Differentiation of Trimethoprim Resistance Genes among *Eschericha coli* Strains from an Environment with Intensive Supply of Antibiotics

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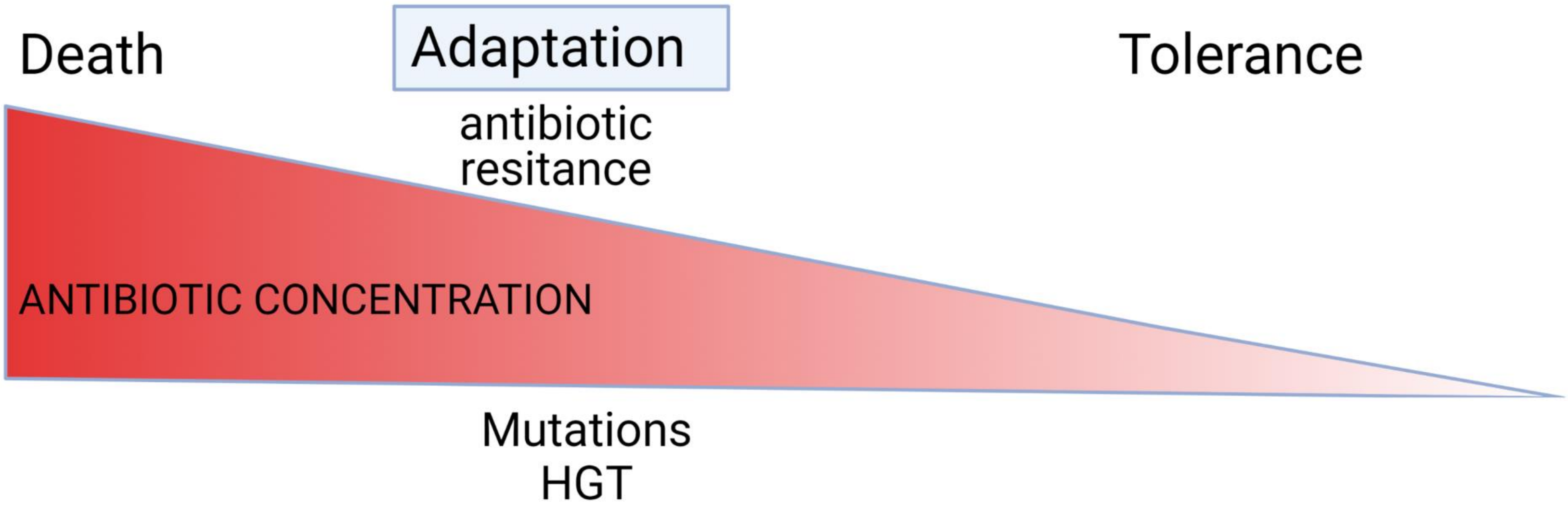
Antibiotic resistance

- One of the main assumptions behind the spread of antibiotic resistance



Death

Adaptation

antibiotic
resitance

Tolerance

ANTIBIOTIC CONCENTRATION

Mutations
HGT

The more often and the more intensively antibiotics are used
the stronger the selection pressure is
and the selection of resistant strains

