Community Reference Laboratory Antimicrobial Resistance

DTU Food
National Food Institute

# Quinolone resistance- updates and collaborative project

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# Background

## (Fluoro)quinolone resistance

- Importance of quinolones
  - Large spectrum antimicrobial class (including Gram positive and Gram negative bacteria)
  - Act on DNA supercoling- inhibit replication (bactericidal effect)
  - Used in human and veterinary medicine
  - Classified as critically important drugs
- Increasing trends in resistance
- Findings of new transferable resistance mechanisms

# Quinolone resistance

- Topoisomerase mutations
- Transferable resistance mechanims
  - Target protection:
    - *qnr genes* (A, B, S, C and D)
    - Many variants (A6, B20, S3, C1, D1)
    - Related to several chromosomally encoded pentapeptide repeat proteins from *Vibrionaceaea*, but also found among Gram positive bacteria as *E. faecalis*
  - Enzymatic modification
    - Aminoglycoside acetyltransferase - *aac(6')Ib-cr*
  - Specific efflux
    - Quinolone efflux pump- *qepA* (2 variants)

# Project qnr CRL-AR

- Objectives
  - Collection of data on quinolone resistance in Salmonella and E. coli in National Reference laboratories in Europe
  - Establishment of a database containing data on quinolone resistance from European countries
  - Retrospective screening for transferable quinolone resistance mechanisms of strains with suspected PMQR phenotype

# Project- methods

- Methods
  - Questionnaire for data collection
  - Data selection and collection
  - Screening for resistance mechanisms
    - Selection of PMQR isolates
    - Data (numbers) on other resistant isolates
    - Testing: PCR, sequencing
  - Collaboration and networking between laboratories/countries

# Screening of databases for PMQR phenotype-CRL retrospective screening

- fluoroquinolone low-level resistance
  - MIC of Cip>= 0,125mg/L *Salmonella*
  - MIC of Cip >=0,06 mg/L *E. coli*

- Nalidixic acid susc/intermediate
  - MIC 4-32 mg/L

# Project update

- Questionnaire /invitation to all NRL's
  - 22 participant laboratories (17 countries)
    - *E. Coli*- 19
    - *Salmonella*- 21
    - Both- 18
  - Good coverage of the European territory
  - Quantitative data: MIC (except one lab semiquantitative data- breakpoint testing)
  - Databases with similar data compared
  - Study period –last 10 Years (if possible more)- some labs have MIC data only for the last years.

