The occurrence of *qnr* genes in Dutch quinolone resistant *Salmonella* isolates

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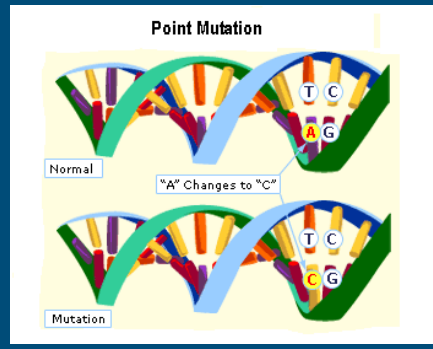
WAGENINGEN UR

Quinolone resistance mechanisms

- Chromosomal mutations
  - Mutations in QRDR region (*gyrA/B*, *parC/E*); altering the target-enzymes (DNA gyrase and type IV topoisomerase)
  - Over-expression of efflux pumps
  - Decrease of expression of outer-membrane porins
- Plasmid mediated quinolone resistance (PMQR)
  - *Qnr:* proteins protect the target enzymes
  - *AAC(6')-1b-cr:* adapted aminoglycoside R gene
  - *qepA*: specific efflux pump

Phenotypes of QRDR mutations

- High level resistant
  - Multiple mutations in the QRDR region
  - Ciprofloxacin MIC: ≥ 8 µg/ml
  - Nalidixic acid MIC: ≥ 64 µg/ml



- Low level resistant
  - Single mutation in the QRDR region
  - Ciprofloxacin MIC: 0.125 - 0.5 µg/ml
  - Nalidixic acid MIC: ≥ 64 µg/ml

PMQR phenotypes

- *qnr*
  - Proteins belong to the pentapeptide-repeat family
  - Bind to the target enzymes DNA gyrase and topoisom.
  - Confers low-level resistance to fluoroquinolones
  - Ciprofloxacin MIC: 0.25 - 1 µg/ml
  - Nalidixic acid MIC: 8 - 32 µg/ml

PMQR phenotypes

- *AAC(6') -1b-cr*
  - Acetylates ciprofloxacin and norfloxacin
  - Confers slightly higher MICs for ciprofloxacin and norfloxacin
  - No change in nalidixic acid MICs
  - Also kanamycin, amikacine and tobramycin resistant!

PMQR phenotypes

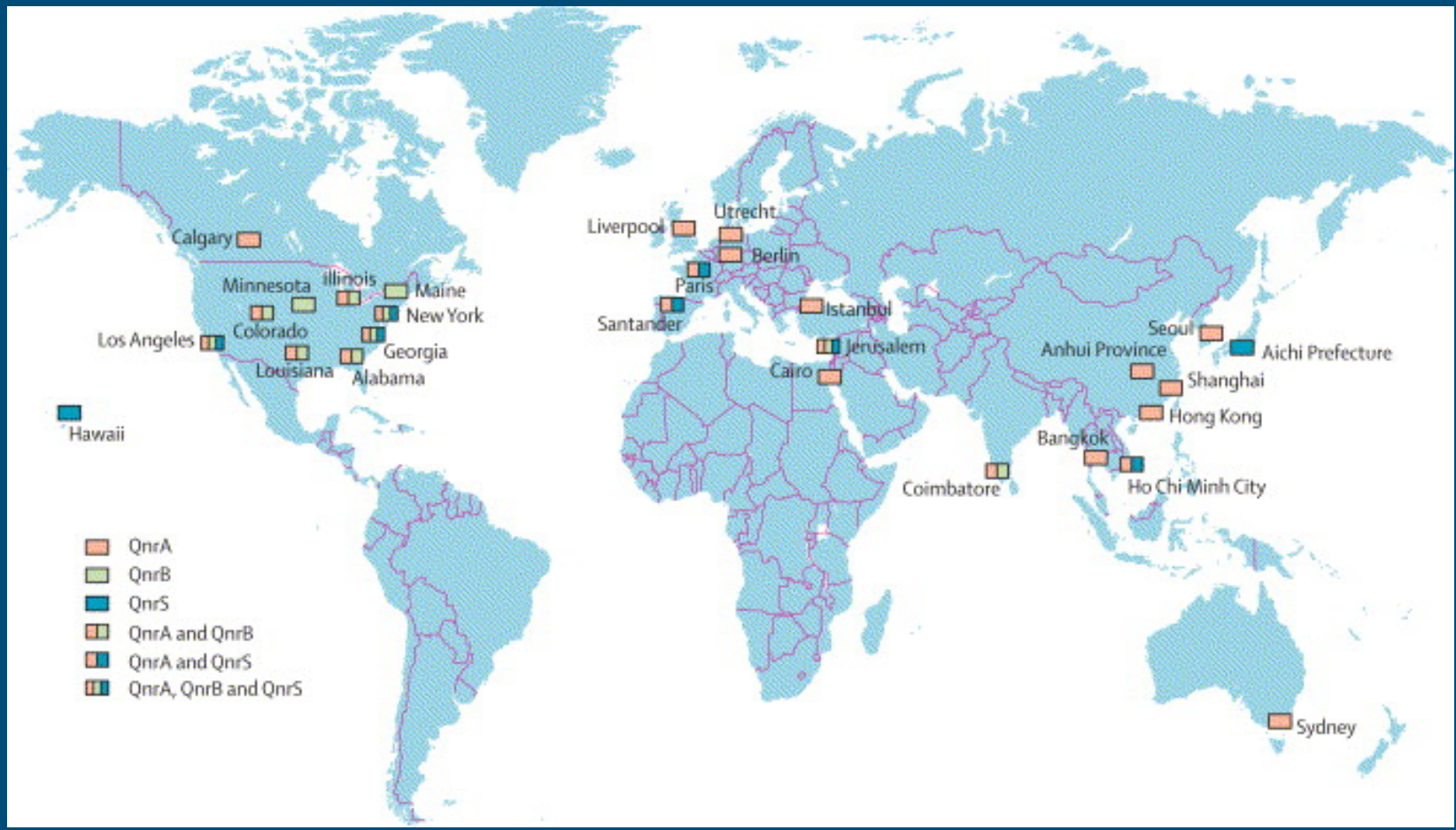
- *qepA*
  - Efflux pump for hydrophyllic fluoroquinolones
  - Confers low-level resistance to ciprofloxacin, norfloxacin and enrofloxacin
  - No change in nalidixic acid MICs