Adapted from: Bernier S.P Front. Microbiol. 2013

Antibiotic resistance

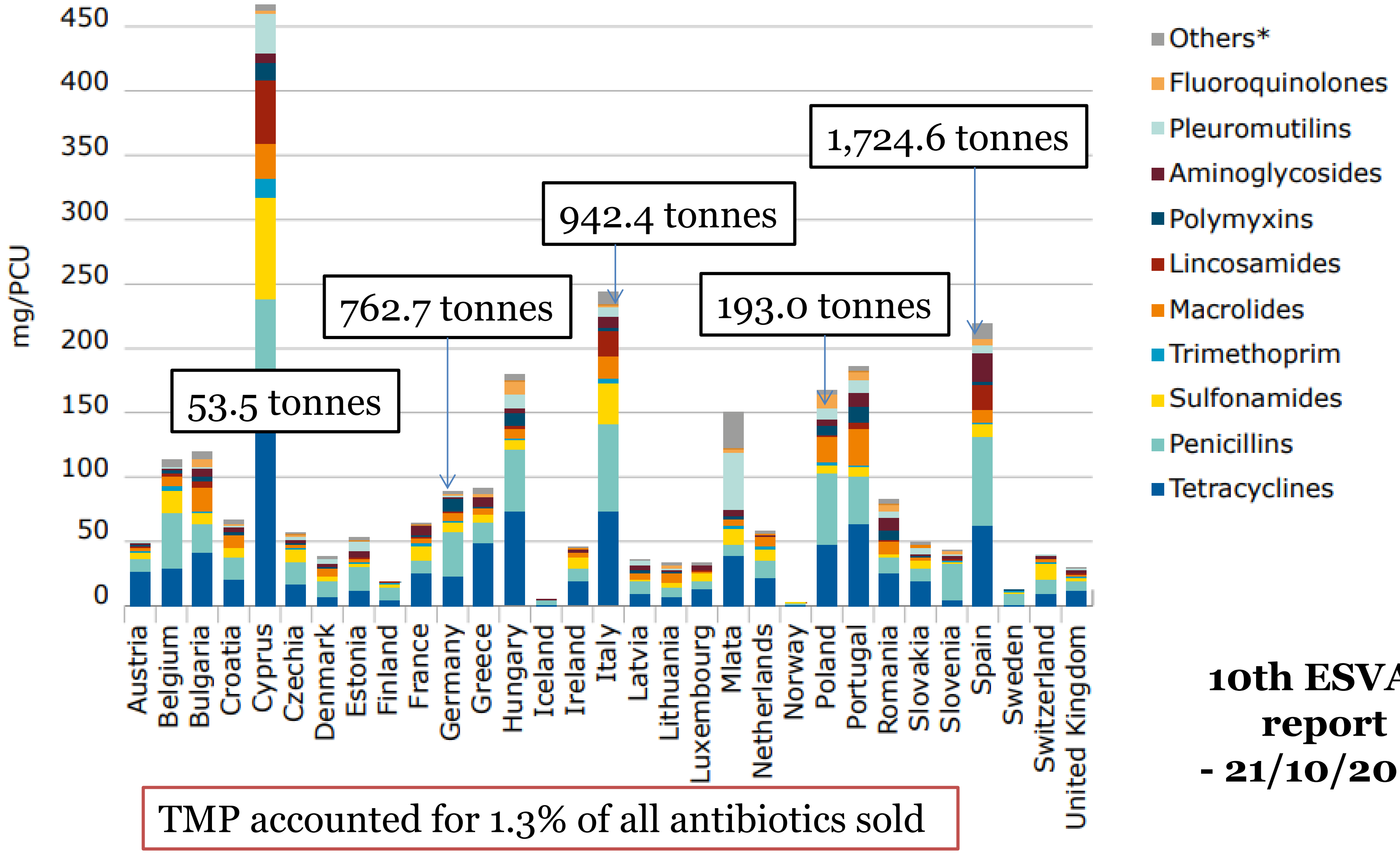
- Half of antibiotics usage have non-human applications;
- Agriculture impact on the development of antibiotic resistance is huge.

- Latest data of European Medicines Agency shows a downward trend and overall decline in antibiotic sales, but still:

✓ sales for food-producing species of the various veterinary antimicrobial classes, in 2018 amounted 6,500.7 tonnes (31 European countries).

European surveillance of veterinary antimicrobial consumption

Figure 1. Sales for food-producing species, in mg/PCU, of the various veterinary antimicrobial classes, for 31 European countries, in 2018 (1 PCU = 1 kg of animal biomass).



