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

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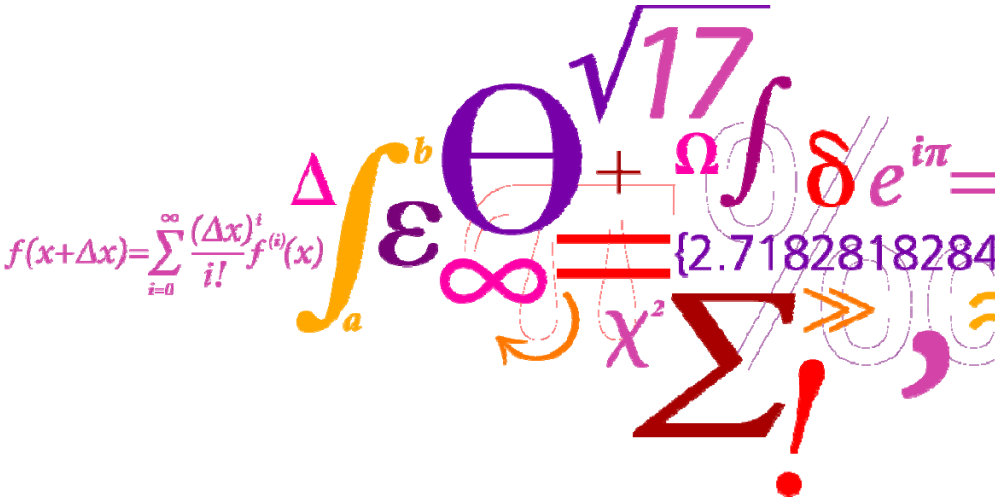
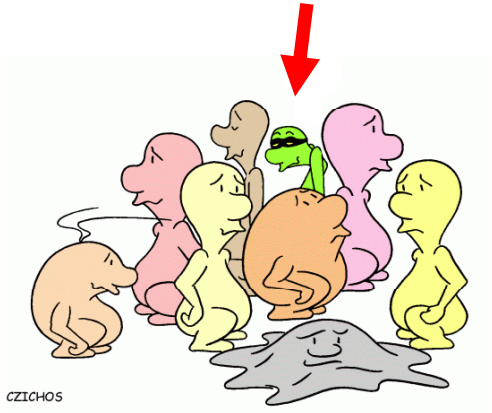


**SALMONELLA**

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

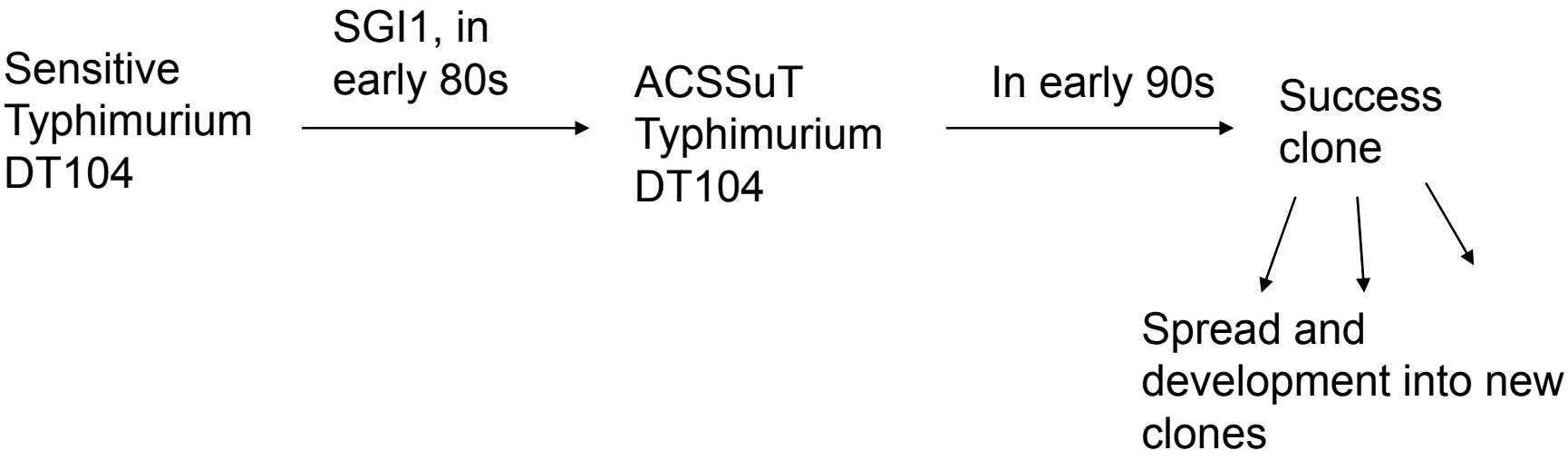
S. Typhimurium
S. Enteritidis

S. Dublin
S. Cholerasis
S. Gallinarium

The success clone:

