



EURL-AR training course on genotypic characterization of antimicrobial resistance

Plasmid Mediated Quinolone Resistance

Kees Veldman



Introduction

- Working as a senior technician at the Central Veterinary Institute in Lelystad (NL) since 1991
- Responsible for the daily routine regarding resistance monitoring (NRL for antibiotic resistance in animals)
- PhD on plasmid mediated quinolone resistance in *Salmonella* and *E. coli*

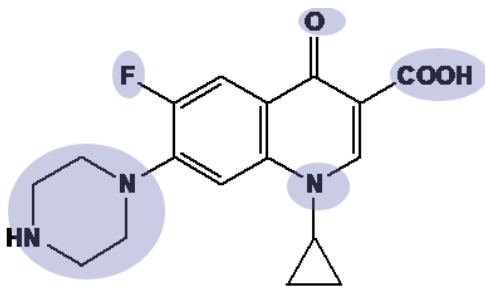
Outline

- General introduction
- Resistance mechanisms
- Phenotypes and genotypes
- MPC and clinical relevance
- Plasmids and genes
- Occurrence
- Links to other resistance genes
- Questions



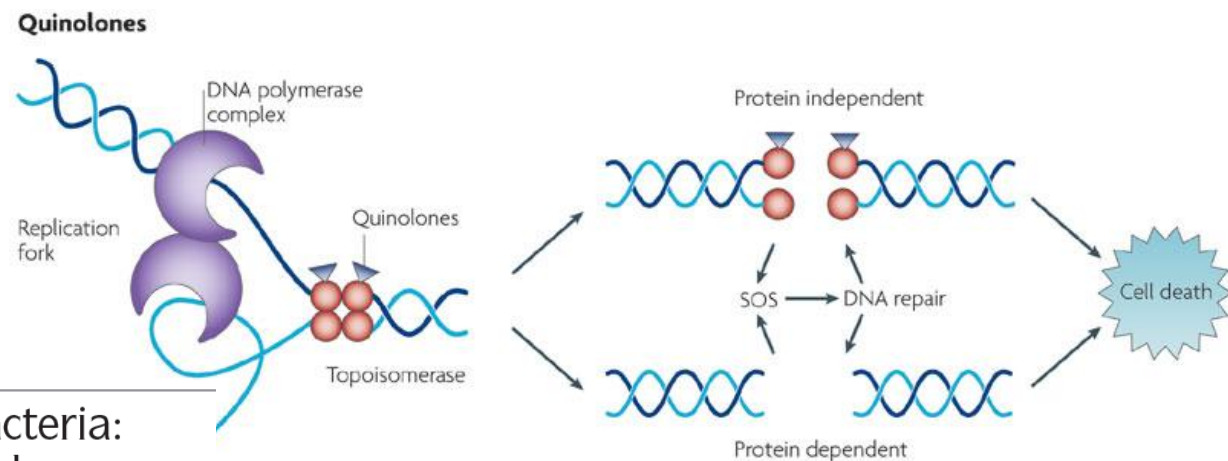
Quinolones

- By WHO classified as group of critically important antimicrobials for treatment of human infections
- Both human (ciprofloxacin) and veterinary (enrofloxacin) applications
- Broad spectrum antibiotics



Mechanism of action

- DNA gyrase (gyrA/B) and DNA topoisomerase IV (parC/E) are important enzymes in the DNA synthesis
- Antibiotic-enzyme-DNA complex traps the enzymes leading to lethal double-stranded DNA breaks



How antibiotics kill bacteria:
from targets to networks

Michael A. Kohanski[†], Daniel J. Dwyer^{*} and James J. Collins^{†*}

'Old' resistance mechanisms

Most important mechanism:

- Target modification by mutations in QRDR region of *gyrA*, *gyrB*, *parC* and *parE*
 - can lead to high-level resistance

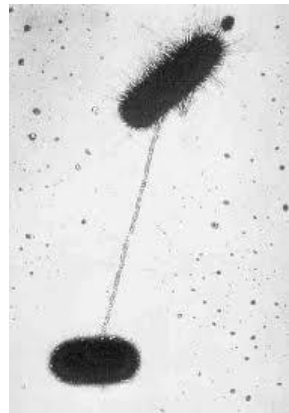
Others mechanisms:

- Upregulated efflux pumps
- Altered permeability of outer membrane porins

- Not transferable

'New' resistance mechanisms

- **Plasmid Mediated Quinolone Resistance**
 - Qnr: proteins protect target
 - qepA: specific quinolone efflux pump
 - OqxAB: multidrug efflux pump
 - aac(6')-1b-cr: altered aminoglycoside acetyltransferase
- Reduced susceptibility to fluoroquinolones
- Transferable!



Qnr family



- First qnr gene (*qnrA*) was described by Martinez-Martinez et al. in 1998 in the Lancet
- Detected in *Klebsiella pneumoniae* from urine specimen in 1994 on plasmid pMG252
- Qnr proteins belong to the pentapeptide repeat family
- Protect DNA gyrase and topoisomerase IV enzymes

Qnr family

- Nowadays five known qnr-families with many variants:
 - qnrA1-7 (first since 1998)
 - qnrS1-5 (first since 2003)
 - qnrB1-42 (first since 2006)
 - qnrC1 (first since 2009)
 - qnrD1 (first since 2009)
- <http://www.lahey.org/qnrStudies/>

QepA

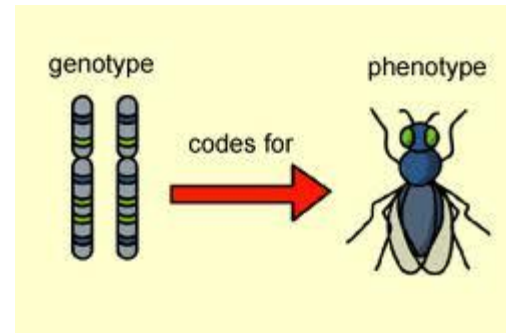
- Specific quinolone efflux pump
 - Slight increase of ciprofloxacin and norfloxacin MICs
- First described in *E. coli* from urine tract in Japan in 2002 by Yamane et al.
- Now two variant described: QepA1 and QepA2