53.5 tonnes

762.7 tonnes

942.4 tonnes

193.0 tonnes

1,724.6 tonnes

TMP accounted for 1.3% of all antibiotics sold

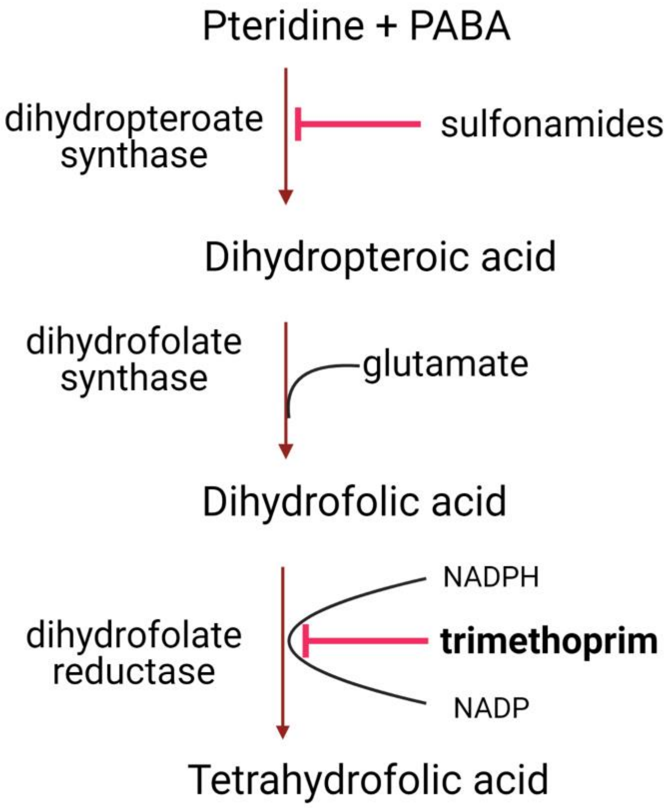
10th ESVAC report - 21/10/2020

Trimethoprim (TMP)

- Structural analog of dihydrofolic acid, inhibits the synthesis of tetrahydrofolic acid.

- In medicine- mostly treatment of urinary tract infections.

- In veterinary - treatment and metaphylaxis of gastrointestinal and respiratory infections; administered, in drinking water, in feed for calves, pigs, poultry (commonly with sulfonamides).



Steps in folate metabolism blocked by sulfonamides and trimethoprim.

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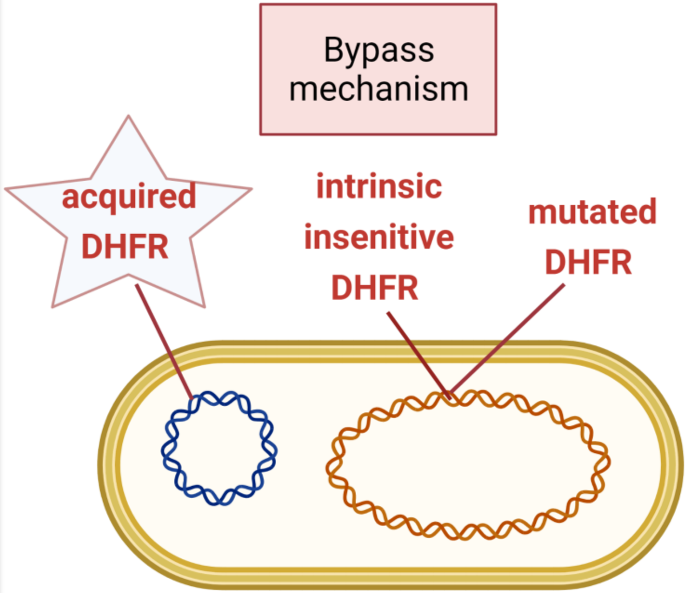
Resistance to trimethoprim

Associated with main mechanisms, including:

- permeability barrier and
- bypass mechanism:
  - ✓ naturally insensitive intrinsic DHFR,
  - ✓ spontaneous chromosomal mutations in DHFR (*folA*) genes involved in the folic acid pathways,
  - ✓ production of acquired, alternative DHFR encoded by *dfr* genes related to integrons, transposons, plasmids.