Qnr genes

- First *qnr* gene was described in *K. pneumoniae* from the USA by Martínez-Martínez in 1998.
- *qnrA(1-6)*, *qnrB(1-19)* and *qnrS(1-3)*
  - *Jacoby et al, qnr Gene Nomenclature: AAC 2008, Epub ahead of print)*
- ***qnrC***
  - Presented at the ECCMID congress in Barcelona (M.H. Wang, Shanghai China)
  - Detected in *P. mirabilis;* clinical isolate from the urine tract
  - on a tranferable plasmid pHS9
  - 48 – 68 % identitity with qnrA, B and S

Global distribution of *qnr* genes

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

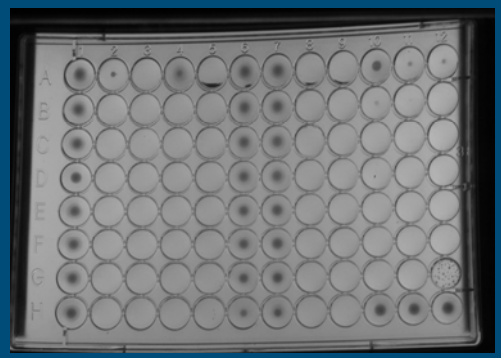


Qnr genes in The Netherlands

- First described by Paauw et al in a multi-resistant *E. cloacae* in 2006.
- Outbreak in the UMCU in 2002 and subsequently that in other centres.
- Located on a large plasmid (incHI2) harboring a complex class I integron with a *qnrA1* gene next to other resistance genes ($bla_{\text{CTX-M-9}}$).
- In dec 2006 we detected the first *qnr* genes in *Salmonella.* (JAC 2008 Feb;61(2):452-3.)

Strain collection

- More than 16.000 *Salmonella* isolated from humans and animals collected from 1999 - 2008. m4
- We test 2000 *Salmonella* isolates per year using the Broth Microdilution Method according to the CLSI.



Selection in database:

(1) high level resistant isolates (n=16)

(2) typical qnr phenotype (n=56)

Methods (PCR and sequencing)

- Mutations in the QRDR
- Presence of *qnr*, *AAC(6')-1b-cr* and *qepA*

- Primers and conditions
  - QRDR -> Eaves et al, AAC, Oct. 2004, 412-4015.
  - *Qnr* -> Gay et al, CID 2006; 43:297-304.
  - *AAC(6')-1b-cr* -> Robicsek et al, Nat. Med.2006;12:83-88
  - *qepA* -> Yamane et al. AAC Sep 2007; 51(9):3354-60

Results QRDR mutations

- High-level resistant isolates
  - Double mutation in *gyrA*
  - Single mutation in *parC*
- Qnr phenotype:
  - no mutations in the QRDR region

Mutations in the QRDR of ciprofloxacin resistant *S.* Kentucky (n=11)

Year of isolation	***gyrA* (Ser83 ->)**	***gyrA* (Asp87 ->)**	***parC* (Ser80 ->)**
2002 (n=1)	Phe	Tyr	Ile
2003 (n=1)	Phe	Tyr	Ile
2004 (n=6)	Phe	Gly	Ile
2005 (n=2)	Phe	Asn	Ile
2006 (n=1)	Phe	Asn	Ile

