

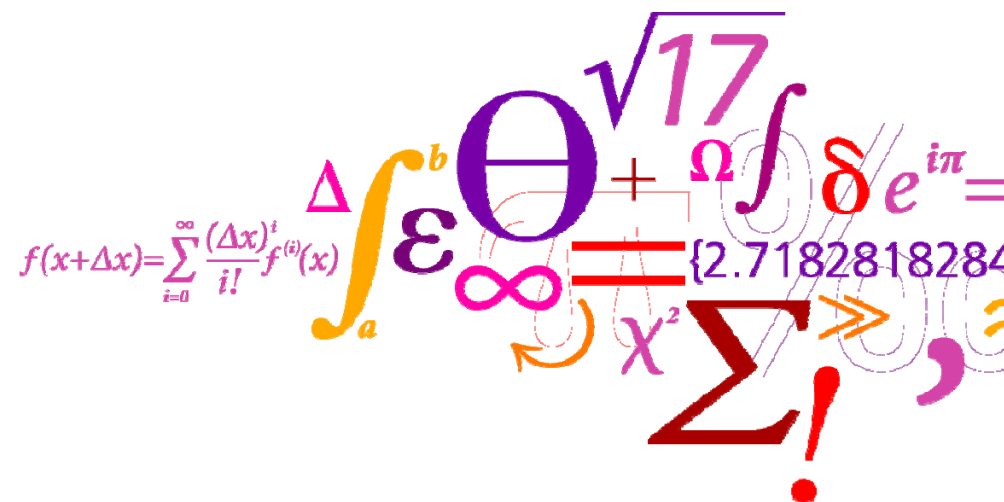
# “Characterization of successful multidrug- and quinolone resistant Salmonella clones”

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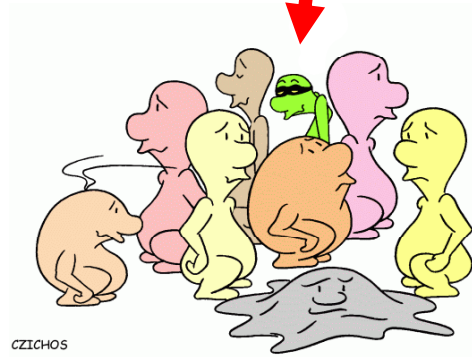


# SALMONELLA

*S. bongori*

*S. enterica*

*enterica arizonae diarizonae houtenae indica salamae*



Only a limited number of *Salmonella* clones are responsible for most of the Salmonellosis cases in industrialized countries (Thorns, 2000)

Over 1500 serovars

*S. Typhi*  
*S. Paratyphi*

*S. Typhimurium*  
*S. Enteritidis*

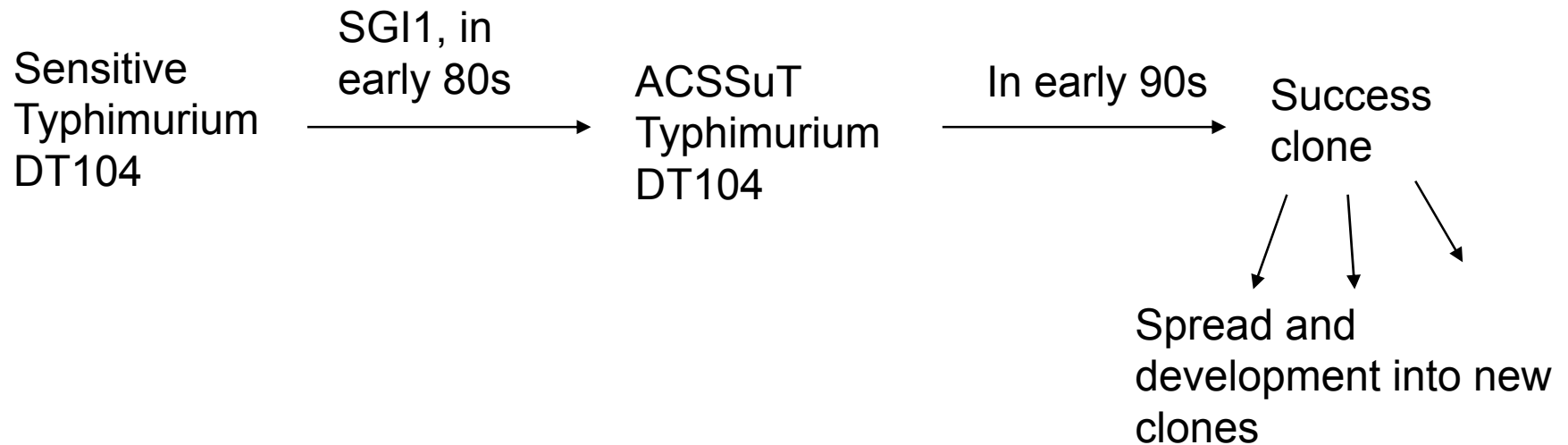
*S. Dublin*  
*S. Choleraesuis*  
*S. Gallinarium*