AAC(6')-1b-cr

- First described in 2003 in qnrA-positive E. coli isolate from China by Robicsec et al.
- Variant of aminoglycoside acetyltransferase AAC(6')-Ib designated AAC(6')-1b-cr (ciprofloxacin resistant variant)
- Modest increase of ciprofloxacin and norfloxacin MICs, but still active against kanamycin, tobramycin and amikacin
- Two base-pair changes responsible for the reduced susceptibility to quinolones: Trp102Arg and Asp179Tyr

Phenotype and genotype

- The quinolone resistance phenotype reveals (part of) the resistance mechanism (= genotype)
- Provided only one resistance mechanism is involved



Phenotype and genotype



- Chromosomal mutations
 - Low-level or high-level resistant against ciprofloxacin:
 - Depending on the number of mutations
 - MICs: 0.125 - > 8 mg/L
 - Resistant against nalidixic acid:
 - MICs: ≥ 64 mg/L

Phenotype and genotype

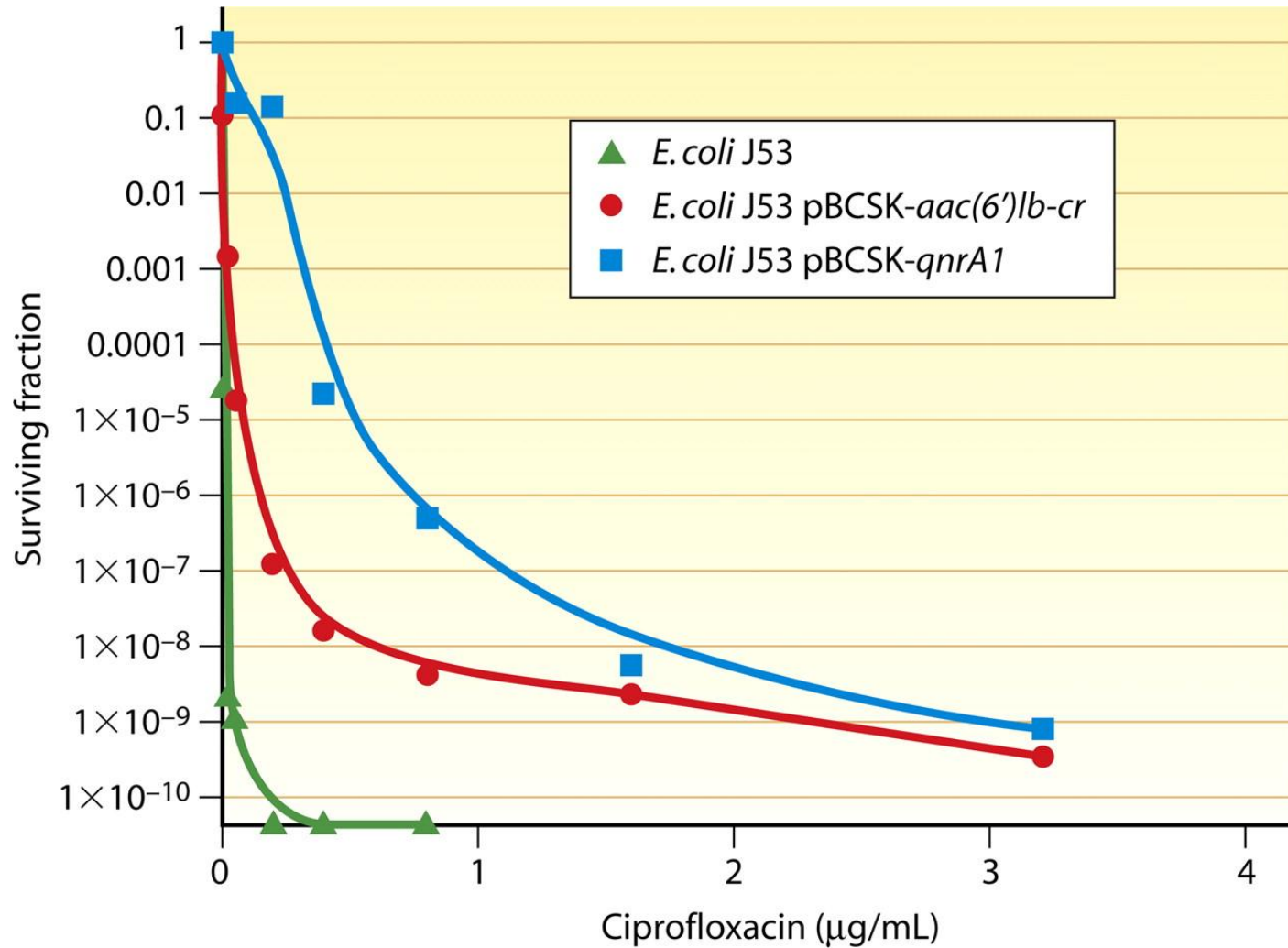
Plasmid mediated quinolone resistance (PMQR)

- Low-level resistant against ciprofloxacin:
 - MICs: 0.25 - 1 mg/L
- Reduced susceptible to nalidixic acid:
 - MICs: 8 – 16 (32) mg/L

Effect on MPC

- PMQR-genes effect the mutant prevention concentration (MPC)
- MPC: lowest concentration to prevent growth of quinolone-resistant mutants from a inoculum of 10^{10} cfu/ml
- When the quinolone concentration $>$ MPC: no single-step mutants will arise

MPC assay



Clinical relevance of PMQR

- PMQR enhances the number of chromosomal mutants selected from a population (in vitro)
- Also keep in mind that PMQR elevates the quinolone MIC of chromosomal mutants
- This might lead to decreased therapeutic efficacy

Detection of PMQR

- Detection with PCR
 - during course: **qnrS1, qnrD1 and aac(6')-1b-cr**
- Selection:
 - Random (no MICs available)
 - All quinolone resistant isolates
 - Isolates with PMQR phenotype
- Followed by sequencing the amplicon or sequencing the full-length of the gene



Plasmids and genes

- Italian research group analyzed Dutch *qnr*-positive *Salmonella*.

