9

RESISTANCE IN ANIMAL PATHOGENS

9. Resistance in animal pathogens

Highlights

Surveillance of antimicrobial resistance in 2024 focused on pathogenic bacteria from acute mastitis in dairy cows and from various organs in pigs and included results obtained through antimicrobial susceptibility testing.

Most pathogenic bacteria from acute mastitis in dairy cows displayed low frequencies of phenotypic resistance. A relatively high proportion of *Streptococcus uberis* isolates displayed resistance to penicillin, which is noteworthy because beta-lactamase sensitive penicillins are the most commonly used drugs for treatment of adult cattle in Denmark. However, there is currently no clinical breakpoint available for penicillin in *S. uberis* and most of the isolates had a MIC value of 0.25mg/L, which is just above the tentative ECOFF of 0.12 mg/L. Thus, it remains unclear whether penicillin is active against these borderline resistant *S. uberis* isolates.

Most pathogenic bacteria isolated from pigs in 2024 displayed similar frequencies of phenotypic resistance as in 2023 (1-year period) and 2019 (5-year period). However, six pathogen-drug combinations were associated with significantly increased resistance, whereas three were associated with significantly decreased resistance. The increased frequency of neomycin resistance in haemolytic *Escherichia coli* is worrisome because it is one of only a few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhoea. The increased frequency of gentamicin resistance in haemolytic *E. coli* is also worrisome because it is considered critically important for human medicine by the World Health Organization. It should also be noted that haemolytic *E. coli* displayed increased resistance to 3rd generation cephalosporins (cefpodoxime), from 4.7% in 2023 to 8.2% in 2024, although this change was nonsignificant.

9.1 Introduction

Surveillance of antimicrobial resistance in 2024 focused on pathogenic bacteria from mastitis in dairy cows and from various organs in pigs and included results obtained through antimicrobial susceptibility testing (AST).

9.2 Acute mastitis in dairy cows

9.2.1 Background

Milk samples for routine diagnosis of clinical mastitis in dairy cows are normally collected by and tested at the local veterinary clinics throughout Denmark. As part of the testing, veterinarians identify common pathogens using simple culture techniques and bacterial identification methods and sometimes perform AST to decide on therapeutic options. Normally, the samples and isolates are discarded after analysis. To establish a future-proof surveillance program for mastitis pathogens, we set up a diagnostic laboratory at Statens Serum Institut and asked veterinarians specialised in cattle to collect milk samples from dairy cows with acute mastitis. In total, 487 milk samples were collected by 15 veterinary clinics across Denmark during 2024. Isolates were identified to the genus or species level using MALDI-TOF. AST data were based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs were unavailable (https://www.vetssi.dk/).

9.2.2 Results

Among the 487 milk samples tested, 82.5% were positive for one or more pathogenic bacteria, while the remaining milk samples were interpreted as negative, either because of lack of growth or unspecific growth. In total, 462 isolates were recovered, of which 85.9% belonged to well-known mastitis pathogens: *Streptococcus uberis* (27.5%), *Escherichia coli* (20.1%), *Staphylococcus aureus* (13.4%), *Streptococcus dysgalactiae* (10.8%), coagulase-negative staphylococci (CoNS) (5.4%), *Trueperella pyogenes* (3.2%), *Streptococcus agalactiae* (2.8%) and *Klebsiella pneumoniae* (2.6%). In addition, we identified a number of bacterial species and yeasts found in less than 2% of the milk samples. Table 9.1 provides an overview of the identified pathogenic bacteria.

AST was performed on 376 bacterial isolates representing well-known mastitis pathogens (Table 9.1). *T. pyogenes* isolates were not tested due to their relatively slow growth and specific methodological requirements for AST. Table 9.2 shows the frequencies of resistant isolates in 2024.

Among streptococci, we observed that 19.7% of the *S. uberis* isolates and 9.1% of the *S. agalactiae* isolates displayed resistance to penicillin. It should be noted, however, that most of these isolates had a MIC value of 0.25mg/L, which is just above the tentative ECOFF for *S. uberis* isolates and the ECOFF for *S. agalactiae* (both 0.12 mg/L). Tetracycline resistance was observed in 19.7% of the *S. uberis* and 14.0% of the *S. dysgalactiae* isolates. Tetracycline resistance was also found in 81.8% of the *S. agalactiae* isolates, which is consistent with a high prevalence of tetracycline resistance genes in this bacterial species (Da Cuna et al. Nat Commun. 2014 Aug 4;5:4544. doi: 10.1038/ncomms5544). Moreover, 18.2% of the *S. agalactiae* isolates were resistant to erythromycin, while 9.1% were resistant to chloramphenicol and clindamycin.