The success clone:



- Resistant to multiple antibiotics
- And quinolones seem to play a specific role

### The most famous example: *S. Typhimurium* DT104



# Resistant and virulent clones

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- Usually non-Typhoid Salmonella cause a self limiting gastroenteritis which is not treated with antibiotics
- Higher risk for hospitalization, bacteremia and mortality is reported for multidrug- and quinolone resistant strains (Threlfall, 1998; Barza 2002; Helms, 2004; Martin, 2004; Varma 2004)
- Hybrid plasmids containing resistance and virulence genes have been found (Threlfall, 1993; Guerra, 2001; Garcia, 2010)
- The special case: quinolone resistant strains
  - Particular risk for a more severe disease outcome
  - Most often caused by point mutations of genes encoding the gyrase
  - Can occur during treatment
- Additional virulence genes?
- Or other genes related to survival or metabolism etc?
- Higher mutation frequency?

Collection of Salmonella isolates and their classification  
into Q, MR, R and S



Pan genomic Salmonella microarray



PCR of selected genes



Disruption of selected genes



Phenotypical analysis of mutants and of isolate collection



# Pan-genomic Salmonella microarray (VLA)



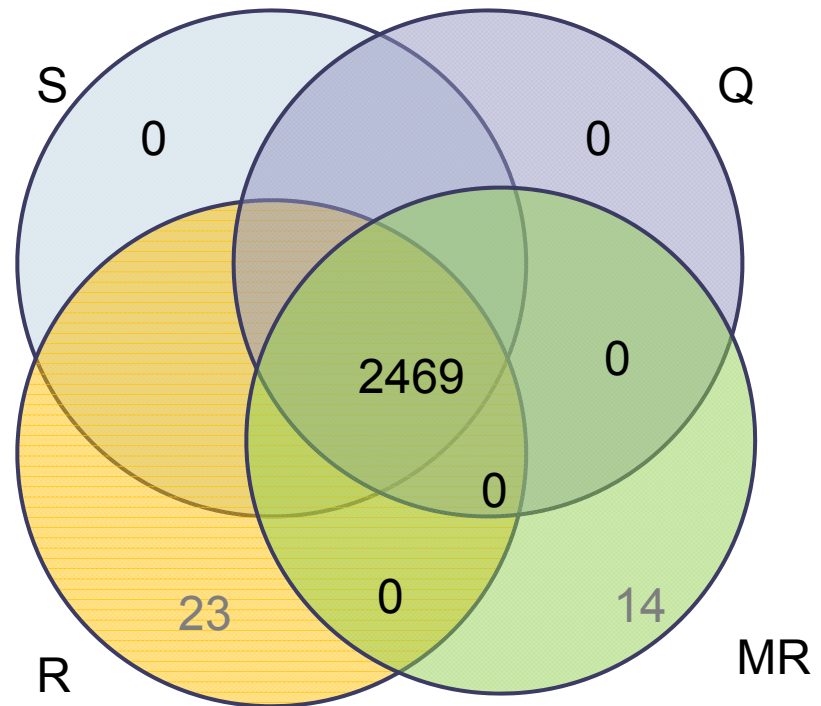
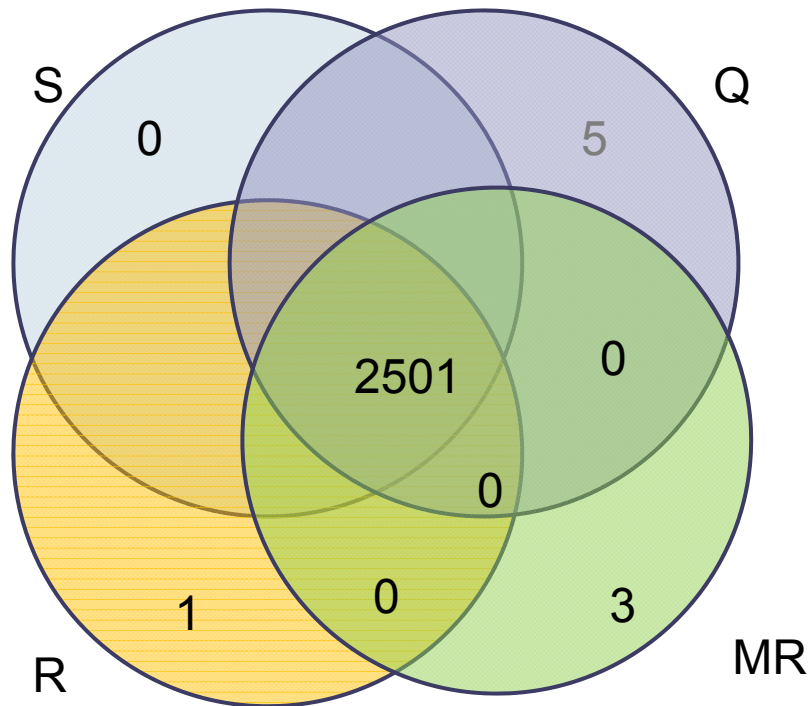
**Test Strains (29)**