Journal of Antimicrobial Chemotherapy (2009) **63**, 274–281
doi:10.1093/jac/dkn470
Advance Access publication 11 November 2008

JAC

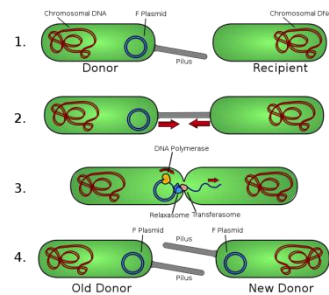
Characterization of plasmids harbouring *qnrS1*, *qnrB2* and *qnrB19* genes in *Salmonella*

Aurora García-Fernández¹, Daniela Fortini¹, Kees Veldman², Dik Mevius^{2,3}
and Alessandra Carattoli^{1*}

- Results:
 - *qnrB2* and *qnrB19* on IncN plasmids
 - *qnrS1* on IncN, ColE, IncR and IncHI2 plasmids

Plasmids and genes

- Different reports about qnr-positive *E. coli*:
 - *qnrS1* on IncX2
 - *qnrB19* on ColE, IncN, IncR and IncX2
- General conclusion:
 - Different qnr genes are present on different types of plasmids in different bacterial species



PMQR in Europe

- In 2008 the EURL-AR initiated a study on the occurrence of PMQR-genes

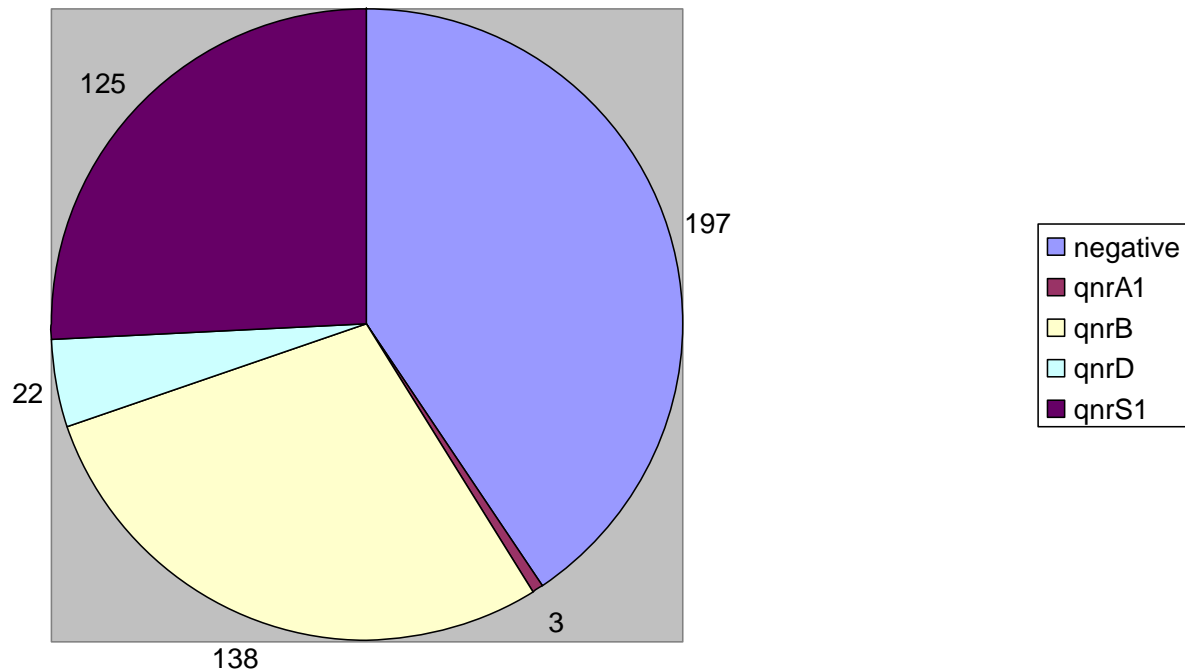
J Antimicrob Chemother 2011; **66**: 1278–1286
doi:10.1093/jac/dkr084 Advance Access publication 9 March 2011

**Journal of
Antimicrobial
Chemotherapy**

**International collaborative study on the occurrence of
plasmid-mediated quinolone resistance in *Salmonella enterica*
and *Escherichia coli* isolated from animals, humans, food
and the environment in 13 European countries**