The relatively high frequency of borderline resistance to penicillin in *S. uberis* is particularly noteworthy because beta-lactamase sensitive penicillins are the most commonly used drugs for treatment of adult cattle in Denmark (Table 4.1).

Among staphylococci, we found that 12.0% of the CoNS and 4.8% of the *S. aureus* isolates were resistant to penicillin.

We observed that 10.1-13.5% of the *E. coli* isolates were resistant to ampicillin, sulfamethoxazole, tetracycline and trimethoprim. A single *E. coli* isolate displayed an extended-spectrum β-lactamase (ESBL) phenotype and was resistant to multiple drugs, including ampicillin, cefotaxime, ceftazidime, chloramphenicol, ciprofloxacin, nalidixic acid, sulphamethoxazole, tetracycline and trimethoprim. Due to the ESBL phenotype (resistance to cefotaxime and ceftazidime), this isolate was further analysed with extra disk diffusion tests and subjected to whole genome sequencing, which confirmed the ESBL phenotype and showed that it belongs to multilocus sequence type 46 and carries $bla_{\text{CTX-M-15}}$.

Finally, 16.7% and 8.3% of the *K. pneumoniae* isolates were resistant to tetracycline and trimethoprim, respectively. In addition, 91.7% of the *K. pneumoniae* isolates were resistant to ampicillin, reflecting that this bacterial species is intrinsically resistant to penicillins.

The remaining pathogenic bacteria from acute mastitis in dairy cows displayed low frequencies of phenotypic resistance (0.0-4.7%).

Table 9.1 Pathogenic bacteria from acute mastitis in dairy cows, Denmark, 2024 DANMAP 2024

Taxon	Culture		AST
	n	%	n
Streptococcus uberis	127	27.5%	127
Escherichia coli	93	20.1%	89
Staphylococcus aureus	62	13.4%	62
Streptococcus dysgalactiae	50	10.8%	50
Coagulase-negative staphylococci	25	5.4%	25
Trueperella pyogenes	15	3.2%	ND
Streptococcus agalactiae	13	2.8%	11
Klebsiella pneumoniae	12	2.6%	12
Coagulase-negative staphylococci in mixed cultures	8	1.7%	ND
Lactococcus garviae	7	1.5%	ND
Enterococcus faecium	4	0.9%	ND
Lactococcus lactis	4	0.9%	ND
Pseudomonas spp.	4	0.9%	ND
Serratia liquefaciens	4	0.9%	ND
Streptococcus spp.	4	0.9%	ND
Enterococcus faecalis	3	0.6%	ND
Helcococcus ovis	3	0.6%	ND
Pantoea agglomerans	3	0.6%	ND
Corynebacterium bovis	2	0.4%	ND
Enterococcus cecorum	2	0.4%	ND
Streptococcus parauberis	2	0.4%	ND
Aerococcus viridans	1	0.2%	ND
Bacillus licheniformis	1	0.2%	ND
Enterococcus spp.	1	0.2%	ND
Helcococcus kunzii	1	0.2%	ND
Lysinibacillus sphaericus	1	0.2%	ND
Mannheimia spp.	1	0.2%	ND
Mycobacterium smegmatis	1	0.2%	ND
Pseudomonas aeruginosa	1	0.2%	ND
Raoultella planticola	1	0.2%	ND
Serratia plymuthica	1	0.2%	ND
Serratia rubidea	1	0.2%	ND
Serratia spp.	1	0.2%	ND
Streptococcus canis	1	0.2%	ND
Streptococcus equinus	1	0.2%	ND
Streptococcus gallolyticus	1	0.2%	ND

Isolates were identified to the genus or species level using MALDI-TOF
Abbreviations: AST, antimicrobial susceptibility testing

Table 9.2 Phenotypic antimicrobial resistance among pathogenic bacteria from acute mastitis in dairy cows, Denmark, 2024

