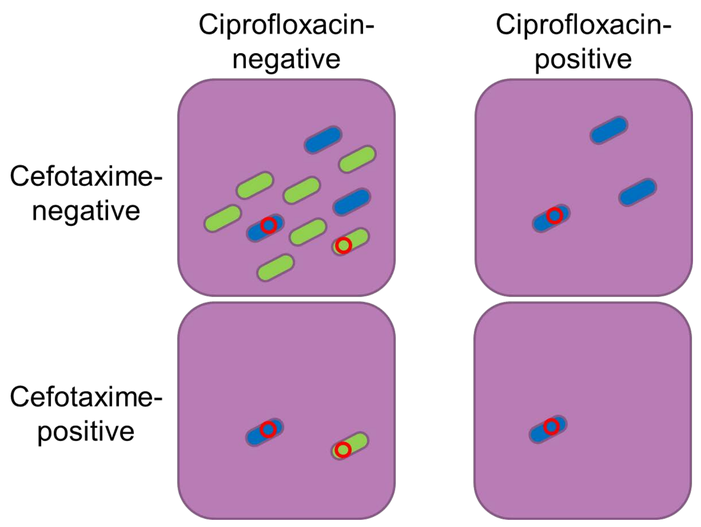
1. Set-up



- 8 isolators with 5 chickens
- All chickens inoculated with 10^8 *E coli*
- Four treatments (2 isolators per treatment)
  - 0% plasmid
  - 0.1% plasmid
  - 10% plasmid
  - 100% plasmid
- Inoculation at age 4 days, sampled twice weekly until age 41 days

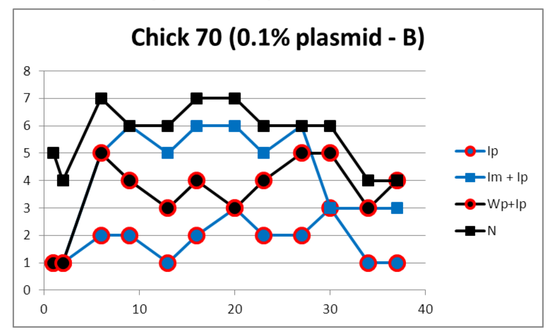
2. Lab analysis

- plating 10-fold dilutions
  - **wild-type**; **inoculum**; **plasmid**

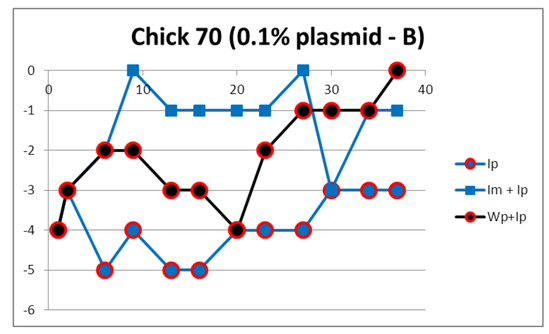


3. Raw data (log scale)

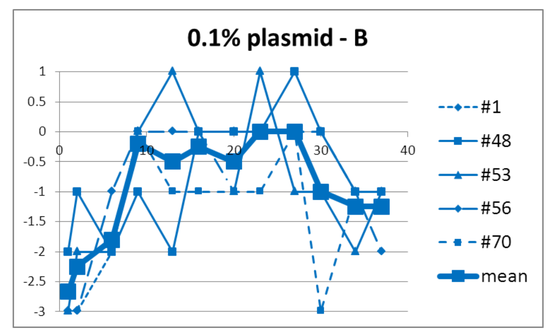
Four counts per sample, …



…changed to relative counts (to *N*)…

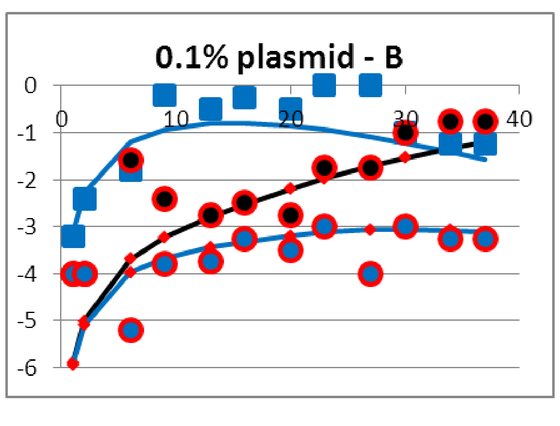


…for five chickens per isolator



Result: analysis per group, not per chicken

Methods & Results II



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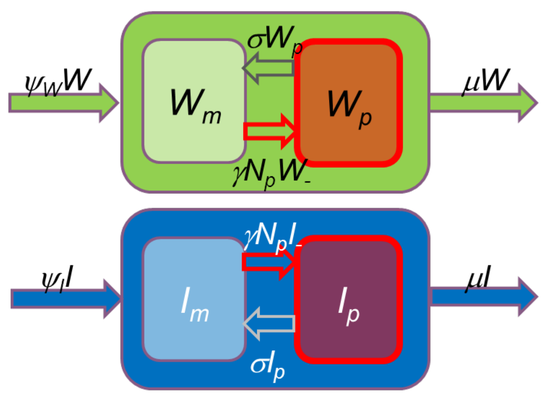