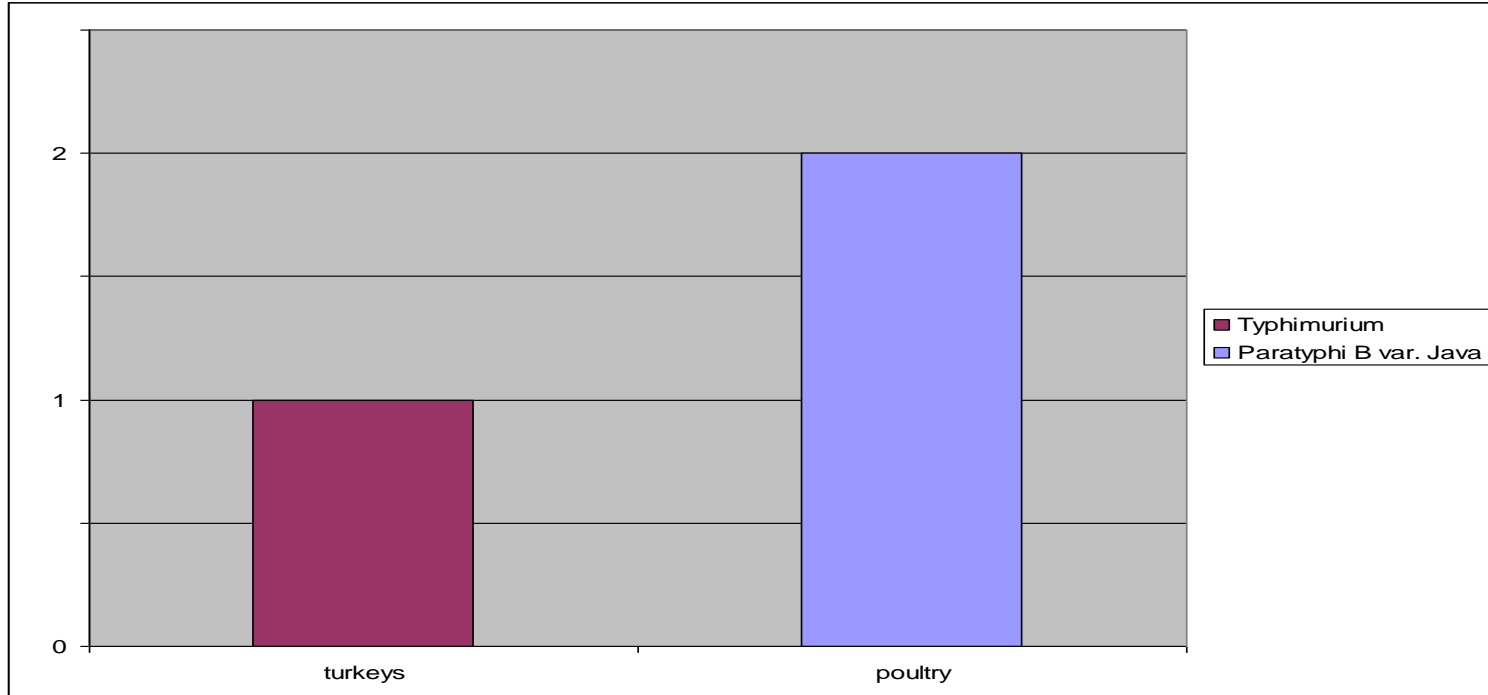
Kees Veldman^{1*}, Lina M. Cavaco², Dik Mevius^{1,3}, Antonio Battisti⁴, Alessia Franco⁴, Nadine Botteldoorn⁵,
Mireille Bruneau⁶, Agnès Perrin-Guyomard⁶, Tomas Cerny⁷, Cristina De Frutos Escobar⁸, Beatriz Guerra⁹,
Andreas Schroeter⁹, Montserrat Gutierrez¹⁰, Katie Hopkins¹¹, Anna-Liisa Myllyniemi¹², Marianne Sunde¹³,
Dariusz Wasyl¹⁴ and Frank M. Aarestrup²

Results of PCR testing of *Salmonella* (n = 485)



qnrB and *qnrS1* were predominant

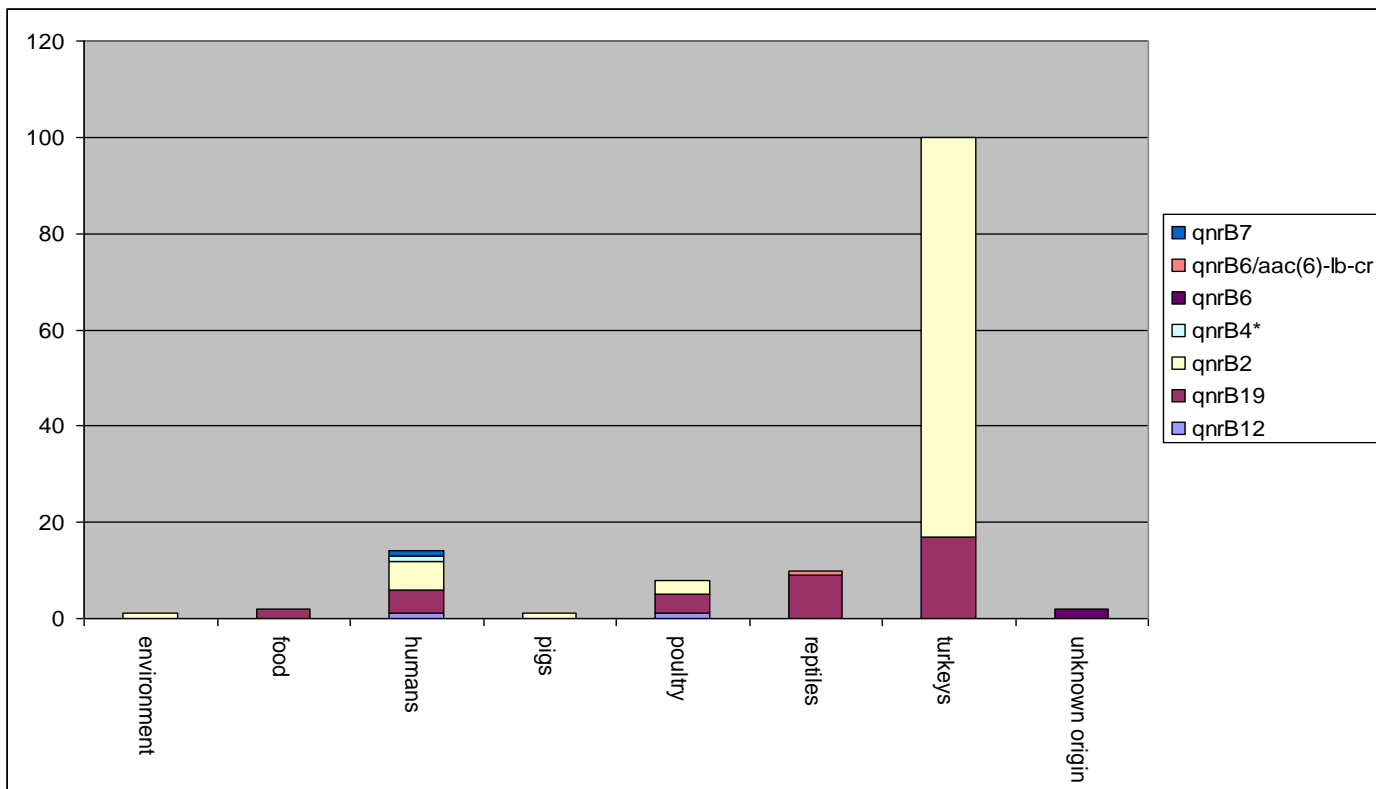
qnrA1-positive *Salmonella* isolates (n=3)