DANMAP 2024

Antimicrobial agent	Ec (n=89)	Kp (n=12)	Sau (n=62)	CoNS (n=25)	Sag (n=11)	Sd (n=50)	Su (n=127)
	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
Amikacin	0.0%	0.0%*	ND	ND	ND	ND	ND
Ampicillin	13.5%	91.7%*	ND	ND	ND	ND	ND
Azithromycin	1.1%	ND	ND	ND	ND	ND	ND
Cefotaxime	1.1%	0.0%*	ND	ND	ND	ND	ND
Cefoxitin	ND	ND	0.0%	ND	ND	ND	ND
Ceftazidime	1.1%	0.0%*	ND	ND	ND	ND	ND
Chloramphenicol	1.1%	0.0%*	0.0%	4.0%	9.1%*	0.0%	0.0%
Clindamycin	ND	ND	0.0%	ND	9.1%*	0.0%	3.1%
Ciprofloxacin	1.1%	0.0%*	0.0%	ND	0.0%*	ND	ND
Colistin	0.0%	0.0%*	ND	ND	ND	ND	ND
Erythromycin	ND	ND	0.0%	4.0%	18.2%*	4.0%	4.7%
Fusidate	ND	ND	0.0%	ND	ND	ND	ND
Gentamicin	0.0%	0.0%*	0.0%	0.0%	0.0%*	ND	ND
Kanamycin	ND	ND	0.0%	ND	ND	ND	ND
Linezolid	ND	ND	0.0%	0.0%	0.0%*	ND	ND
Meropenem	0.0%	0.0%*	ND	ND	ND	ND	ND
Mupirocin	ND	ND	0.0%	ND	ND	ND	ND
Nalidixic acid	2.2%	ND	ND	ND	ND	ND	ND
Penicillin	ND	ND	4.8%	12.0%	9.1%*	ND	19.7%
Quinopristin/dalfopristin	ND	ND	0.0%	ND	ND	ND	ND
Rifampin	ND	ND	0.0%	0.0%	ND	ND	ND
Streptomycin	ND	ND	0.0%	ND	ND	ND	ND
Sufamethoxazole	12.4%	ND	ND	ND	ND	ND	ND
Tetracycline	10.1%	16.7%*	0.0%	0.0%	81.8%*	14.0%	19.7%
Tiamulin	ND	ND	0.0%	ND	ND	ND	ND
Tigecycline	0.0%	0.0%*	ND	ND	ND	ND	ND
Trimethoprim	11.2%	8.3%*	0.0%	ND	ND	ND	ND
Vancomycin	ND	ND	0.0%	0.0%	0.0%*	0.0%	0.0%

Data were based on epidemiological cut-offs (ECOFFs) or clinical breakpoints when ECOFFs were unavailable (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterices and should be interpreted with caution
Abbreviations: Ec, *Escherichia coli*; Kp, *Klebsiella pneumoniae*; Sau, *Staphylococcus aureus*; CoNS, coagulase-negative staphylococci; Sag, *Streptococcus agalactiae*; Sd, *Streptococcus dysgalactiae*; Su, *Streptococcus uberis*; R, resistant; ND, not determined

9.3 Pigs

9.3.1 Background

The Veterinary Laboratory, The Danish Agriculture and Food Council, performed AST of isolates belonging to *Actinobacillus pleuropneumoniae*, *Bordetella bronchiseptica*, haemolytic and non-haemolytic *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Staphylococcus hyicus* and *Streptococcus suis*. Table 9.3 shows the frequencies of resistant isolates in 2024, while all results from 2016-2024 can be found on DK-VET's homepage (https://www.vetssi.dk/). AST data were based on epidemiological cut-offs (ECOFFs) and clinical breakpoints when ECOFFs are unavailable (https://www.vetssi.dk/).

9.3.2 Results

Most pathogenic bacteria isolated from pigs in 2024 displayed similar frequencies of phenotypic resistance as in 2023 (1-year period) and 2019 (5-year period). However, six pathogen-drug combinations were associated with significantly increased resistance, whereas three were associated with significantly decreased resistance. Table 9.4 and Figure 9.1 show all significant changes in phenotypic resistance over a 1-year period (2024 vs. 2023) and a 5-year period (2024 vs. 2019).

Haemolytic *E. coli* from pigs displayed significantly increased resistance to amoxicillin-clavulanic acid (2024 vs. 2023 and 2024 vs. 2019), florfenicol (2024 vs. 2019), gentamicin (2024 vs. 2023 and 2024 vs. 2019), neomycin (2024 vs. 2019) and spectinomycin (2024 vs. 2019).