DHFR - dihydrofolate reductase

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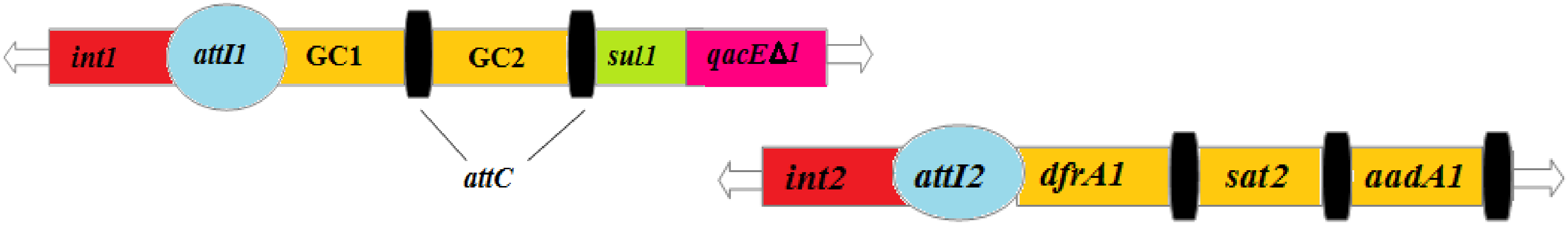
Trimethoprim resistance genes

- *Dfr* genes are found mostly:
  - As gene cassettes within variable parts of class 1 and class 2 integrons,
  - with common regions ISCR (insertion sequence common region).



- Association with the mobile genetic elements contributes to the rapid spread of resistance to trimethoprim among bacteria

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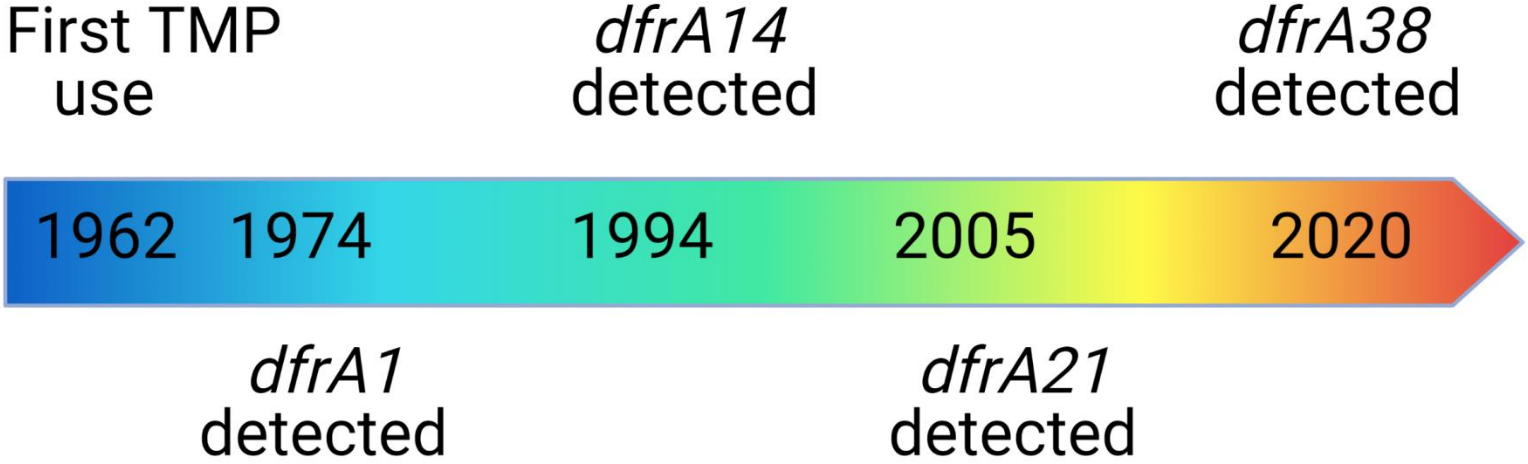
Trimethoprim resistance genes