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

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

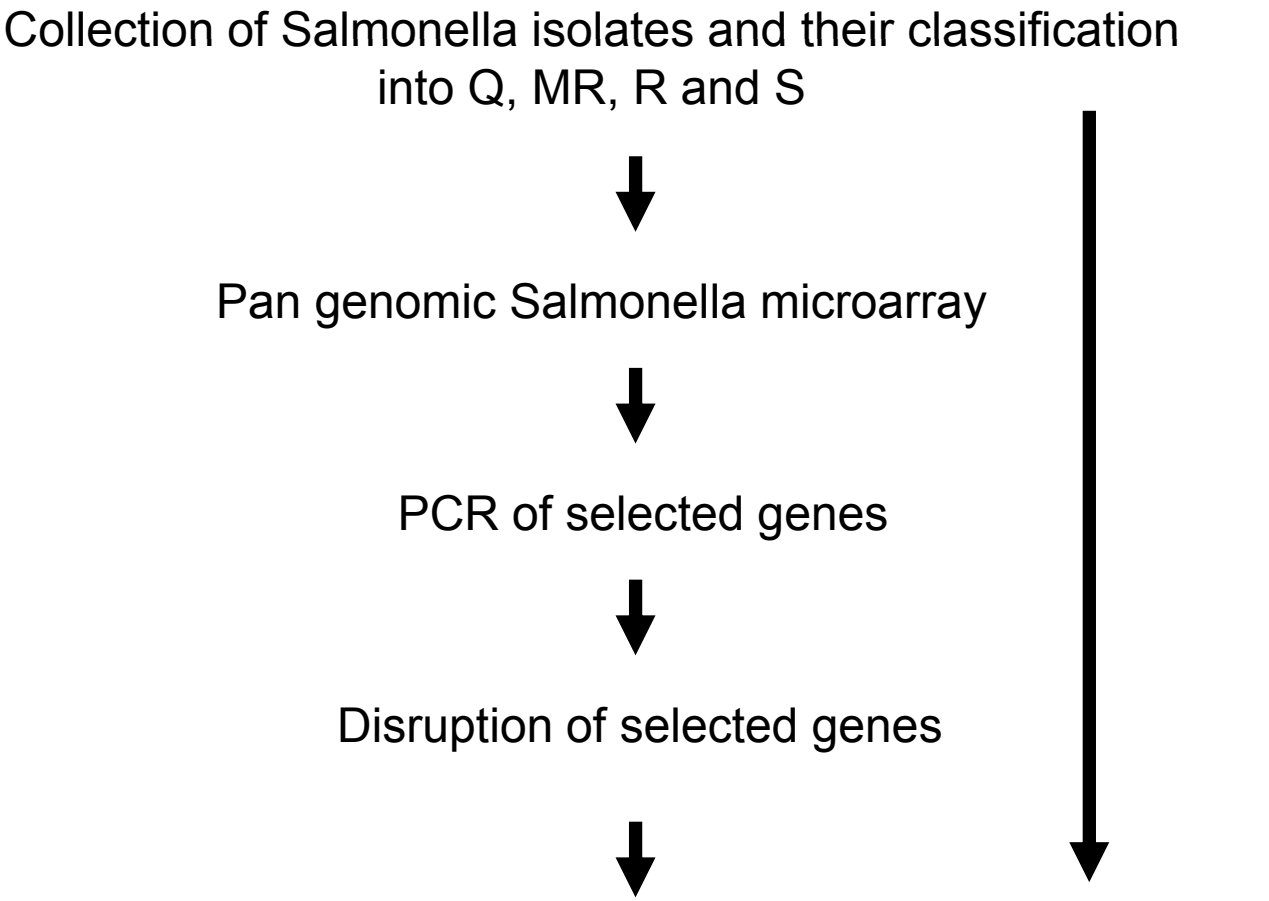


# Resistant and virulent clones



- 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

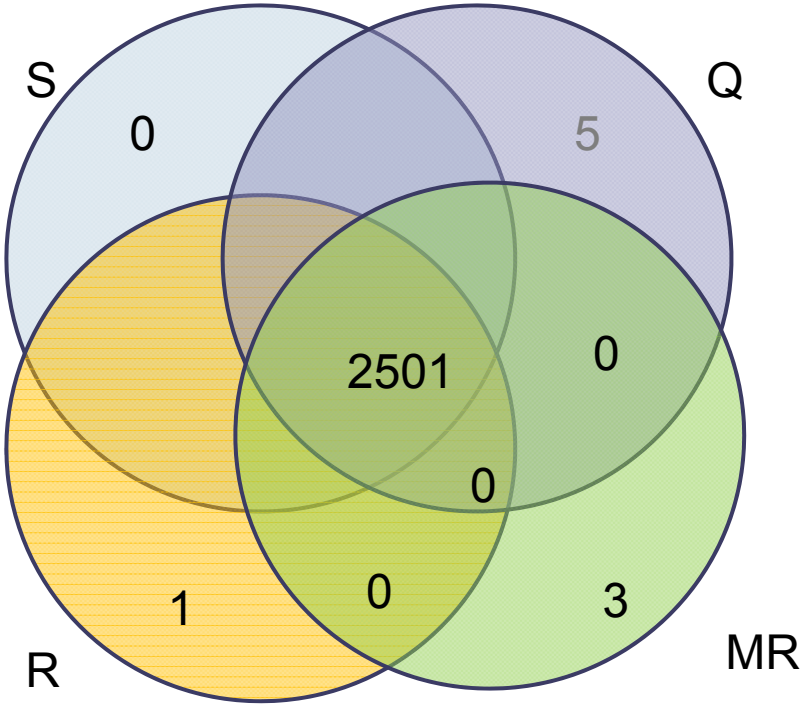
# Pan-genomic Salmonella microarray (VLA)

**Test Strains** (29)

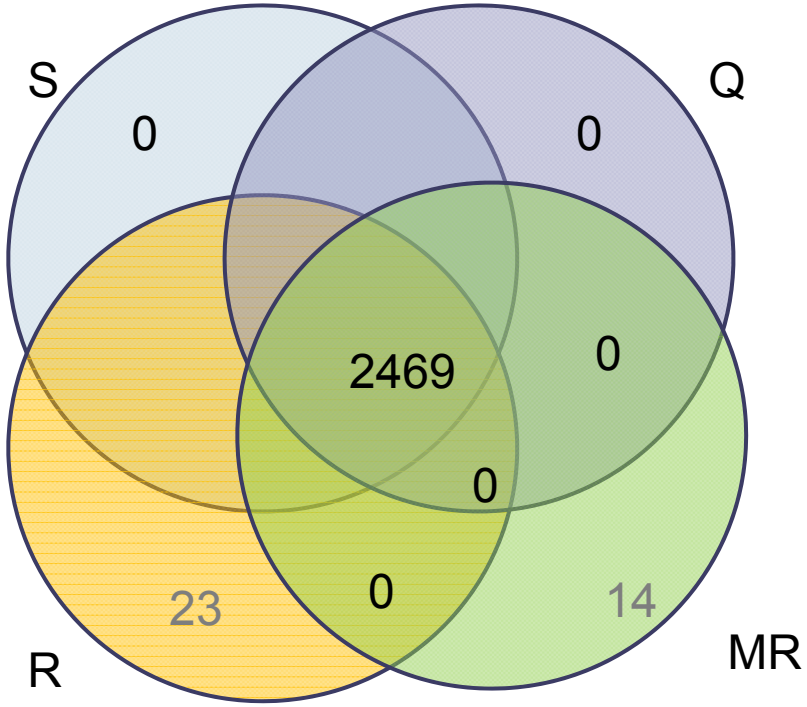
**Control strains** (8 sequenced strains)

**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 **MR and Q** strains | | | |
|---|---|---|---|---|
| | Typhimurium n=6 | | non-Typhimurium n=6 | |
| mobile genetic elements | 4 gene clusters and 9 single genes[1] | **56%** | 4 gene clusters and 5 single genes[1] | **77%** |
| putative or predicted virulence genes | 5 single genes | 9% | 0 genes | 0% |
| genes with other function[2] | 11 single genes | 20% | 7 single genes | 15% |
| unkown | 8 single genes | 15% | 4 single genes | 8% |
| total | | 54 | | 47 |

[1]**the largest fraction were phage and prophage genes**

[2]including metabolic, transporter and regulatory genes and genes involved in stress tolerance