Table 9.3 Phenotypic antimicrobial resistance among pathogenic bacteria from pigs, Denmark, 2024 DANMAP 2024

Antimicrobial agent	Ap (n=78)	Bb (n=35)	H-Ec (n=246)	NH-Ec (n=177)	Kp (n=18)	Se (n=32)	Sh (n=7)	Ss (n=106)
	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)	R (%)
Amoxicillin	0.0%	ND	74.4%	78.0%	ND	84.4%	85.7%*	ND
Amoxicillin-clavulanic acid	ND	ND	22.4%	23.9%	11.1%*	6.3%	ND	ND
Cefpodoxime	ND	ND	8.2%	3.4%	ND	ND	ND	ND
Cefquinome	ND	ND	ND	ND	0.0%*	ND	ND	ND
Ceftiofur	0.0%	ND	ND	ND	ND	ND	ND	ND
Colistin	ND	ND	0.0%	0.0%	0.0%*	ND	ND	ND
Doxycyclin	0.0%	ND	74.0%	63.8%	33.3%*	81.3%	ND	34.9%
Enrofloxacin	1.3%	ND	13.8%	6.8%	ND	ND	0.0%*	0.0%
Florfenicol	0.0%	0.0%	23.6%	22.0%	16.7%*	12.5%	14.3%*	0.0%
Gentamicin	ND	ND	46.5%	14.8%	11.1%*	18.8%	ND	ND
Lincomycin	ND	ND	ND	ND	ND	ND	85.7%*	ND
Neomycin	ND	ND	60.0%	19.3%	16.7%*	ND	ND	ND
Penicillin	0.0%	ND	ND	ND	ND	ND	100.0%*	0.9%
Spectinomycin	ND	ND	66.9%	44.9%	ND	59.4%	ND	ND
Streptomycin	ND	ND	82.1%	79.1%	ND	90.6%	ND	ND
Tetracycline	ND	ND	75.5%	63.6%	33.3%*	81.3%	ND	ND
Tiamulin	0.0%	ND	ND	ND	ND	ND	28.6%*	ND
Tildipirosin	0.0%	0.0%	ND	ND	ND	ND	ND	ND
Tilmicosin	0.0%	ND	ND	ND	ND	ND	85.7%*	ND
Trimethoprim-sulfamethoxazole	0.0%	ND	62.6%	65.0%	50.0%*	43.8%	42.9%*	13.2%
Tulathromycin	3.8%	2.9%	ND	ND	ND	ND	ND	ND
Tylosin	ND	ND	ND	ND	ND	ND	85.7%*	ND