- Two main families of *dfr* genes: *dfrA* and *dfrB;*
- Almost 40 genes *dfrA* have been identified so far:
  - ✓ first *dfrA1* - detected in 1974, induced by an R-factor mediating high resistance to trimethoprim,
  - ✓ last one- *dfrA38* - found in *Acinetobacter baumannii* isolate in 2020.


First TMP use

dfrA14 detected

dfrA38 detected

1962 1974 1994 2005 2020

dfrA1 detected

dfrA21 detected

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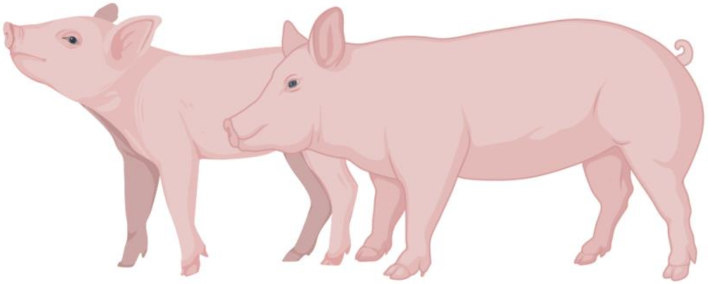
Aim of the study

Assessment of *dfrA* genes differentiation in commensal *Escherichia coli* strains isolated from pigs from a breeding farm with intensive supply of antibiotics in metaphylaxis program.

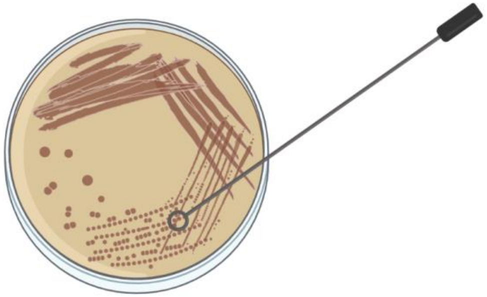
Material

- Fecal samples of 50 pigs (piglets and sows) form one farm in Lubuskie province in Poland;
- Herds after medical metaphylaxis program (after weaning);
- Animals treated with medicated fodder: amoxicillin, trimethoprim, sulfamethoxazole for 4 weeks.



E. coli identification

- Biochemical testing.



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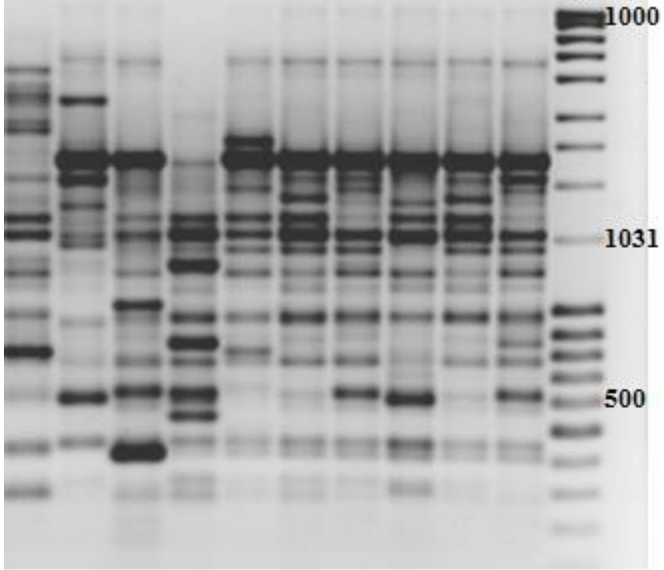
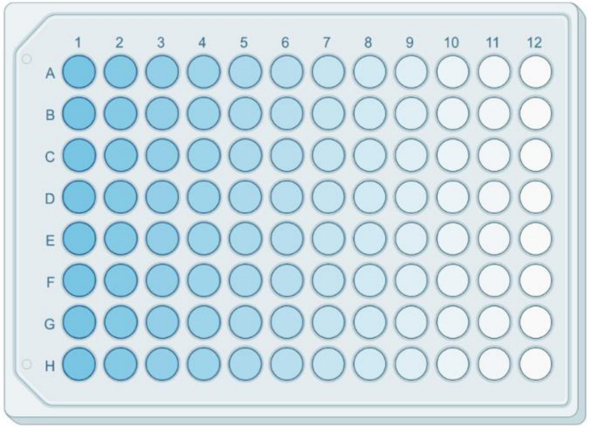
E. coli strains