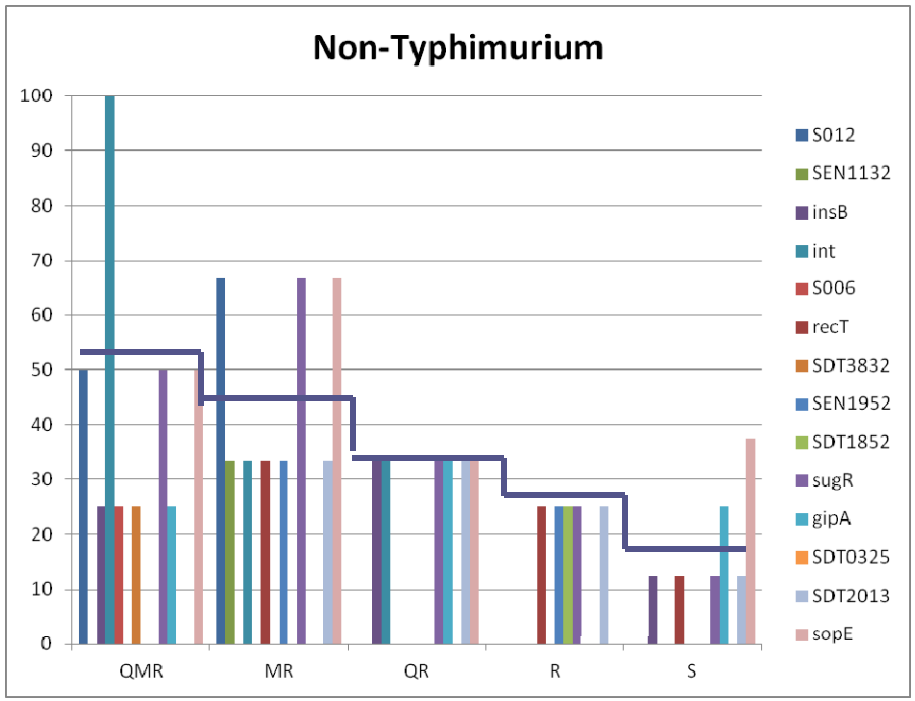
| | genes dominating in **Q** strains | |
|---|---|---|
| | n=3 | |
| mobile genetic elements | 2 gene clusters and 4 single genes[1] | **50%** |
| putative or predicted virulence genes | 1 single gene | 4% |
| genes with other function[2] | 9 single genes | 32% |
| unkown | 4 single genes | 14% |
| total | | 28 |

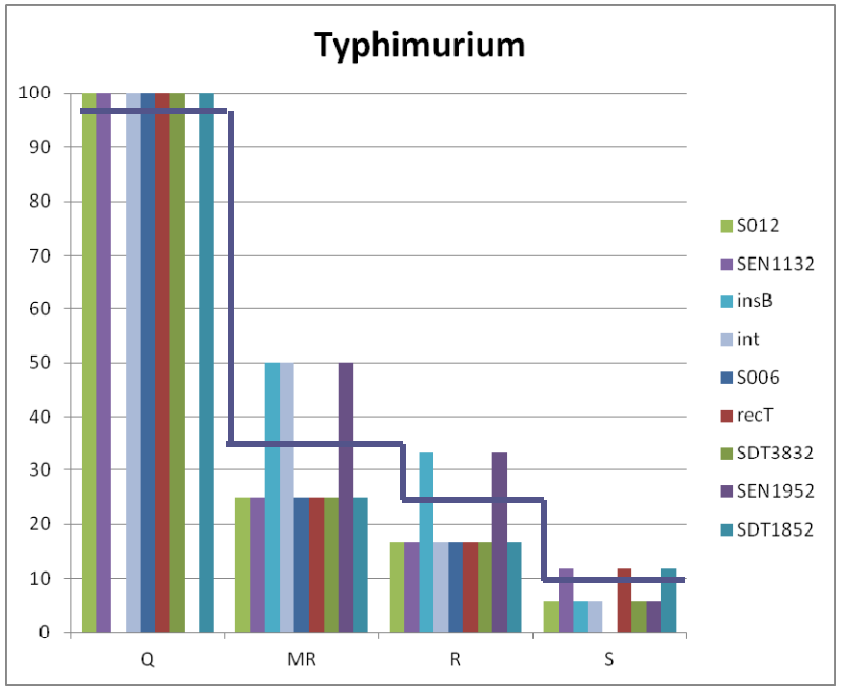
| | genes dominating in **S** strains | | | |
|---|---|---|---|---|
| | Typhimurium n=10 | | non-Typhimurium n=7 | |
| mobile genetic elements | 1 gene cluster 4 single genes | 24% | 0 genes | 0% |
| putative or predicted virulence genes | 0 genes | 0% | 0 genes | 0% |
| genes with other function[2]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

