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SUMMARY DANMAP 2023

Use of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark

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1. Introduction

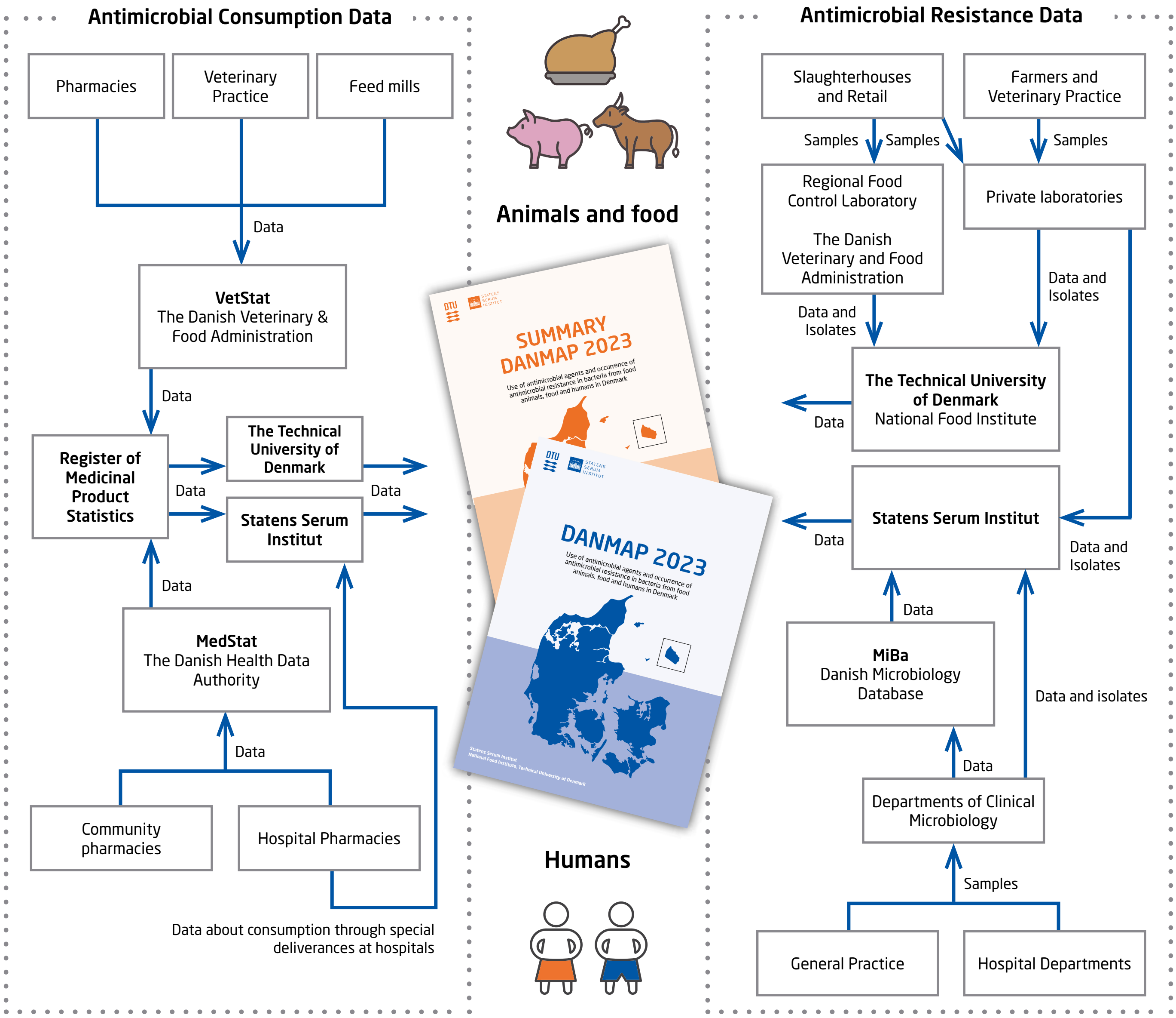
The Danish integrated Antimicrobial Resistance Monitoring and Research Programme (DANMAP) is a governmentally financed surveillance programme that collects and presents antimicrobial use (AMU) and antimicrobial resistance (AMR) data in humans and animals in Denmark. The programme was established in 1995 and is based on clinical data from humans and clinical and survey data from animals.

DANMAP is based on the concept of 'One Health' – a collaborative, multidisciplinary initiative across human and animal sectors. The programme is managed by a collaborating team from the National Food Institute at the Technical University of Denmark, and the National AMR Reference Laboratory at Statens Serum Institut. The work is supported by internal and external technical experts and receives contributions from all Danish Clinical Microbiology departments, the Danish Veterinary and Food Administration and the Danish Health Data Authority.

This summary report 2023 complements the more comprehensive DANMAP report 2023. It features the most important findings from the four main areas under surveillance and includes new perspectives on One Health and antimicrobial resistance. The summary aims to inform healthcare professionals, scientists, decision-makers, and everybody with an interest in antimicrobial use and resistance and the monitoring of these.

More information about the surveillance programme can be found at www.danmap.org, where you also find the full DANMAP 2023 with further data and analyses, including textboxes on topics of special interest this year.