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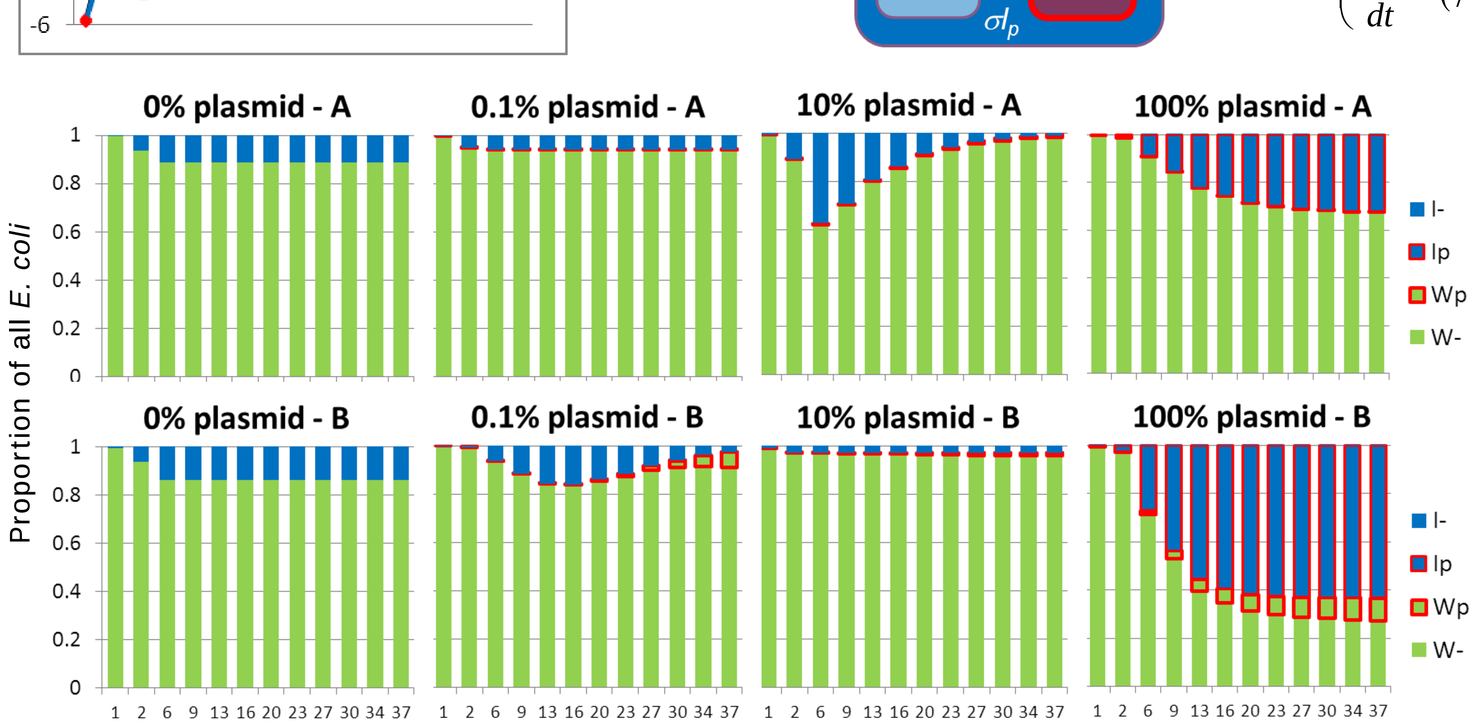
$$\begin{pmatrix} \frac{dw_-}{dt} = (\psi_W - \psi_I)\, i w_- - \gamma N_p w_- + \sigma w_p \\ \frac{dw_p}{dt} = (\psi_W - \psi_I)\, i w_p + \gamma N_p w_- - \sigma w_p \\ \frac{di_-}{dt} = (\psi_I - \psi_W)\, w i_- - \gamma N_p i_- + \sigma i_p \\ \frac{di_p}{dt} = (\psi_I - \psi_W)\, w i_p + \gamma N_p i_- - \sigma i_p \end{pmatrix}$$



Conclusions

1. Conjugation rate 10-100x higher
   -> local high densities in gut?
2. Plasmid loss only in isolator 0.1%-A, so fitness disadvantage may occur
   -> not clear why these differences
3. Inoculum was disappearing in isolators 0.1%-B and 10%-A, so new *E. coli* strains do not always establish
   -> not clear why these differences
   -> plasmid has spread to wild-type
4. Variation within groups lower than between groups, so there is dependency
   -> what about larger groups?