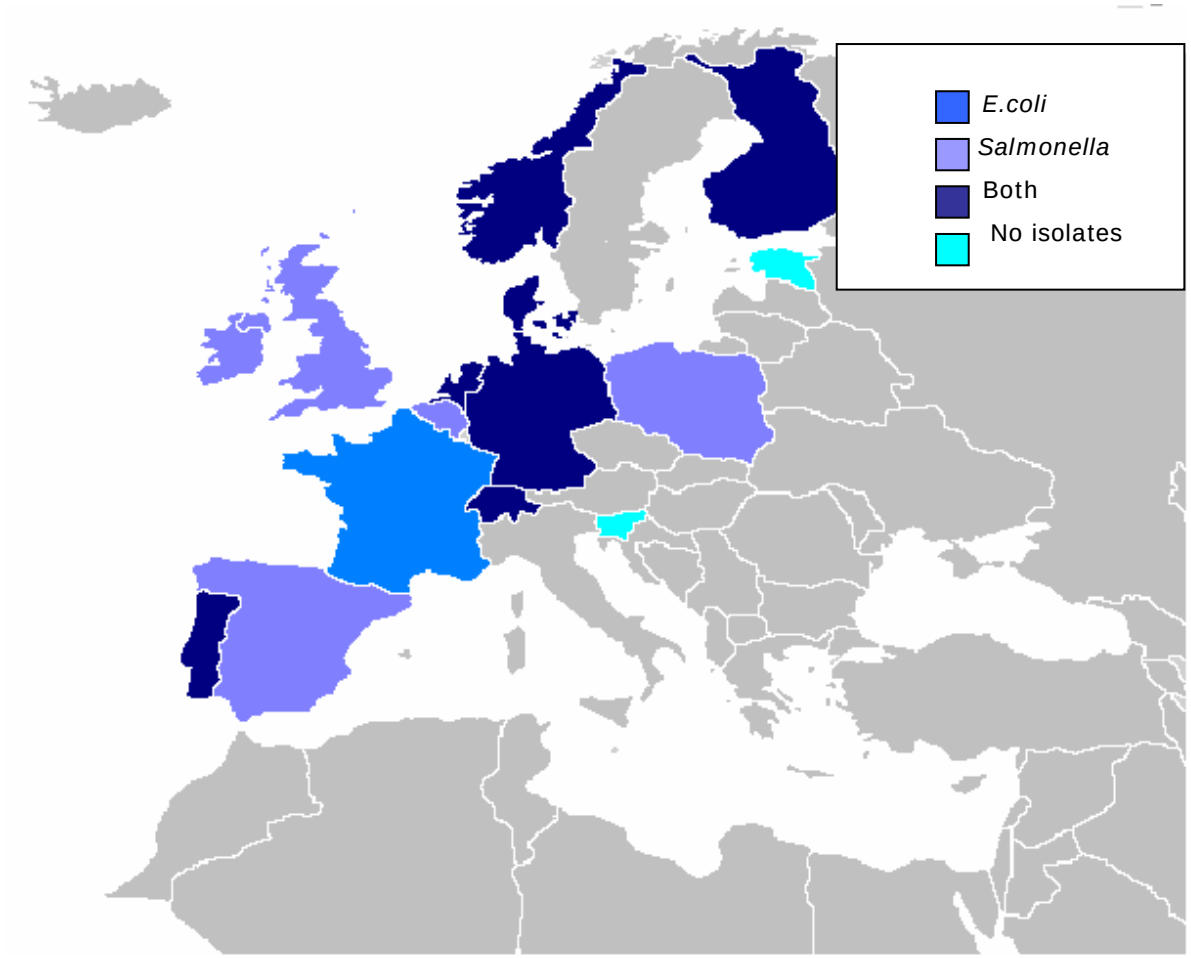
# Project update

- Network/collaboration
  - Protocol distribution
    - Protocols for PCR for *qnr*, *aac(6')*Ib and *qepA* were sent, but missing *qnrC*- will be added asap- if time allows we'll try to work on a multiplex protocol
    - Positive controls are available- contact us if need some
    - Assistance may be requested anytime
  - Assistance in other questions related to quinolone resistance detection

# Project update

- Data collection
  - 3 labs did not send any data yet
  - 16 labs have sent data on PMQR strains
  - 3 have sent only data on resistant strains, but did not find any PMQR suspected strains

# Data from PMQR suspected isolates



# Data

- Quinolone resistance over time
  - Most labs had recent data only, but we received data up to 1981
  - Some differences between countries/laboratories
    - Prevalence of resistance
    - Numbers of isolates tested and their origin