**Control strains (8  
sequenced strains)**

**microarray:** covering 8  
genomes (ca. 6000 genes)

Typhimurium n=16

Non-Typhimurium n=13



A gene was defined to be linked to a resistance pattern

- 1) When present in 100 % of the Q or MR strains and absent in minimum 70 % of the S strains and
- 2) When absent in 100% of S strains and present in minimum 60 % of the Q or MR strains

genes dominating in <b>MR and Q</b> strains				
	Typhimurium n=6		non-Typhimurium n=6	
mobile genetic elements	4 gene clusters and 9 single genes <sup>1</sup>	<b>56%</b>	4 gene clusters and 5 single genes <sup>1</sup>	<b>77%</b>
putative or predicted virulence genes	<b>5 single genes</b>	9%	0 genes	0%
genes with other function <sup>2</sup>	11 single genes	20%	7 single genes	15%
unkown	8 single genes	15%	4 single genes	8%
total		54		47

<sup>1</sup>the largest fraction were phage and prophage genes

<sup>2</sup>including metabolic, transporter and regulatory genes and genes involved in stress tolerance

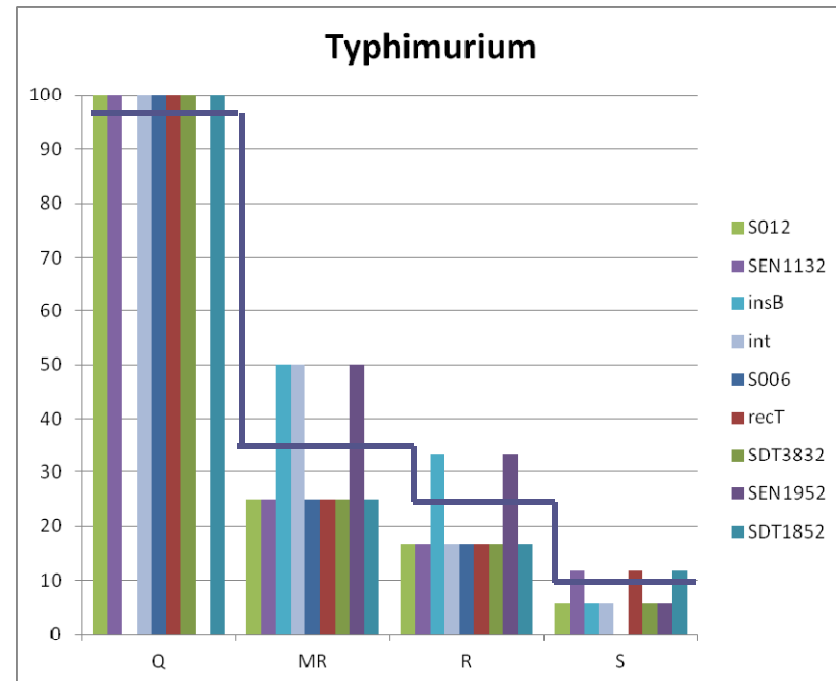
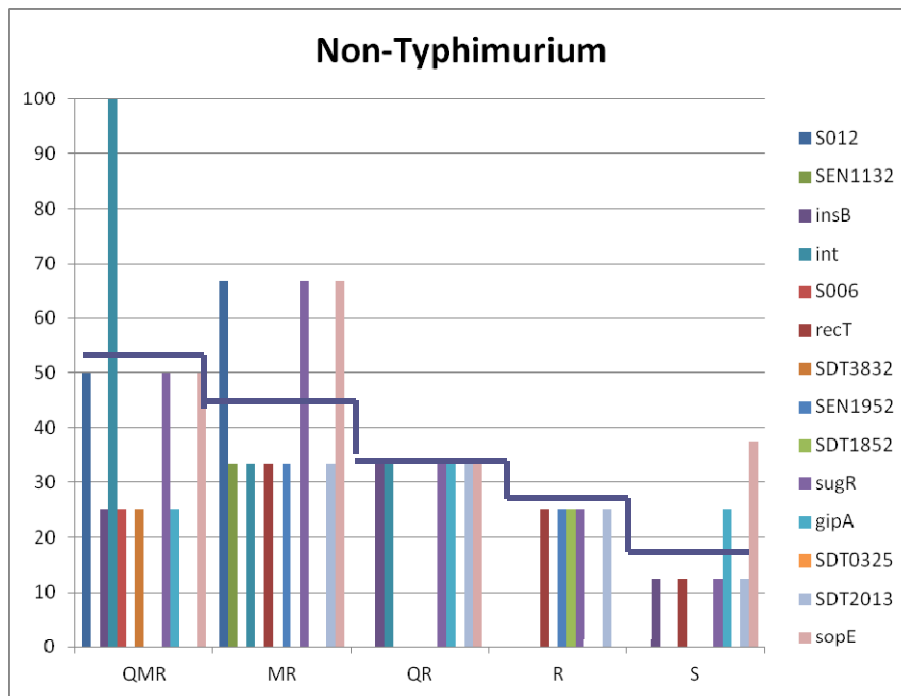
genes dominating in <b>Q</b> strains		
	n=3	
mobile genetic elements	2 gene clusters and 4 single genes <sup>1</sup>	<b>50%</b>
putative or predicted virulence genes	<b>1 single gene</b>	4%
genes with other function <sup>2</sup>	9 single genes	32%
unkown	4 single genes	14%
total		28

genes dominating in <b>S</b> strains				
	Typhimurium n=10		non-Typhimurium n=7	
mobile genetic elements	1 gene cluster 4 single genes	24%	0 genes	0%
putative or predicted virulence genes	0 genes	0%	0 genes	0%
genes with other function <sup>2</sup>	1 gene cluster 18 single genes	<b>61%</b>	1 single gene	25%
unkown	5 single genes	15%	3 single genes	<b>75%</b>
total		33		4

# PCR

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- 23 genes
- 52 additional Salmonella strains
- 59 % of PCR results proved to be consistent with the microarray data