Mutations in the QRDR of ciprofloxacin resistant *Salmonella* other than S. Kentucky (n=5)

Serovar	**Year**	***gyrA* (Ser83)**	***gyrA* (Asp87)**	***parC* (Gly78)**	***parC* (Ser80)**	***parC* (Asp101)**
S.Swartzengrund	2000	Phe	Gly	-	Arg	Glu
S. Typhimurium pt 380	2001	Phe	Asn	-	Arg	-
S. Paratyphi A	2002	Phe	Gly	-	Arg	-
S. Reading	2004	Phe	Gly	Asp	-	-
S. Enteritidis	2005	Tyr	Asn	Asp	-	-

Detection of *qnr* genes

- Selection of 56 isolates
  - ciprofloxacin MIC: 0.25 – 1 µg/ml
  - nalidixic acid MIC: 8 – 32 µg/ml
- 51/56 positive in the *qnr* PCR
- 5 isolates negative for *qnr*, *AAC(6')-1b-cr* and *qepA*

Overview of identified *qnr* genes in Dutch *Salmonella* (2003-2008)

Serovar	Number	Source	Genes
S. Paratyphi B var Java	1	chicken products	*qnrA1*
S. Bredeney	1	broiler	*qnrB2*
S. Concord	3	human	*qnrB2*
S. Senftenberg	1	human	*qnrB2*
S. Paratyphi B var Java	2	chicken products	*qnrB5'*
S. Typhimurium ft 507	1	human	*qnrB5'*
S. Typhimurium ft 510	1	human	*qnrB5'*
S. Mbandaka	1	human	*qnrB7*
S. Anatum	1	human	*qnrS1*
S. Corvallis	32	human	*qnrS1*
S. Infantis	1	meat products	*qnrS1*
S. Kentucky	2	human	*qnrS1*
S. Montevideo	1	human	*qnrS1*
S. Saintpaul	1	human	*qnrS1*
S. Stanley	2	human	*qnrS1*

Summary

- Five different *qnr* genes (qnrA1, qnrB2, qnrB5', qnrB7 and qnrS1) in 14 different serovars.
  - QnrB5' has 99 % homology with qnrB5;codon Ser210 (Ser210Asn) and 3 silent muations
- Most *qnr* genes were detected in human clinical isolates (46/51 = 90%).
- In 2005-2008 identified five *qnr* positive *Salmonella* isolated from broilers or meat products (chicken):
  - 1 x S. Bredeney; *qnrB2*
  - 3 x S. Paratyphi B variant Java; 1 x *qnrA1* and 2 x *qnrB5'*
  - 1 x S. Infantis; *qnrS1*

Qnr and ESBL genes in the Netherlands

- *Qnr* genes are often associated with ESBLs
  - CTX-M-9, CTX-M-15, SHV-5, SHV-7, SHV-12, VEB-1 etc.
- In Dutch *Salmonella* an increase of ESBL genes (poultry in 2003) and *qnr* genes (humans starting in 2003 and poultry in 2005).
- In 2007 we detected the first *Salmonella* isolates with both *qnr* and ESBL genes.

Both qnr and ESBL positive Salmonella

- 3 MDR *S*. Concord isolates;
  - *qnrB2*, $bla_{\text{TEM-1,}}$ $bla_{\text{CTX-M-15}}$ and $bla_{\text{SHV-12}}$
- 1 MDR *S*. Senftenberg isolate;
  - *qnrB2*, $bla_{\text{TEM-1,}}$and $bla_{\text{SHV-12}}$
- 1 *S*. Paratyphi B variant Java isolate;
  - *qnrA1* and $bla_{\text{CTX-M-9}}$

General conclusions

- Although a variety of *qnr* genes have been identified in several serovars, these genes are still rare in *Salmonella*.
- *Qnr* genes are mainly present in human clinical isolates, but also in isolates of animal origin (especially poultry).
- Since 2007 both *qnr* and ESBL positive *Salmonella* isolates are present in The Netherlands.
- First report of *qnr* positive S. Paratyphi variant Java.

Qnr and surveillance

- It is possible do a retrospective prevalence study by checking your database for potential *qnr* positive isolates provided that:
  (1) a quantitative method is used for susceptibility testing
  (2) both nalidixic acid and a fluoroquinolone are tested
    - MIC ciprofloxacin: 0.25 -1 µg/ml
    - MIC nalidixic acid: 8 – 32 µg/ml
- Confirm all isolates with PCR.

Take home message

When it is present..

and you know how to look…

Take home message

You will find it!

Nest of a Garganey (Anas querquedula)

Acknowledgements

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