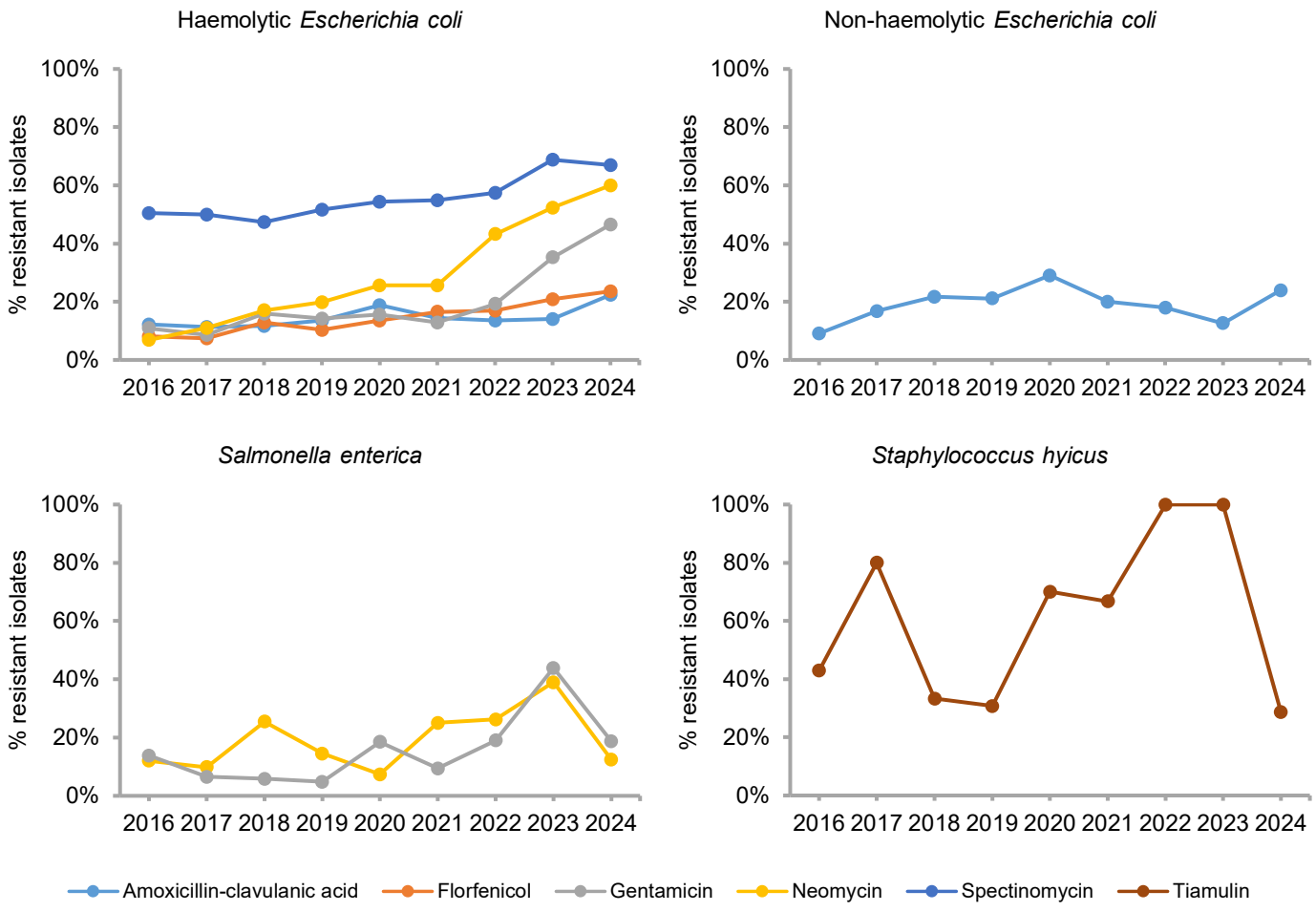
Data were based on epidemiological cut-offs (ECOFFs) or clinical breakpoints when ECOFFs were unavailable (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterices and should be interpreted with caution
Abbreviations: Ap, *Actinobacillus pleuropneumoniae*; Bb, *Bordetella bronchiseptica*; H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Kp, *Klebsiella pneumoniae*; Se, *Salmonella enterica*; Sh, *Staphylococcus hyicus*; Ss, *Streptococcus suis*; R, resistant; ND, not determined

Table 9.4 Statistically significant temporal changes in antimicrobial resistance phenotypes among pathogenic bacteria from pigs, Denmark, 2024 vs. 2023 and 2024 vs. 2019 DANMAP 2024

Patho-gen	Antimicrobial agent	2016 R (%)	2017 R (%)	2018 R (%)	2019 R (%)	2020 R (%)	2021 R (%)	2022 R (%)	2023 R (%)	2024 R (%)	2024 vs. 2023 Trend (p value)	2024 vs. 2019 Trend (p value)
H-Ec	Amoxicillin-clavulanic acid	12.1%	11.3%	11.7%	13.5%	18.8%	14.5%	13.5%	14.1%	22.4%	↗ (0.0134)	↗ (0.0103)
H-Ec	Florfenicol	8.1%	7.4%	12.9%	10.3%	13.6%	16.5%	16.9%	20.8%	23.6%	→ (0.4678)	↗ (0.0001)
H-Ec	Gentamicin	10.9%	8.6%	15.9%	14.3%	15.6%	12.8%	19.3%	35.2%	46.5%	↗ (0.0084)	↗ (0.0000)
H-Ec	Neomycin	6.9%	10.9%	17.0%	19.8%	25.6%	25.6%	43.2%	52.3%	60.0%	→ (0.0826)	↗ (0.0000)
H-Ec	Spectinomycin	50.5%	50.0%	47.3%	51.6%	54.4%	55.0%	57.4%	68.8%	66.9%	→ (0.6455)	↗ (0.0005)
NH-Ec	Amoxicillin-clavulanic acid	9.1%	16.8%	21.7%	21.1%	29.1%	20.0%	18.0%	12.6%	23.9%	↗ (0.0063)	→ (0.5357)
Se	Florfenicol	12.1%	9.8%	25.5%	14.5%	7.4%	25.0%	26.2%	39.0%	12.5%	↘ (0.0167)	→ (1.0000)
Se	Gentamicin	13.8%	6.6%	5.9%	4.8%	18.5%	9.4%	19.0%	43.9%	18.8%	↘ (0.0267)	→ (0.0579)
Sh	Tiamulin	42.9%*	80.0%*	33.3%*	30.8%*	70.0%*	66.7%*	100.0%*	100.0%*	28.6%*	↘ (0.0070)	→ (1.0000)