S. Paratyphi variant Java isolates from poultry meat (Belgium and the Netherlands)

S. Typhimurium isolate from a turkey (Germany)

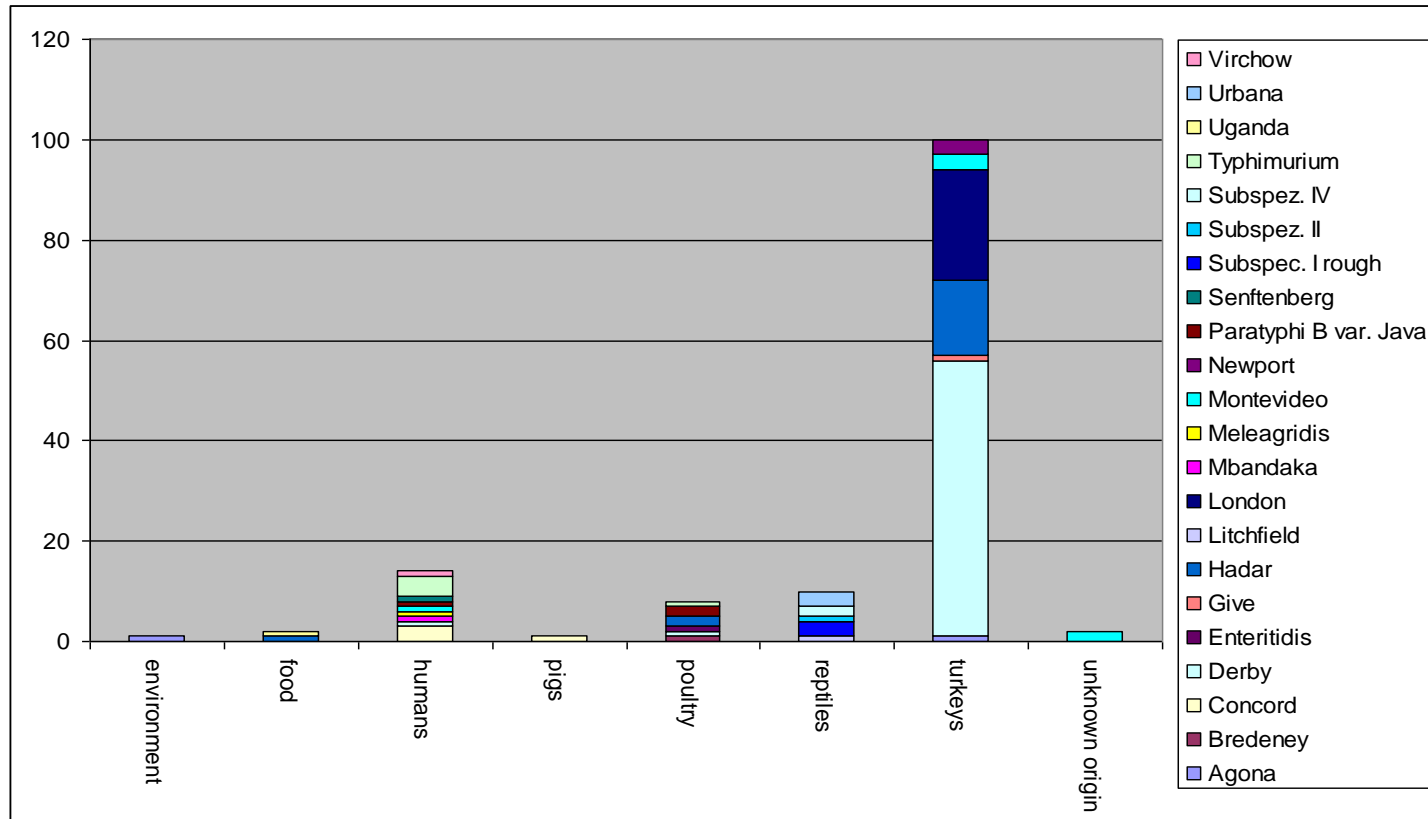
qnrB-variants in *Salmonella* per source (n = 138)



qnrB2 in turkeys

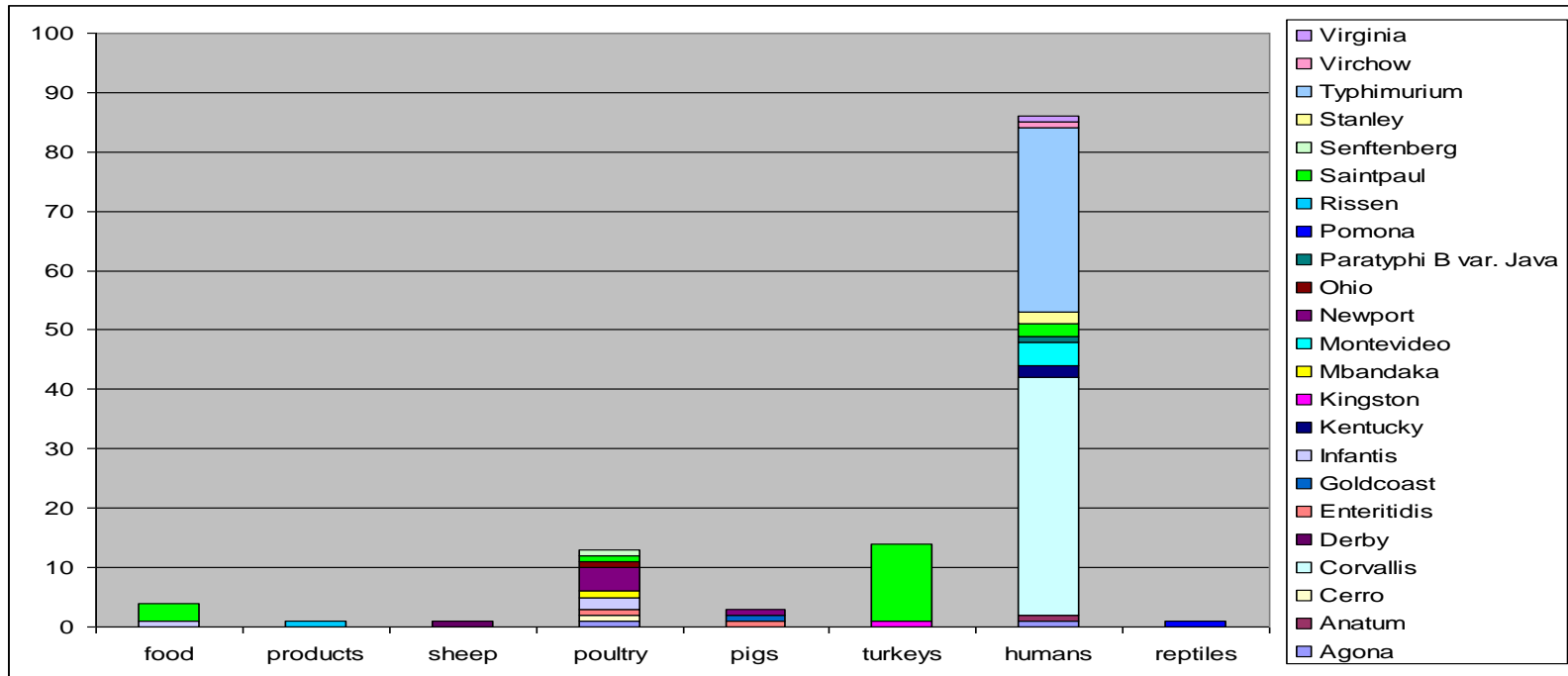
qnrB19 in humans, chicken, turkey and reptiles