## Mobile genetic elements

Phage genes, SGI1 genes, insB

11 genes

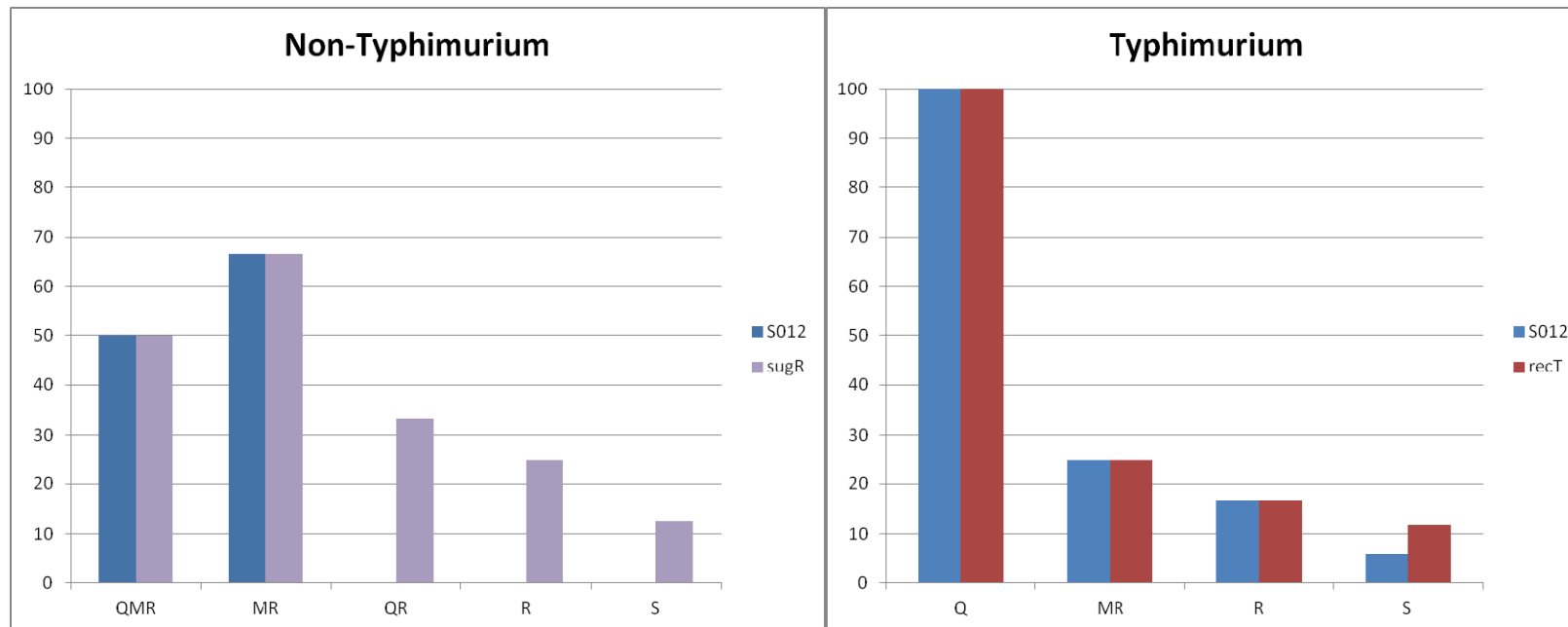
## Virulence genes

sugR, gipA, sopE

3 genes

Knock out of 3 genes:

recT	Phage encoded recombination and repair protein
sugR	Putative ATP-dependent Clp protease of SPI3
S012	Putative pilus assembly protein of SGI1



# $\lambda$ -red mutation assay after Datsenko and Wanner (2000)

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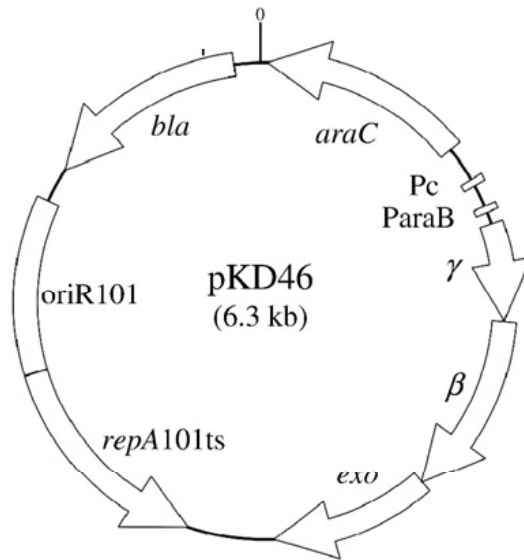
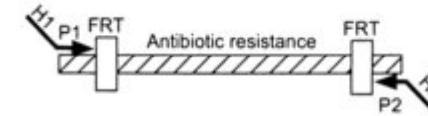


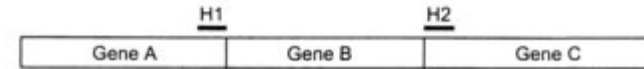
Fig. Doublet et al, 2008

Transformation of pKD46 into amp sensitive *Salmonella* followed by growth in the presence of L-Arabinose when preparing competent cells