# PCR



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

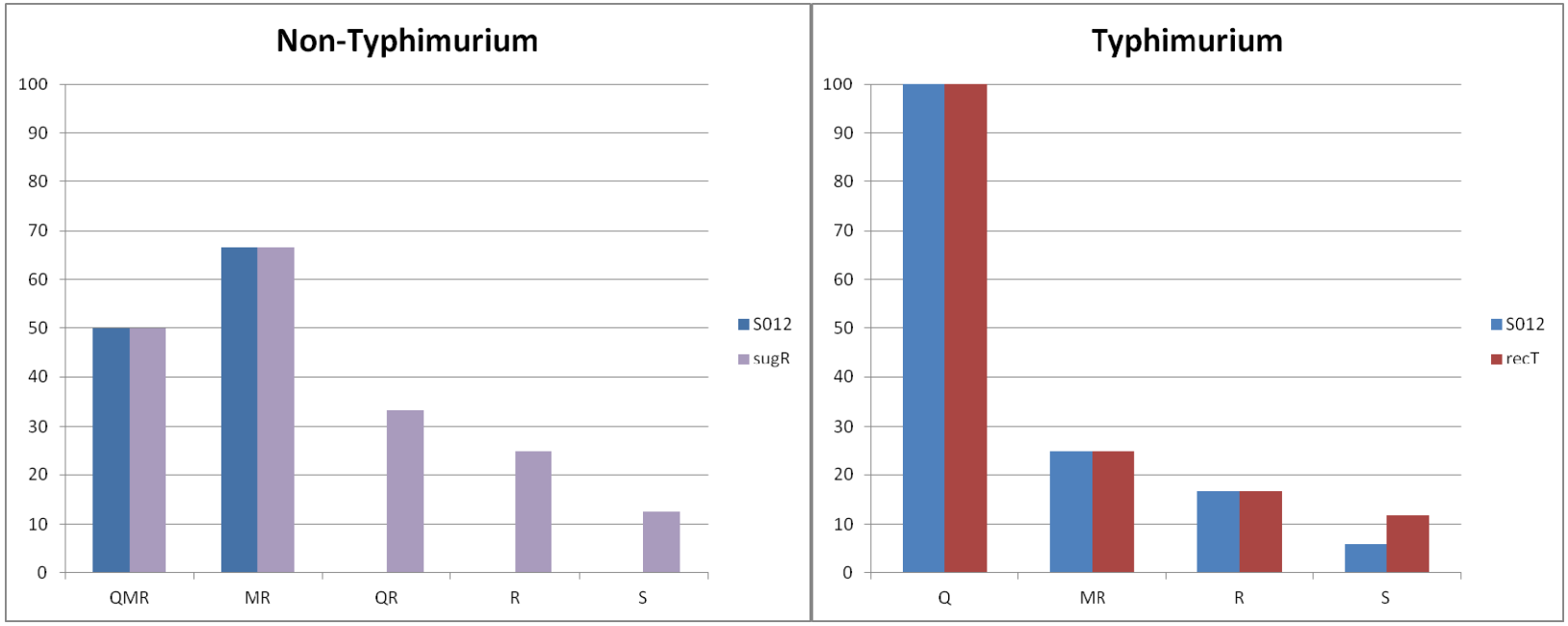



| Mobile genetic elements | Virulence genes |
|---|---|
| Phage genes, SGI1 genes, insB | sugR, gipA, sopE |
| 11 genes | 3 genes |