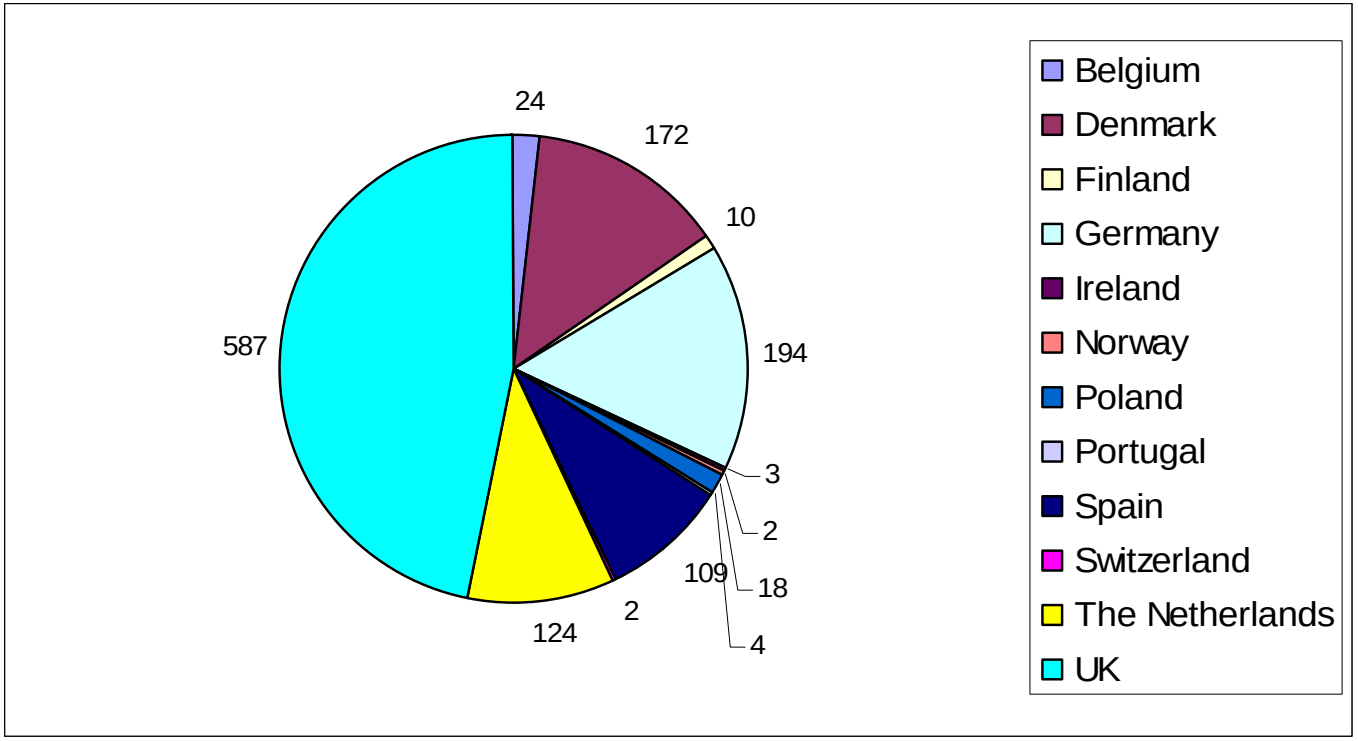
# Data

- Data on resistant isolates (CIP and NAL R)
  - Large number of isolates screened
    - *Salmonella* 670.000 screened over 42.000 resistant (13%)
    - *E. coli* 34.000 screened with 2900 resistant (5%)

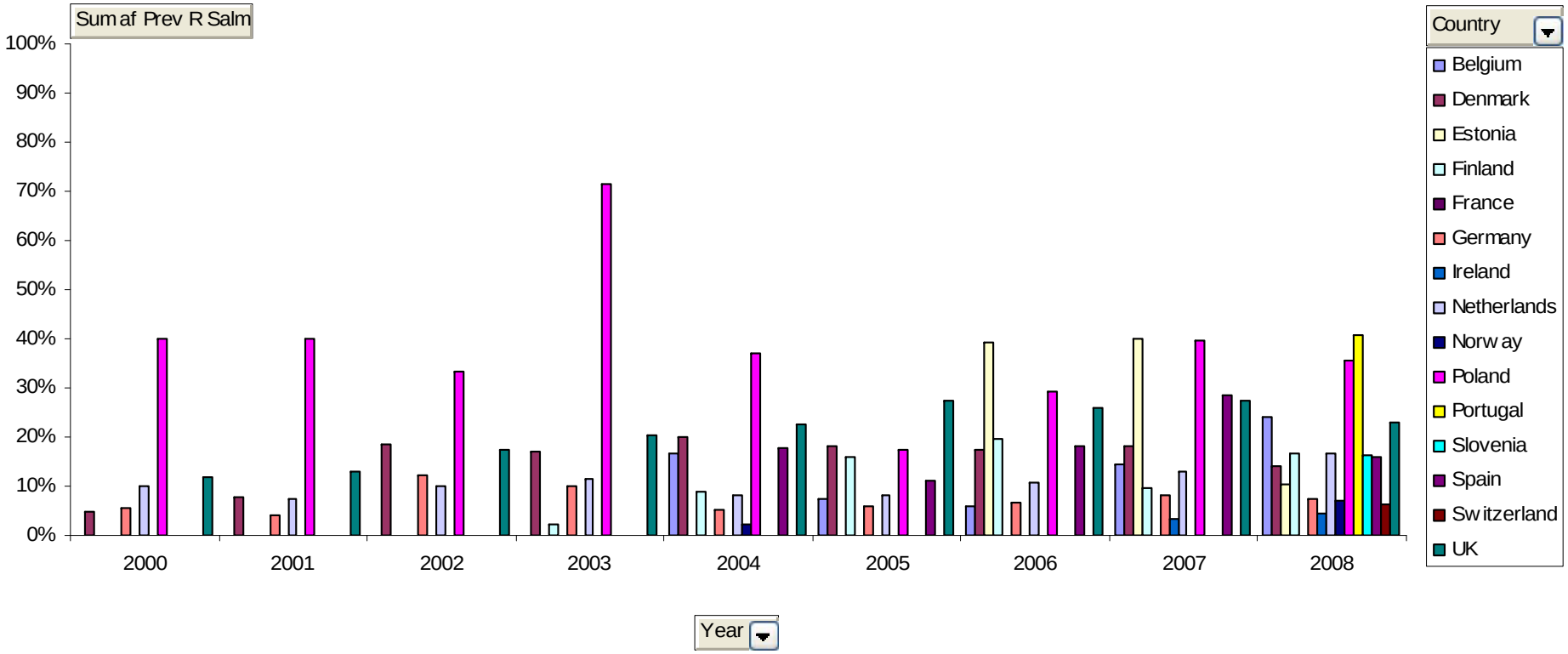
- PMQR suspected isolates (after 1993)
    - *Salmonella* 670.000 screened over 1200 PMQR phenotype
    - *E. coli* 34.000 screened with 316 PMQR phenotype
  - In rather low prevalence but widespread
  - Further data analysis not performed, awaiting test results