Step 1. PCR amplify FRT-flanked resistance gene



Step 2. Transform strain expressing  $\lambda$  Red recombinase



Step 3. Select antibiotic-resistant transformants

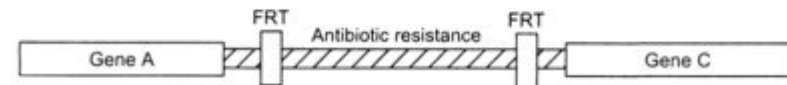


Fig. Datsenko and Wanner, 2000

Control PCR

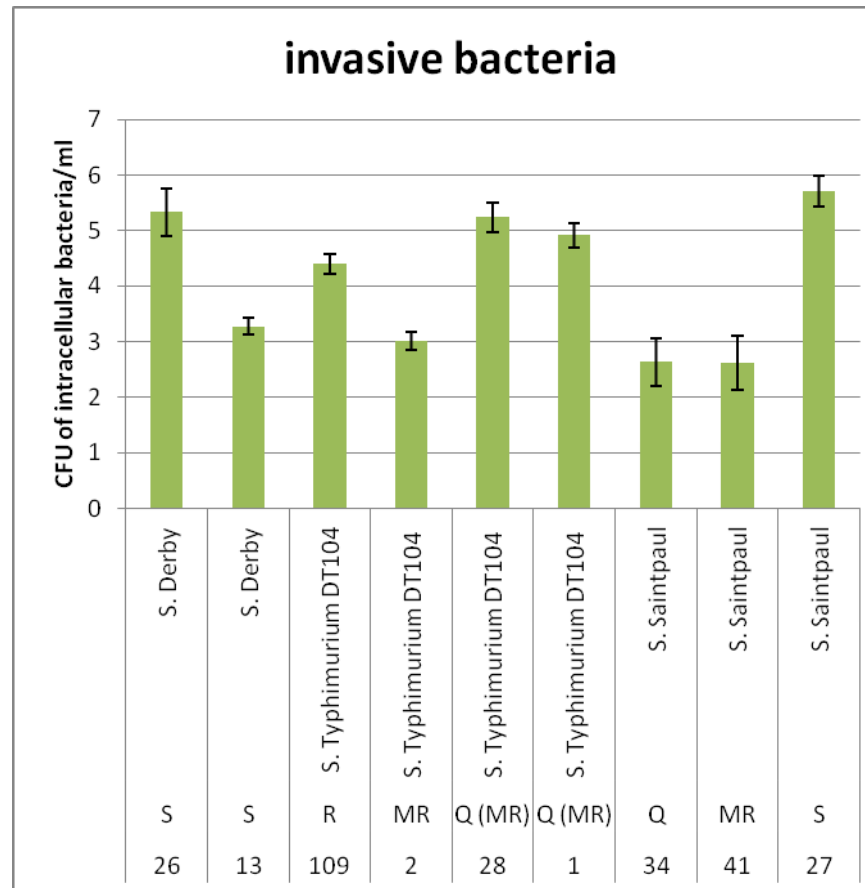
# Phenotypical assays

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- **Adhesion and invasion** of intestinal epithelial cells

Mutants: **No difference**

Strain collection: ?