- BOX-PCR fingerprint analysis



Antibiotic susceptibility testing

- MIC of TMP tested by microdilution method.
- Tested range: 0.25-32 mg/l.
- Results interpreted according to EFSA.



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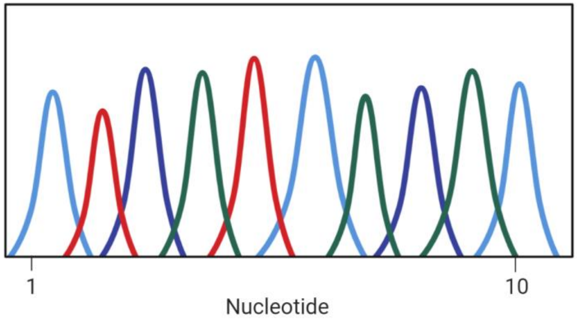
Detection of trimethoprim resistance *dfrA* genes

- PCR method for:
  *dfrA5, dfrA7, dfrA14, dfrA24, dfrA26**
- PCR-RFLP with primers complementary to the *dfr* gene groups:
  - group A1 (*dfrA1, dfrA15, dfrA15b, dfrA16, dfrA16b, dfrA28*)
  - group A12 (*dfrA12, dfrA13, dfrA21, dfrA22*)**.
- Sequence analysis of PCR products after non-specific cut.

PvuI, AluI, TruI, MspI, TasI.



*Brolund; **Seputiene

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Resultes

- A total 164 *E.coli* strains were isolated from feces of 50 animals;
- from 2 to 6 non-identical isolates per animal samples.

Resistance to TMP

- 150 (92%) isolates were resistant to TMP (resistant strains derived from samples of 49 individuals).
- In samples from 10 pigs, both sensitive both resistant *E. coli* to TMP were detected.
- For resistant strains with high MIC values (>32 mg/L).

Resultes

Prevalence of *dfrA* genes

- Six different genes were detected:

dfrA1, dfrA5, dfrA7, dfrA12, dfrA14, dfrA21

Table 1. Distribution of trimethoprim resistance genes among *E. coli* isolates from pigs.

dfrA1	***dfr12***	***dfr7***	***dfr5***	***dfrA14***	***dfr21***
105 (65%)	60 (36.6%)	28 (17.1%)	18 (11%)	15 (9.1%)	14 (8.5%)

Resultes

Prevalence of *dfrA* genes

- 59 (36.6%) of the strains carried 2 to 4 different *dfrA* genes in different combinations.
- 32 strains (20.1%) – carried 2 *dfrA* genes;
- 19 strains (11.6%) - carried 3 genes,
- 6 (3.7%) carried 4 genes,
- 1 (0.6%) strain carried five TMP resistance genes.

Table 2. Distribution of multiple trimethoprim resistance genes among *E. coli* isolates from pigs.