Antimicrobial resistance phenotypes that remained at the same level during 2023-2024 and 2019-2024 were excluded (https://www.vetssi.dk/)
Percentages based on small sample sizes (n<20) are indicated by asterices and should be interpreted with caution
Abbreviations: H-Ec, haemolytic *Escherichia coli*; NH-Ec, non-haemolytic *Escherichia coli*; Se, *Salmonella enterica*; Sh, *Staphylococcus hyicus*; R, resistant

Figure 9.1 Statistically significant temporal changes in antimicrobial resistance phenotypes among pathogenic bacteria from pigs, Denmark, 2024 vs. 2023 and 2024 vs. 2019 DANMAP 2024



The percentages of tiamulin-resistant *Staphylococcus hyicus* isolates are based on small sample sizes (n<20) and should therefore be interpreted with caution

The increased frequency of neomycin resistance in haemolytic *E. coli* (60.0%) is worrisome because it is one of only a few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhoea. Furthermore, haemolytic *E. coli* also displayed medium to high frequencies of resistance to the other first-choice drugs, including amoxicillin-clavulanic acid (22.4%), spectinomycin (66.9%), trimethoprim-sulfamethoxazole (62.6%) and streptomycin (82.1%). *E. coli* isolates from 2023 and 2024 were not tested for susceptibility to the remaining first-choice drug ampicillin, but it should be noted that we observed a high frequency of ampicillin resistance in haemolytic *E. coli* from 2022 (60.9%) (DANMAP 2022). The rapid increase in neomycin resistance coincided with increased use of neomycin in weaners (DANMAP 2023) following two recent decisions to restrict the use of alternative drugs in pigs: 1) the Danish Yellow Card initiative to reduce the use of colistin in 2016 and 2) the European Union-wide ban of medicinal zinc in 2022.

The increasing frequency of gentamicin resistance in haemolytic *E. coli* (46.5%) is also worrisome because it is considered critically important for human medicine by the World Health Organization. We have previously shown that most of the gentamicin resistance genes found in haemolytic *E. coli* also confer resistance to apramycin, which is increasingly used in weaners (DANMAP 2023). In contrast, the use of gentamicin in pigs is negligible and cannot explain the rapidly increasing resistance to this drug (DANMAP 2023). Together, these observations suggest a causal relationship between increased use of apramycin and increased resistance to gentamicin in haemolytic *E. coli*.

It should also be noted that haemolytic *E. coli* displayed increased resistance to 3rd generation cephalosporins (cefpodoxime), from 4.7% in 2023 to 8.2% in 2024, although this change was nonsignificant.

Non-haemolytic *E. coli* displayed significantly increased resistance to amoxicillin-clavulanic acid (2024 vs. 2023). In addition, *S. enterica* displayed significantly decreased resistance to florfenicol (2024 vs. 2023) and gentamicin (2024 vs. 2023), while *S. hyicus* displayed significantly decreased resistance to tiamulin (2024 vs. 2023). However, the results for *S. hyicus* are based on <20 isolates and should be interpreted with caution.

9.4 Conclusions and perspectives

Pathogenic bacteria from acute mastitis in dairy cows generally displayed low frequencies of phenotypic resistance.

The increasing frequency of neomycin, gentamicin and cefpodoxime resistance in haemolytic *E. coli* from pigs is worrisome and should be monitored closely in the coming years.

Interpretation of resistance to antimicrobial agents of veterinary importance are often based on tentative ECOFFs and ECOFFs as animal-specific clinical breakpoints for many drugs are currently lacking (e.g., penicillin resistance in *S. uberis* and neomycin resistance in *E. coli*). ECOFFs are based on microbiological studies and do not necessarily indicate whether a drug will be clinically active. Future studies should therefore seek to establish clinical breakpoints in animals by considering what happens to the drug within a specific animal and body site (pharmacokinetics).

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