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 |



# λ-red mutation assay after Datsenko and Wanner (2000)



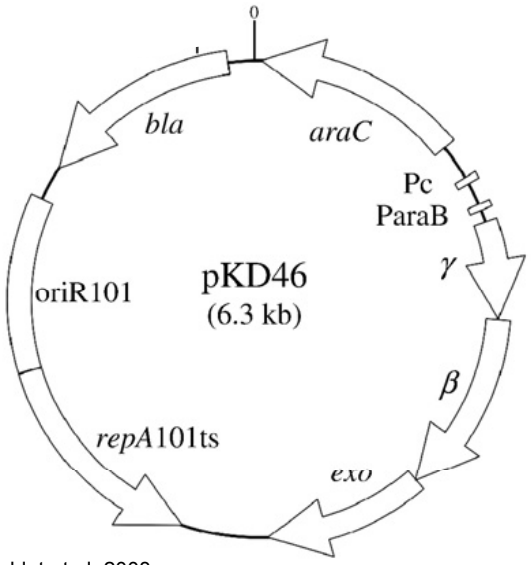



Fig. Doublet et al, 2008

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

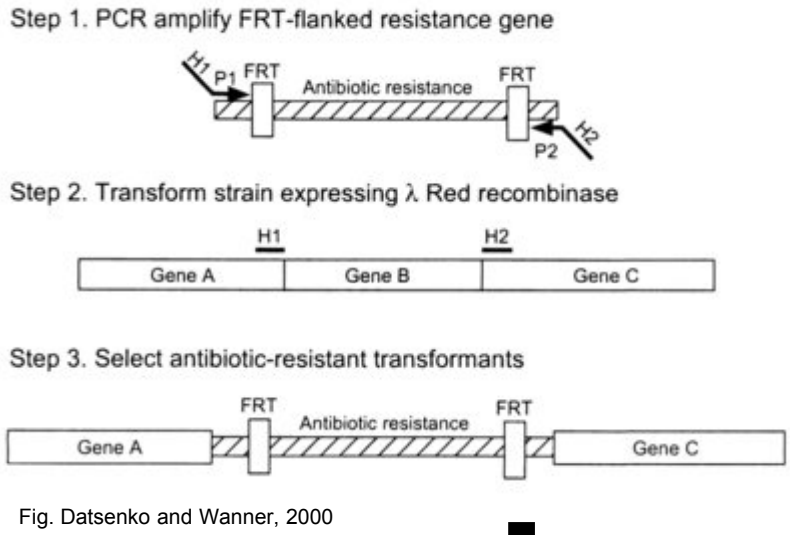



Fig. Datsenko and Wanner, 2000

Control PCR

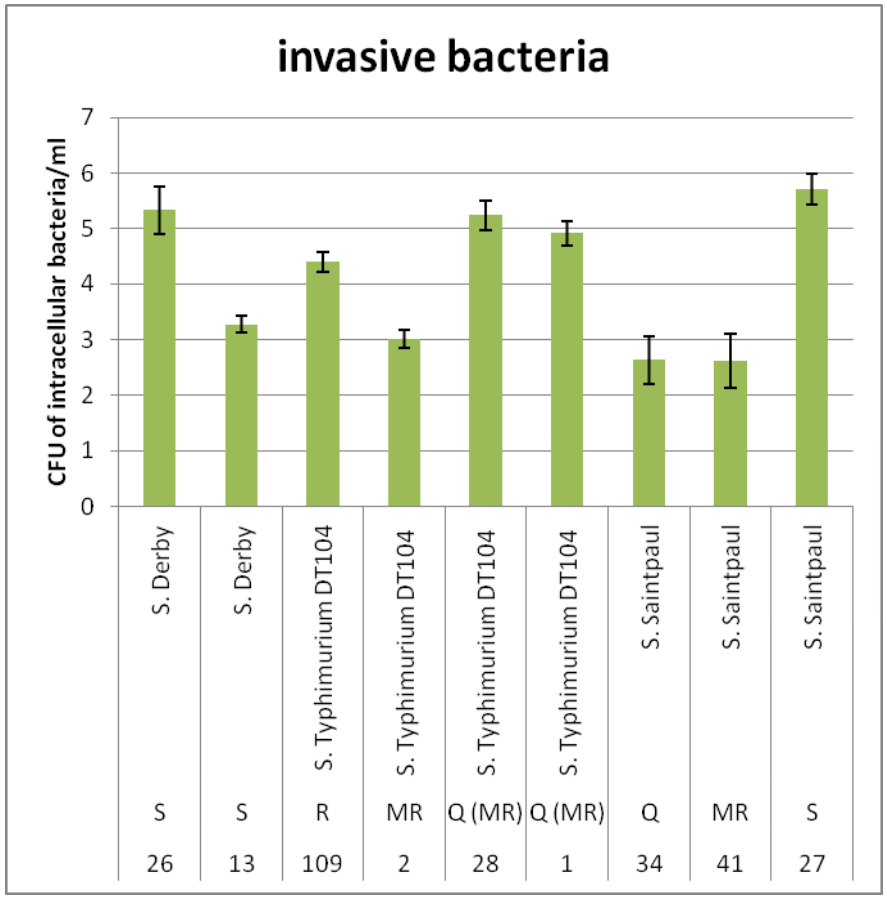
# Phenotypical assays



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

Mutants: **No difference**

Strain collection: ?