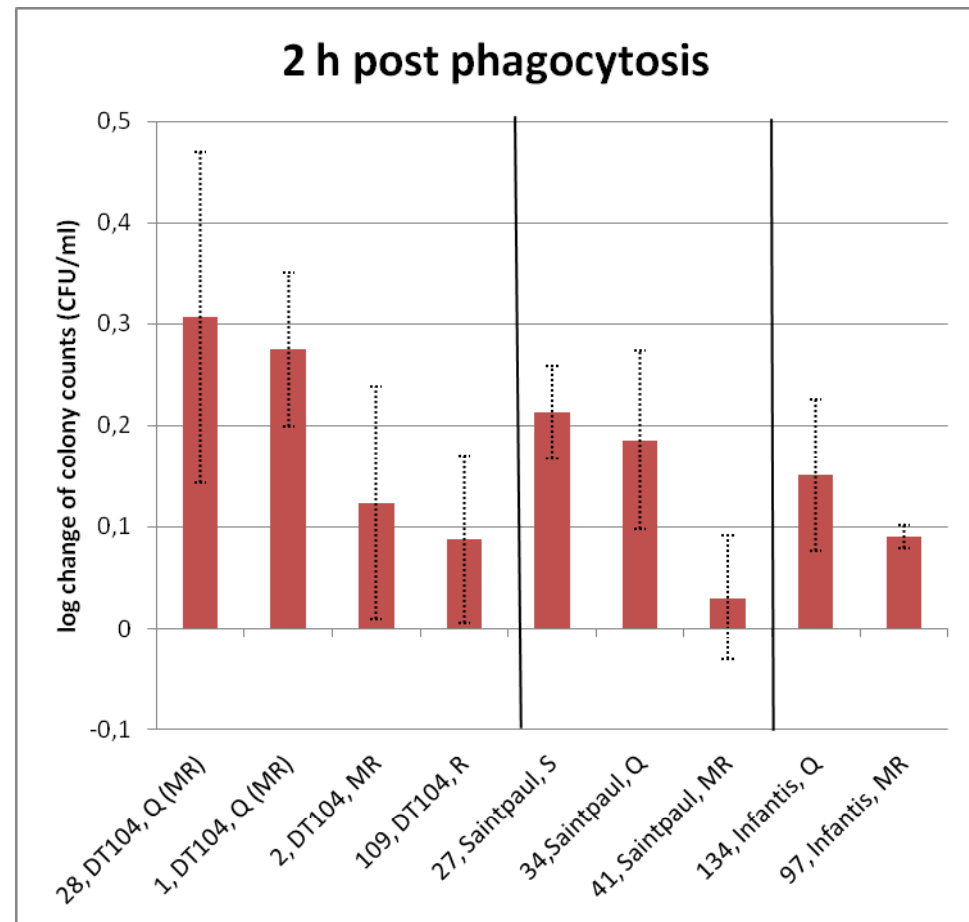
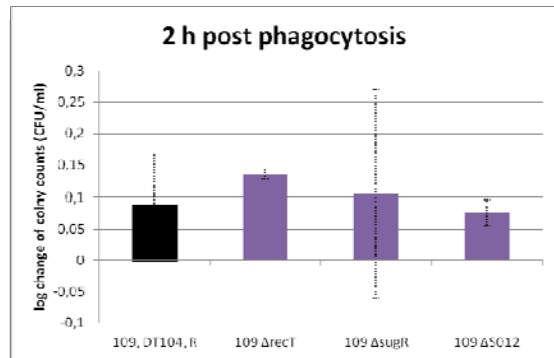
# Phenotypical assays

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- **Survival in macrophages** (in presence of ciprofloxacin)

Mutants: **No significant difference**

Strain collection: ?