qnrB-positive *Salmonella* isolates (n=138)



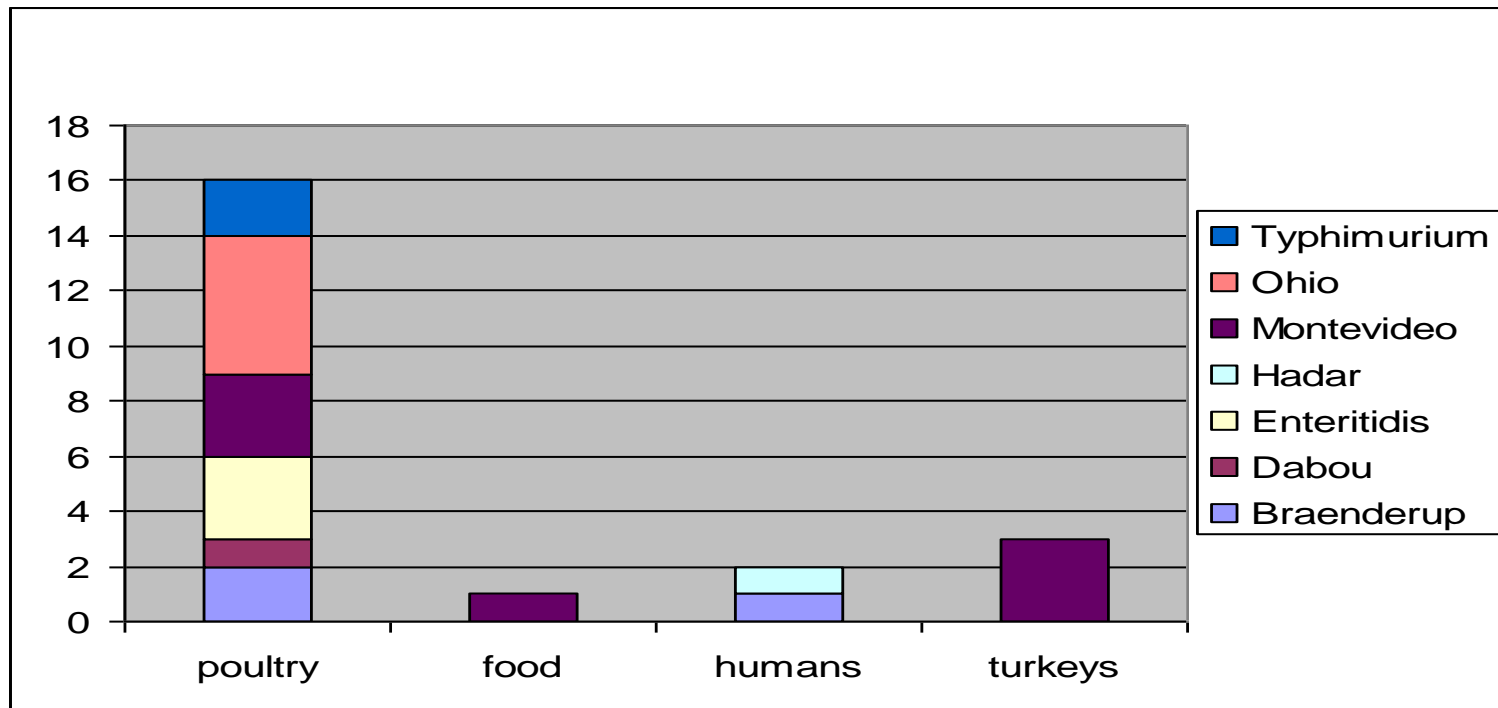
Variants of *qnrB* are identified in 22 different *Salmonella* serotypes
72 % *qnrB*- positive isolates identified in turkeys (*S. Derby*, *S. Hadar*, *S. London*)

qnrS1-positive *Salmonella* isolates (n= 125)



qnrS1 genes in *S. Corvallis* and *S. Typhimurium* (most humans)
qnrS1-positive *S. Saintpaul* in turkey isolates from Denmark, Poland and Germany and in human isolates from the UK and the Netherlands