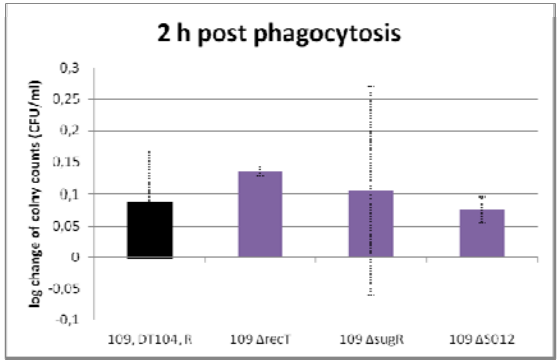
# Phenotypical assays

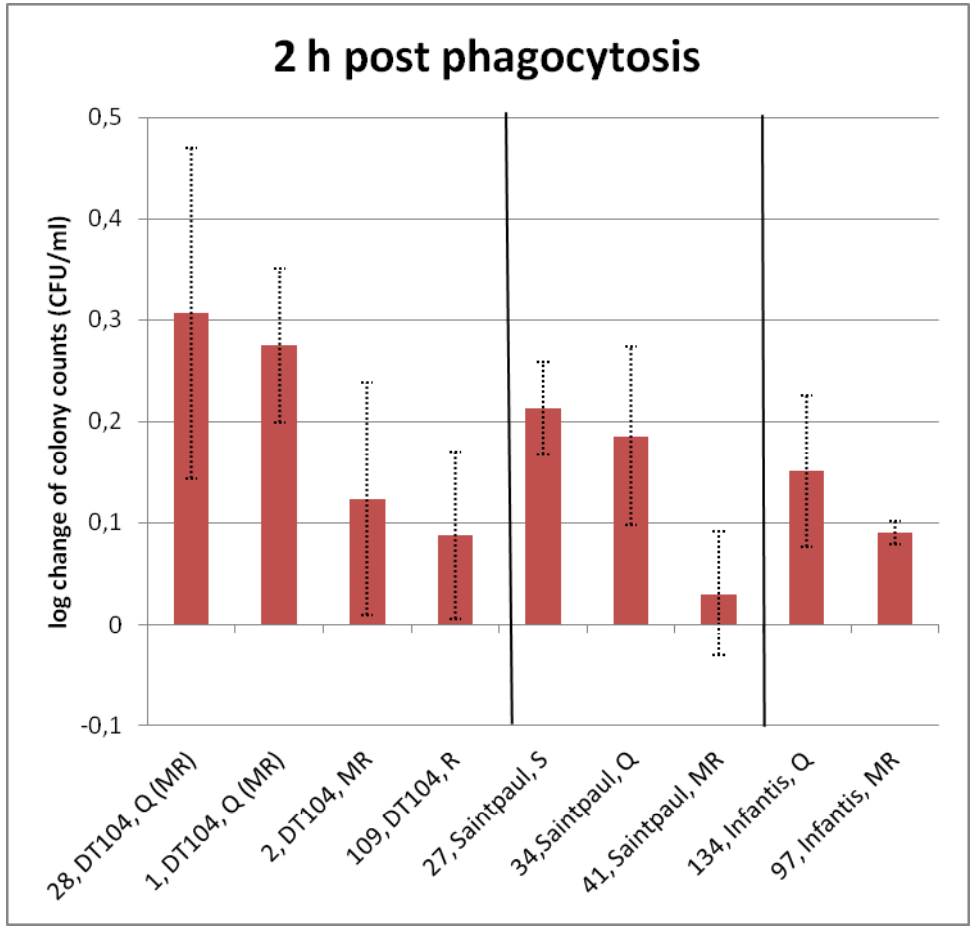


- **Survival in macrophages** (in presence of ciprofloxacin)

Mutants: **No significant difference**

Strain collection: ?