Number of strains with multiple *dfrA* genes					
18	*dfrA1*	*dfrA12*			
7	*dfrA1*	*dfrA7*			
3	*dfrA1*	*dfrA5*			
1	*dfrA1*	*dfrA21*			
1	*dfrA5*	*dfrA7*			
1	*dfrA5*	*dfrA14*			
1	*dfrA7*	*dfrA12*			
1	*dfrA12*	*dfrA21*			
7	*dfrA1*	*dfrA12*	*dfrA21*		
5	*dfrA1*	*dfrA5*	*dfrA14*		
1	*dfrA1*	*dfrA5*	*dfrA7*		
2	*dfrA1*	*dfrA7*	*dfrA12*		
1	*dfrA1*	*dfrA12*	*dfrA14*		
2	*dfrA5*	*dfrA7*	*dfrA14*		
1	*dfrA5*	*dfrA12*	*dfrA14*		
2	*dfrA1*	*dfrA7*	*dfrA12*	*dfrA21*	
1	*dfrA1*	*dfrA7*	*dfrA12*	*dfrA14*	
2	*dfrA1*	*dfrA12*	*dfrA14*	*dfrA21*	
1	*dfrA1*	*dfrA5*	*dfrA7*	*dfrA14*	
1	*dfrA1*	*dfrA5*	*dfrA7*	*dfrA12*	*dfrA21*

Intra-individual differentiation

Among *E. coli* strains from 46 of animal samples 2 to 6 different genes were detected:

- In *E. coli* from 17 animals- 2 different genes,
- *E. coli* from 19 – 3 genes,
- *E. coli* from 6 – 4 genes,
- *E. coli* from 3 – 5 genes,
- *E. coli* from 2 animals, 6 different genes were detected.

Table 3. Intra-individual differentiation of *dfrA* genes detected in *E. coli* strains.

1 gene	**2 genes**	**3 genes**	**4 gene**	**5 gene**	**6 genes**
		per animal sample			
3 (6%)	17 (34%)	19 (38%)	6 (12%)	3 (6%)	1 (2%)

Sequence analysis of *dfrA1* genes

- Restriction analysis of PCR products of *dfrA1* group:

✓ in 5 cases digestion with *PvuI* and *TasI* indicated *dfrA1*

✓ but TruI and AluI gives non-specific products

- PCR products were sequenced and analyzed in NCBI Nucleotide BLAST.
- Alignment revealed the highest identity score with *dfrA1* at 89.27% (GB: NG_047685.1).
- Numerous single nucleotide changes and several deletions were detected (Figure 2.)

Results

- Figure 2. Alignment result with *dfrA1* sequence GB: NG_047685.1.

```
dfrA      1 AATGGAGAC-TC-GGTATGGCCCTGAGATTCC-TGGAGTGCCAAACGTG-ACAGC
dfrA1     1 AATGGAGTTATCGGGAATGGCCCTGATATTCCATGGAGTGCCAAAGGTGAACAGC

dfrA     52 TCCGGTTTAAAGCTATTACCTATAACCAATGCCTGTTGGTTGGACGCCAGACTTT
dfrA1    56 TCCTGTTTAAAGCTATTACCTATAACCAATGGCTGTTGGTTGGACGCAAGACTTT

dfrA    107 TGAATCAGTGGGAGCTTTACCTGACCGATAGTATGCGGTCGTAACACGTTCAAGT
dfrA1   111 TGAATCAATGGGAGCATTACCCAACCGAAAGTATGCGGTCGTAACACGTTCAAGT

dfrA    162 TTTACATCTTACAATGAGAACGTATTGTTCTTTCCATG-GTTAAAGATGCTTTAA
dfrA1   166 TTTACATCTGACAATGAGAACGTATTGATCTTTCCATCAATTAAAGATGCTTTAA

dfrA    216 CCGACCGAAAGAAAATAACGGATCATGTCATTGATTCAGGTGGTGGGGAGATATA
dfrA1   221 CCAACCTAAAGAAAATAACGGATCATGTCATTGTTTCAGGTGGTGGGGAGATATA

dfrA    271 CAAAAGCCTGATCGATCTGGTTGATACTCCACATA-ATCTACAATATACCTCCAG
dfrA1   276 CAAAAGCCTGATCGATCAAGTAGATACACTACATATATCTACAATAGACATCGAG

dfrA    325 CCGGAAGGCGATGTTTACTTTCCTGAAATCTCAAGCAATTTTAGGCCAGTTTTTA
dfrA1   331 CCGGAAGGTGATGTTTACTTTCCTGAAATCCCCAGCAATTTTAGGCCAGTTTTTA

dfrA    380 CCCTCGACTTCACCTCTGACACACATCATAATTACCCA-TCT-GCAAAAGGGT--
dfrA1   386 CCCGAGACTTCGCCTCTAACATAAATTATAGTTACCCAATCTGGCAAAAGGGTTA

dfrA    431 AC--AT
dfrA1   441 ACAAGT
```