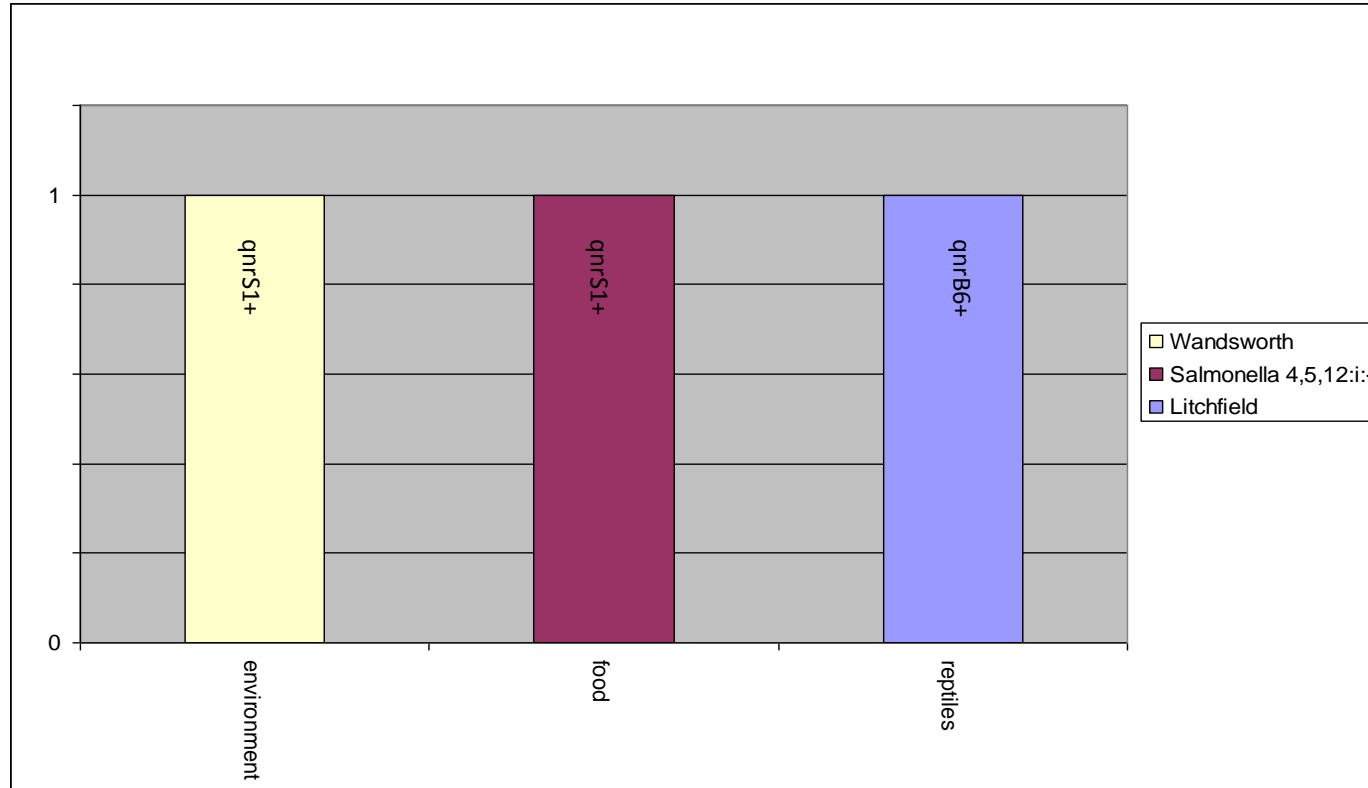
qnrD-positive *Salmonella* isolates (n = 22)



22 *qnrD*-positive *Salmonella* in 7 serotypes from 3 different countries (Italy, Spain and the Netherlands)

qnrD genes in chicken (n = 16), turkeys (n = 3), humans (n = 2) and food (n = 1)

aac(6')-1*b-cr*-positive *Salmonella* isolates (n=3)



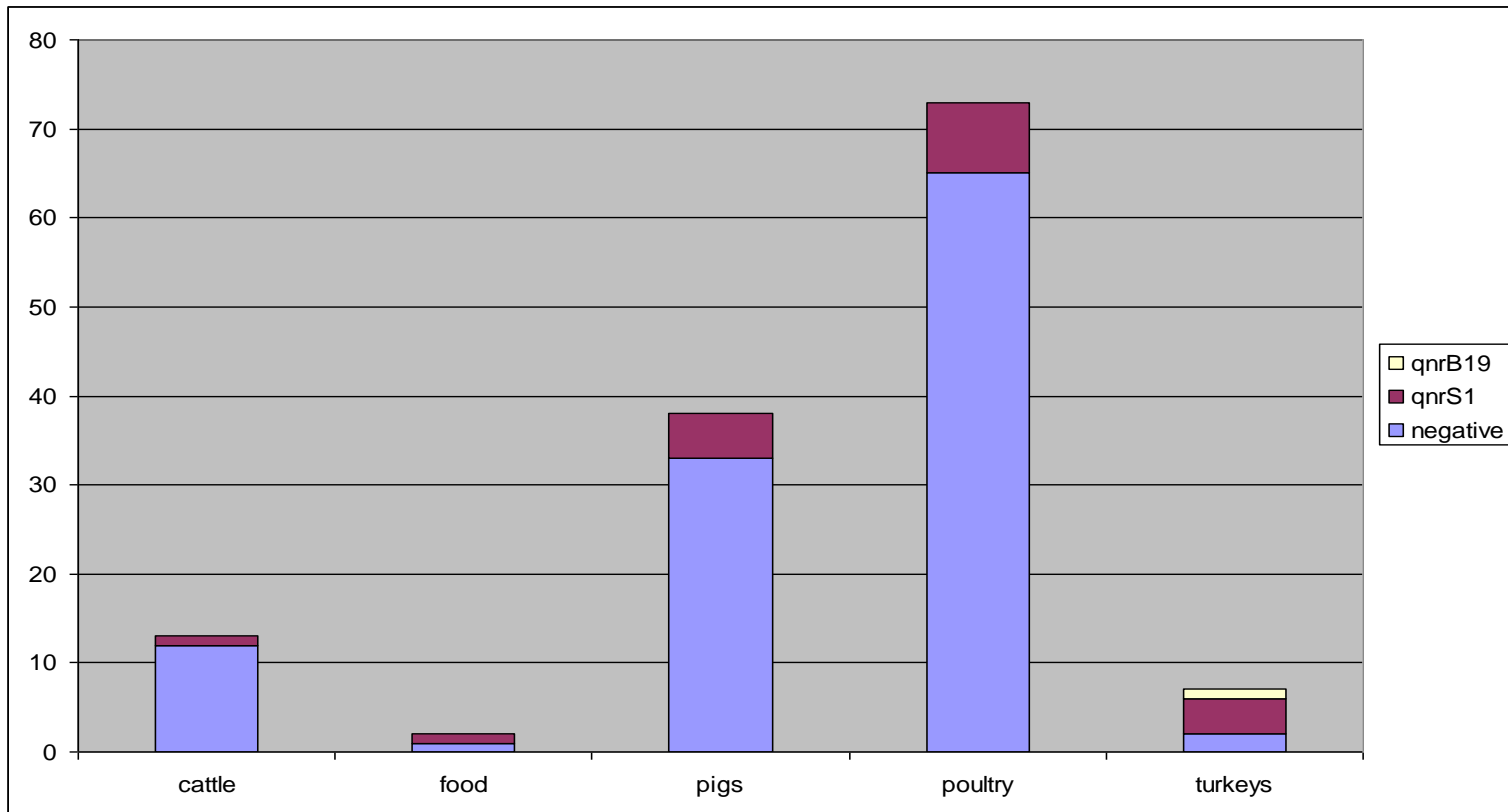
aac(6')-1*b-cr* was only detected in combination with *qnr*