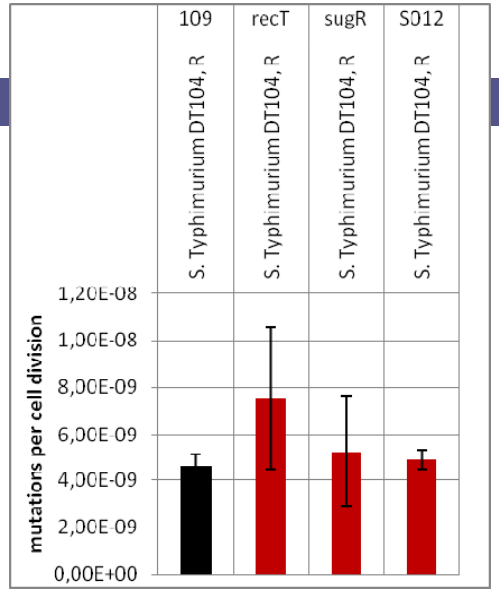
# Phenotypical assays

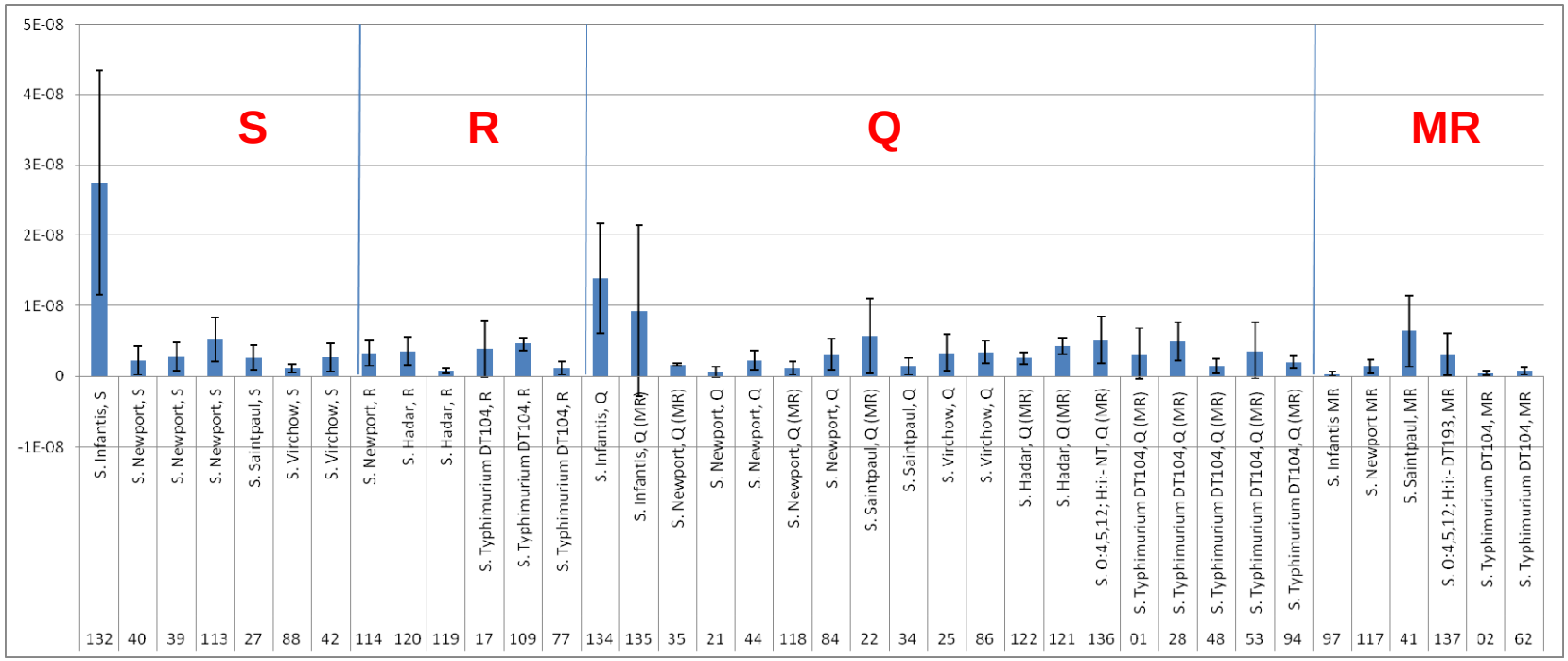


- **Spontaneous mutation frequency**

Mutants: **No significant difference**

Strain collection: **no difference**



# Phenotypical assays



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

  Mutants: **No difference**

# Discussion – Limitations of the study



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 unlimitied amount of possible assays (when is it time to give up?)

# Discussion - Results



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 extend)
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)

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# Thank you!