# PMQR *Salmonella*

- 1249 suspected strains reported from 14 labs in 12 countries
- Many serotypes reported

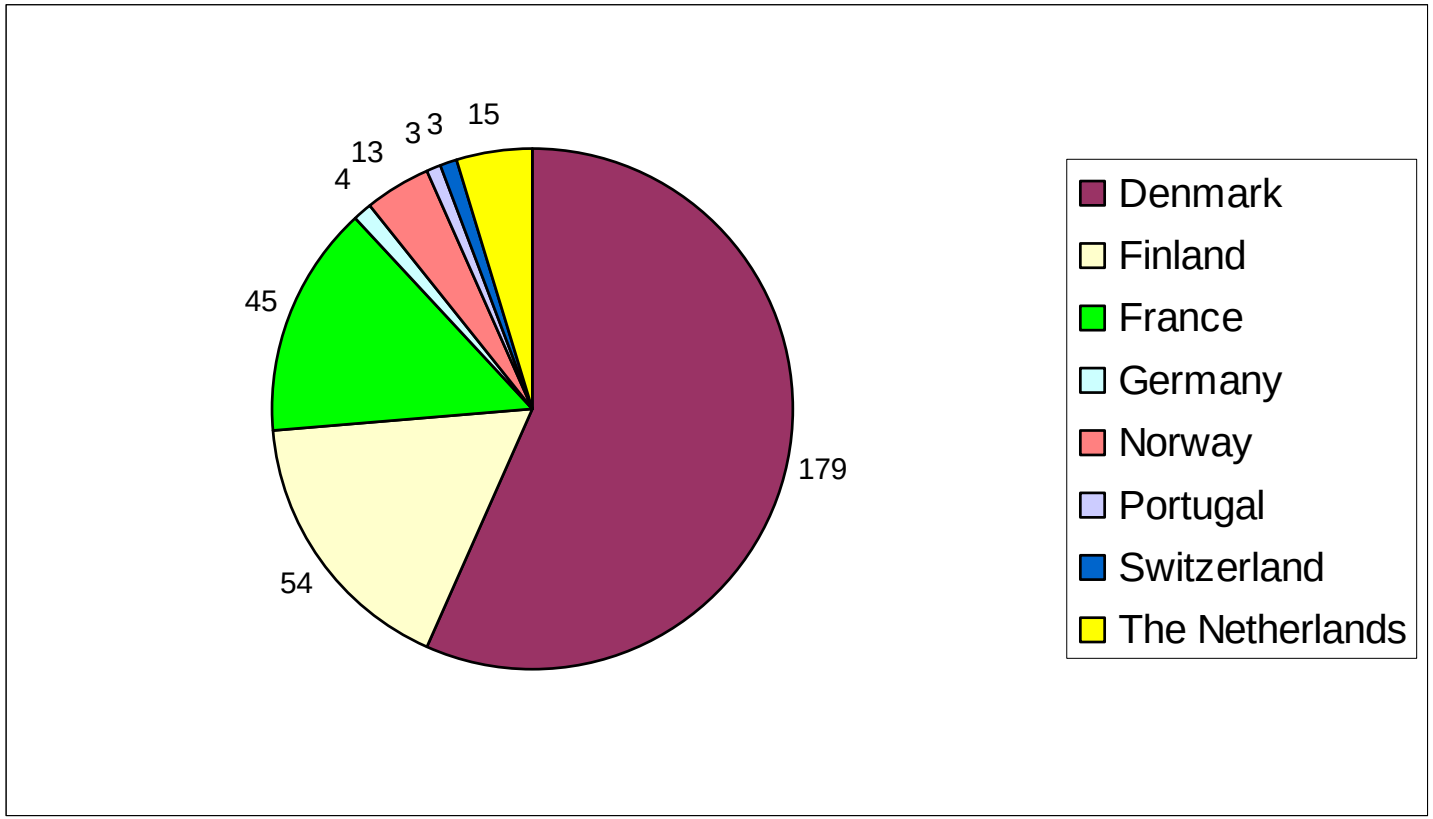


# Prevalence of resistant strains over time- *Salmonella*

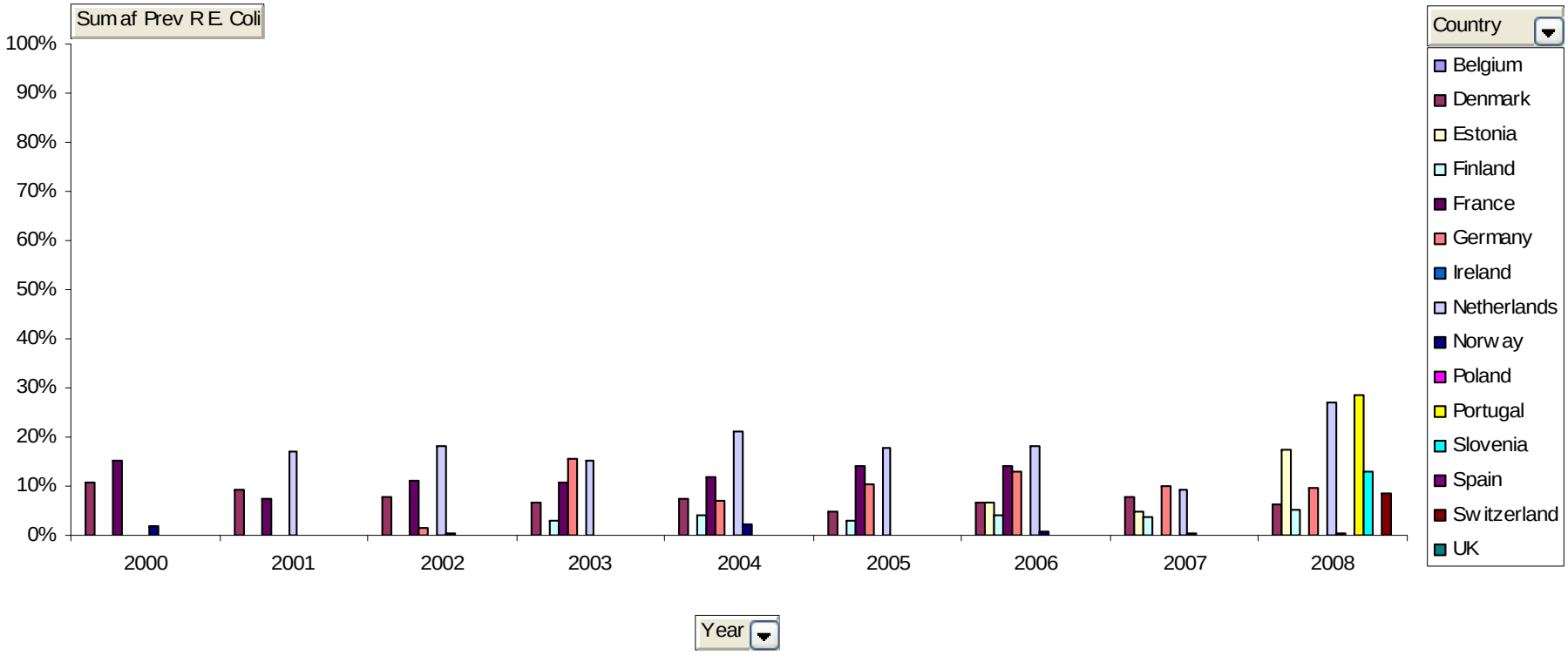


# PMQR *E. coli*

- 316 suspected isolates reported from 8 labs in 8 countries



# Prevalence resistant strains over time- *E. coli*



# Discussion about lab work

- **What are the consequences of the high number of PMQR isolates selected (1249 *Salmonella* and 316 *E. coli)*??**
  - How to check the PMQR isolates?
    - Who will do it?
      - Local NRLs or centrally (eg. DTU, CVI?)
    - When (deadline)?
    - How - Method for testing
      - PCR
      - Alternatives- Southern blot/ hybridisation, other ??

# Discussion about lab work

- **In the current selection NAL R isolates are excluded, however, PMQR may be present as well:**

**Should we additionally study the presence of PMQR genes in nalidixic acid resistant isolates ??**

# Data analysis and report

- **Analysis of data (Who, When, How??)**
- **Publication / Report:**
  - Agree on a collective report/ publication, which we consider to be a very important pan-European output from this network

# Funding options??

- Importance of the results of this project and the large numbers of isolates included in the MIC-database
  - potential wide-spread of PMQR

- Should we explore the options for **extra funding**??
  - to support local analysis at NRLs, or
  - central analysis at selected labs
  - further follow up studies

# Thank you for your collaboration!!!