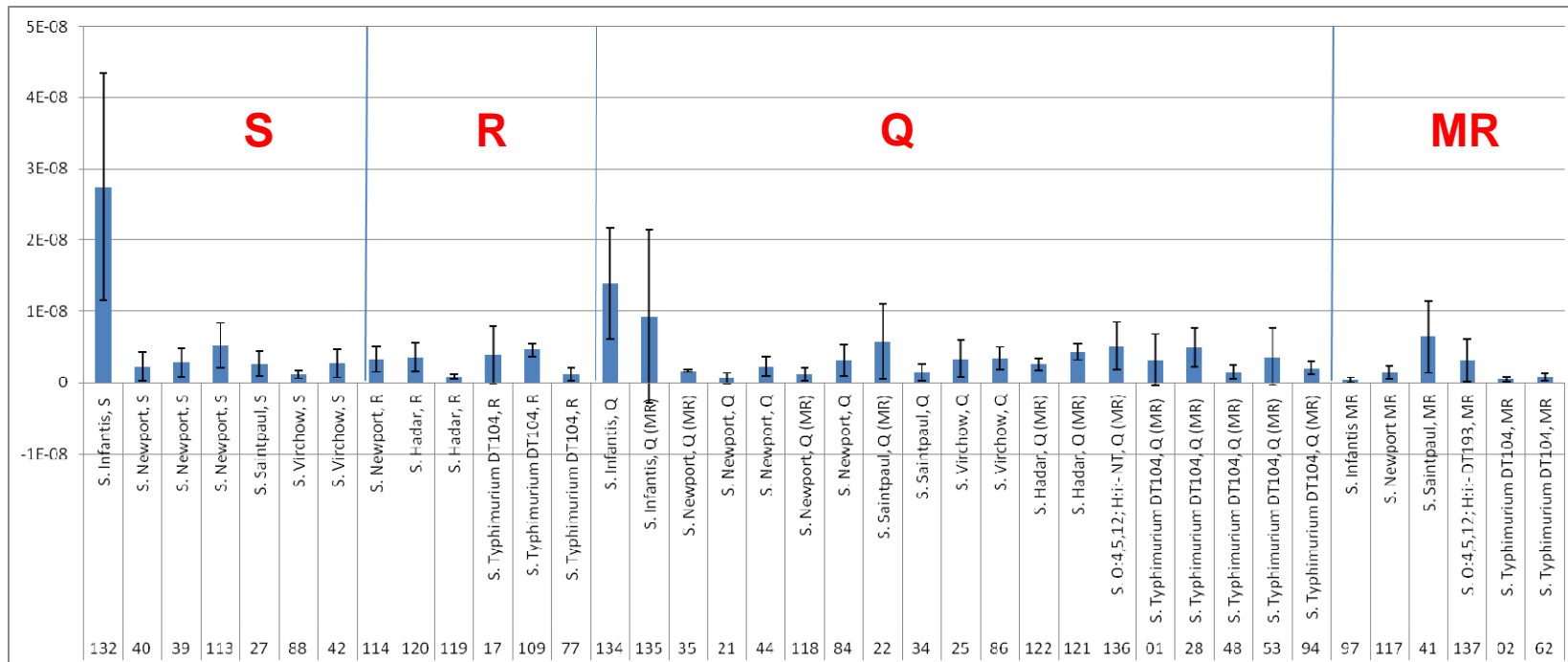
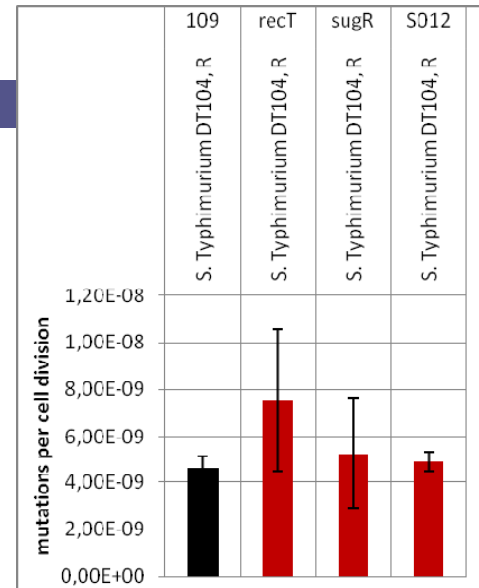
# Phenotypical assays

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## □ Spontaneous mutation frequency

Mutants: **No significant difference**

Strain collection: **no difference**



# Phenotypical assays

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- **Conjugation frequency** with *S. Agona*, *A. faecalis* and *E. coli* as donors

Mutants: **No difference**

# Discussion – Limitations of the study

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## DNA/DNA microarrays

- Divergent genes will be shown as absent (or evtl .as present in case of single point mutations)
- Only a limited amount of genes can be detected
- Present genes are not necessarily expressed in the cell (could be pseudogenes)

## Mutants

- It might be difficult to find a phenotype when only single genes are disrupted

## phenotypical assays:

- Very time consuming and not possible with too many isolates
- Almost unlimited amount of possible assays (when is it time to give up?)

# Discussion - Results

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1. Neither Q nor MR strains have higher or lower spontaneous mutation frequencies but many carry a gene (*recT*) that seems to reduce mutations
2. All strains perform very similar in cell culture based virulence studies
3. Ciprofloxacin did not seem to enhance virulence (survival in macrophages)
4. Many MR and Q strains seem to have additional virulence traits located on mobile genetic elements, mainly on prophages (they are also present in S strains but to a lesser extent)
5. These prophages might contribute to virulence themselves
6. MR and Q strains might be characterized by very dynamic genomes that help to adapt very fast to new environmental conditions (indicated by prophages, genomic islands, insertion elements)

## Acknowledgements:

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# Thank you!