PMQR in Europe

E. coli

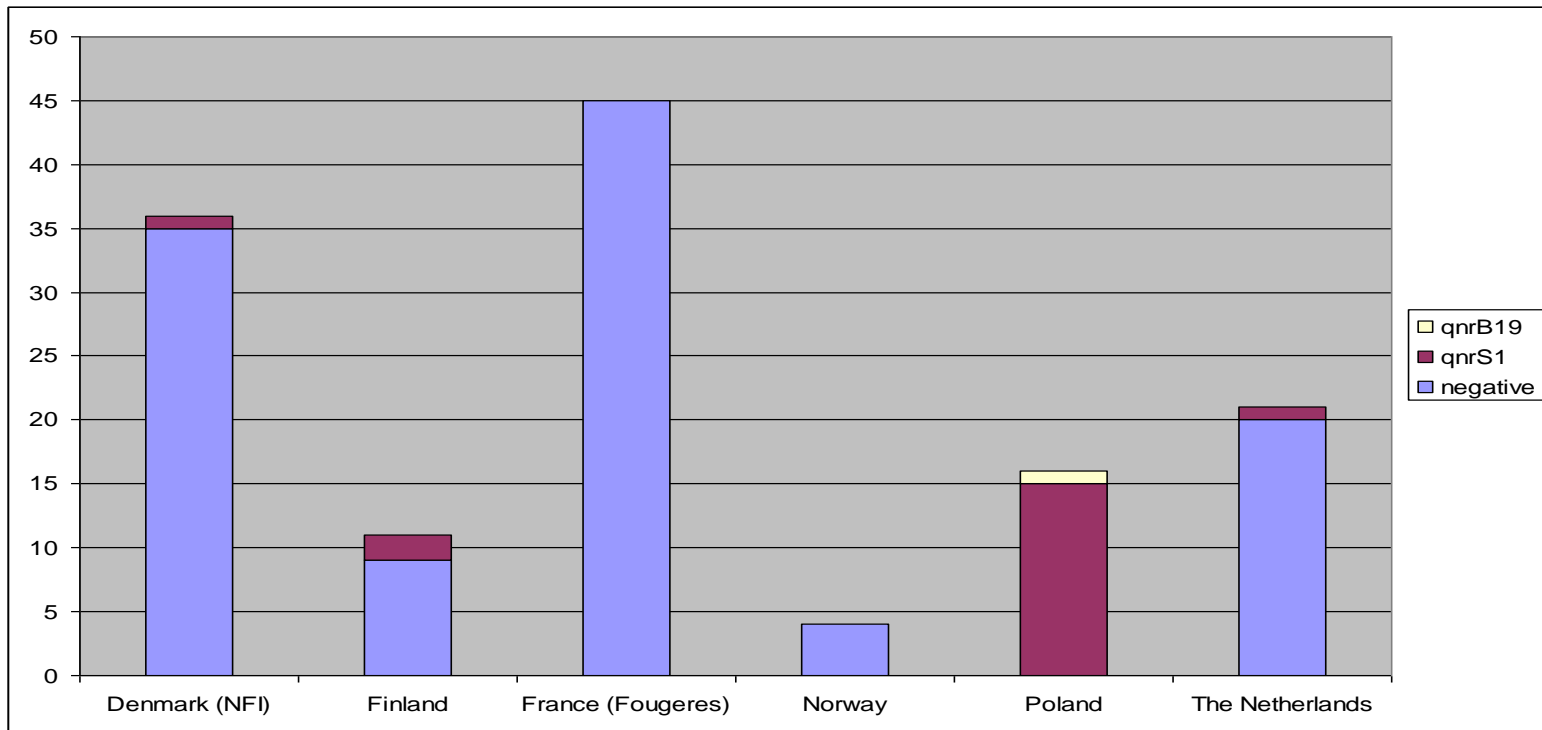
- No data on human isolates available
- In animals still rare but we detected in four different countries

Results PMQR-suspected *E. coli* (n = 133)



Qnr-positive *E. coli* seem to be rare, but were identified from 5 different sources

Results PMQR-suspected *E. coli* (n = 133)



Qnr-positive *E. coli* identified in 4 different countries
High incidence of *qnrS1*-positive *E. coli* (n = 15) from Poland

Worldwide spread



(Robicsek et. al, Lancet Infect. Dis. 5 2006;6: 629-40)

Worldwide spread

- After 2006: several reports from Africa, South-America and other parts of the world
- Nowadays PMQR genes can be found in all parts of the world



Origin of qnr genes

- Scientists found PMQR genes in different aqueous environments
 - *qnrA3* and *qnrA5* in *Shewanella algae* (Poirel et al.)
 - *qnrS2* in different *Aeromonas* species in the Seine river in France (Cattoir et al.)
 - qnrB-like genes in the Sargasso sea (Sanchez et al.)
- These findings suggest some qnr genes have an aqueous origin

Links to other genes

- PMQR genes are often linked to ESBL genes
- In The Netherlands PMQR + ESBL in:
 - *E. cloacae*/*E. hormaechei* from humans, cats and dogs
 - MDR *S. Concord* and *S. Senftenberg* from humans
 - *E. coli* from poultry
 - *Citrobacter freundii* from turtle

Questions?