Nucleotide BLAST (https://blast.ncbi.nlm.nih.gov), T-Coffee (http://tcoffee.crg.cat), BOXSHADE (https://embnet.vital-it.ch/cgi-bin/BOX)

Conclusions

Environment with Intensive Supply of Antibiotics Conclusions

- Great diversity of the trimethoprim resistance genes,

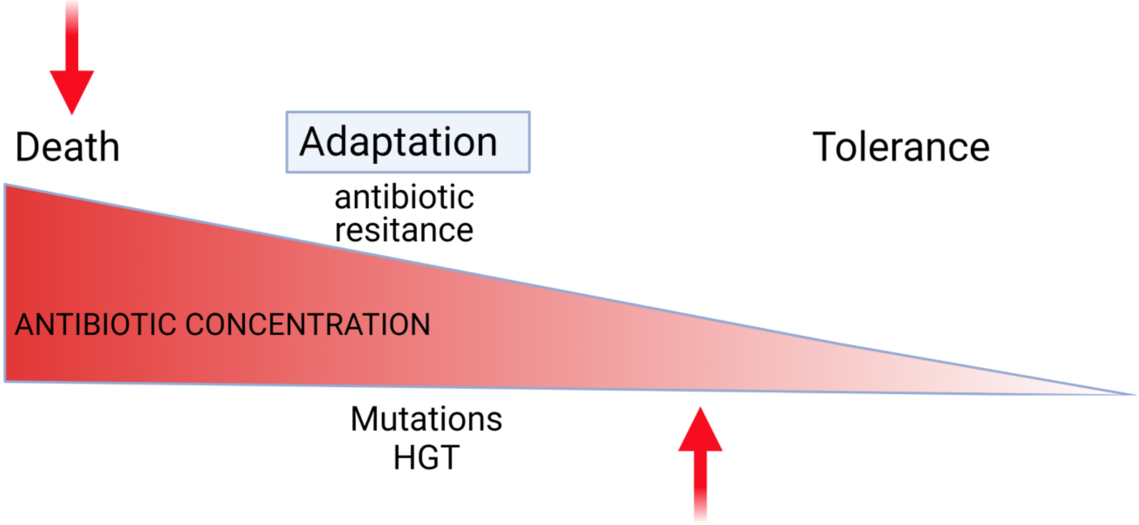
✓ both within the tested animal population and

✓ in the individual host.

✓ Nucleotide changes within *dfrA1* genes, highlight the potential for alterations leading to the emergence of new resistance gene variants.

The key issue- Antibiotics stewardship

Use of an antibiotic at a concentration above the MIC, would limit the emergence of resistant mutants



Death

Adaptation

antibiotic resitance

Tolerance

ANTIBIOTIC CONCENTRATION

Mutations
HGT

Exposure to very low antibiotic concentrations can select for low-level resistant mutants, but- strains evolve for high-level resistance

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Antibiotics stewardship

- Rational and responsible use of antibiotics in animal husbandry.
- Developing and using vaccines that replace antibiotics to prevent bacterial infections in pigs.
- Increased awareness of the threat of antimicrobial resistance, responsible-use campaigns.

Thank you for your attention

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