Figure 1.1 Organisation of DANMAP regarding data and data flow



2. Antimicrobial consumption in animals

The surveillance of antimicrobial consumption in animals is based on sales data from pharmacies, veterinary practices and feed mills. In the following sections, antimicrobial consumption data are presented at national level as well as at different animal species levels.

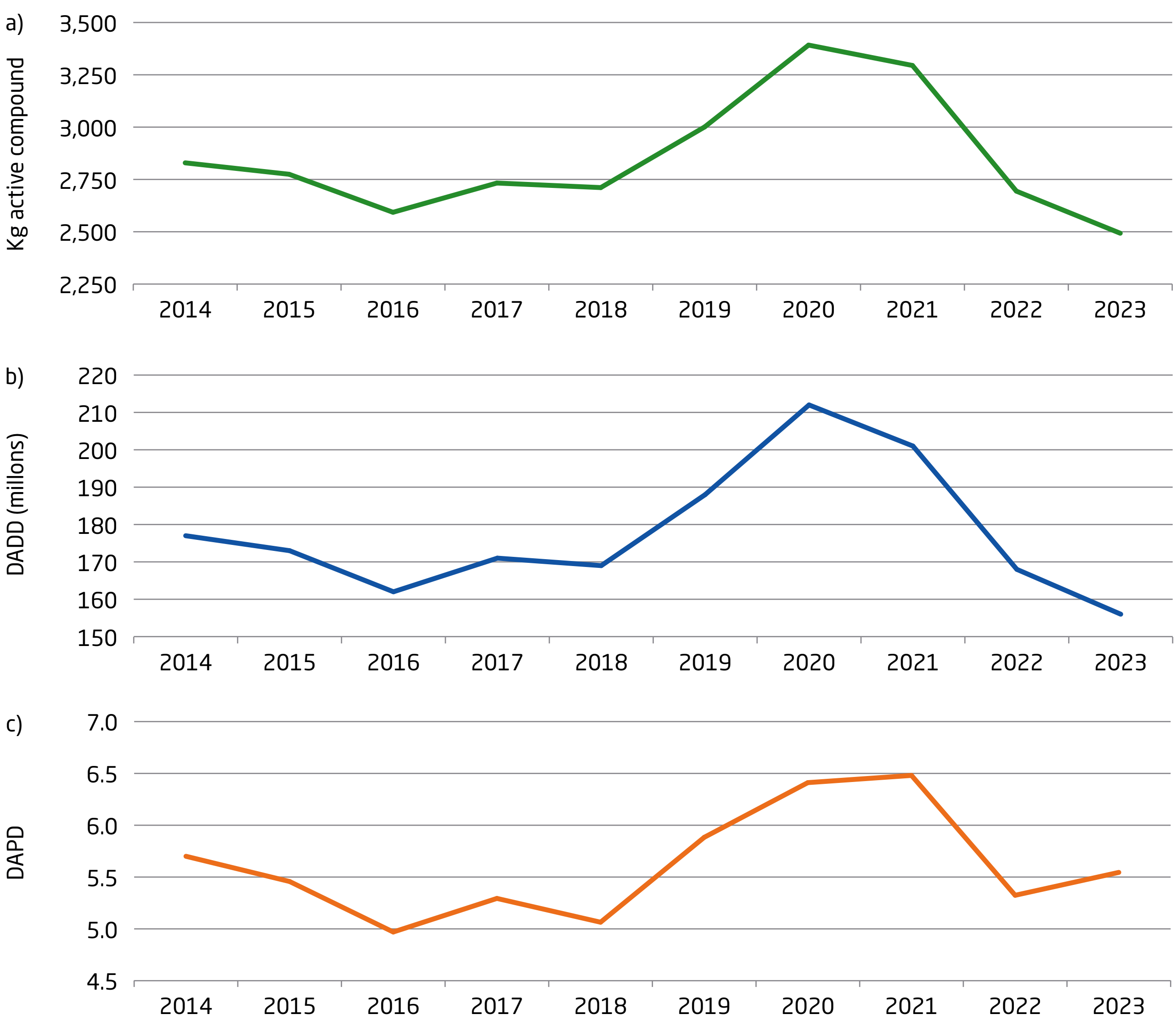
Metrics for measuring antimicrobial consumption in animals

Kg active compound: Provides an overall crude comparison of antimicrobial consumption in the veterinary and human sectors. Importantly, it does not account for changes in population sizes, changes in usage patterns or how potent the compounds are, (Figure 2.1 a.).

DADD (Defined animal daily dose): The assumed average maintenance dose per day for a drug used for its main indication in the appropriate animal species, (Figure 2.1 b.).

DAPD (DADD per 1,000 animals per day): This metric takes into account differences in body-mass and lifespan, (Figure 2.1 c.). It provides an estimate of the proportion of animals treated daily with a particular antimicrobial active compound. For example, 10 DAPDs indicate that an estimated 1% of the given population, on average, receives a certain treatment on a given day (see DANMAP 2023, Materials and Methods).

Figure 2.1 Oral consumption of the macrolide tilmicosin in weaners measured as kg active compound, DADD (millions) and DAPD, Denmark, 2014-2023

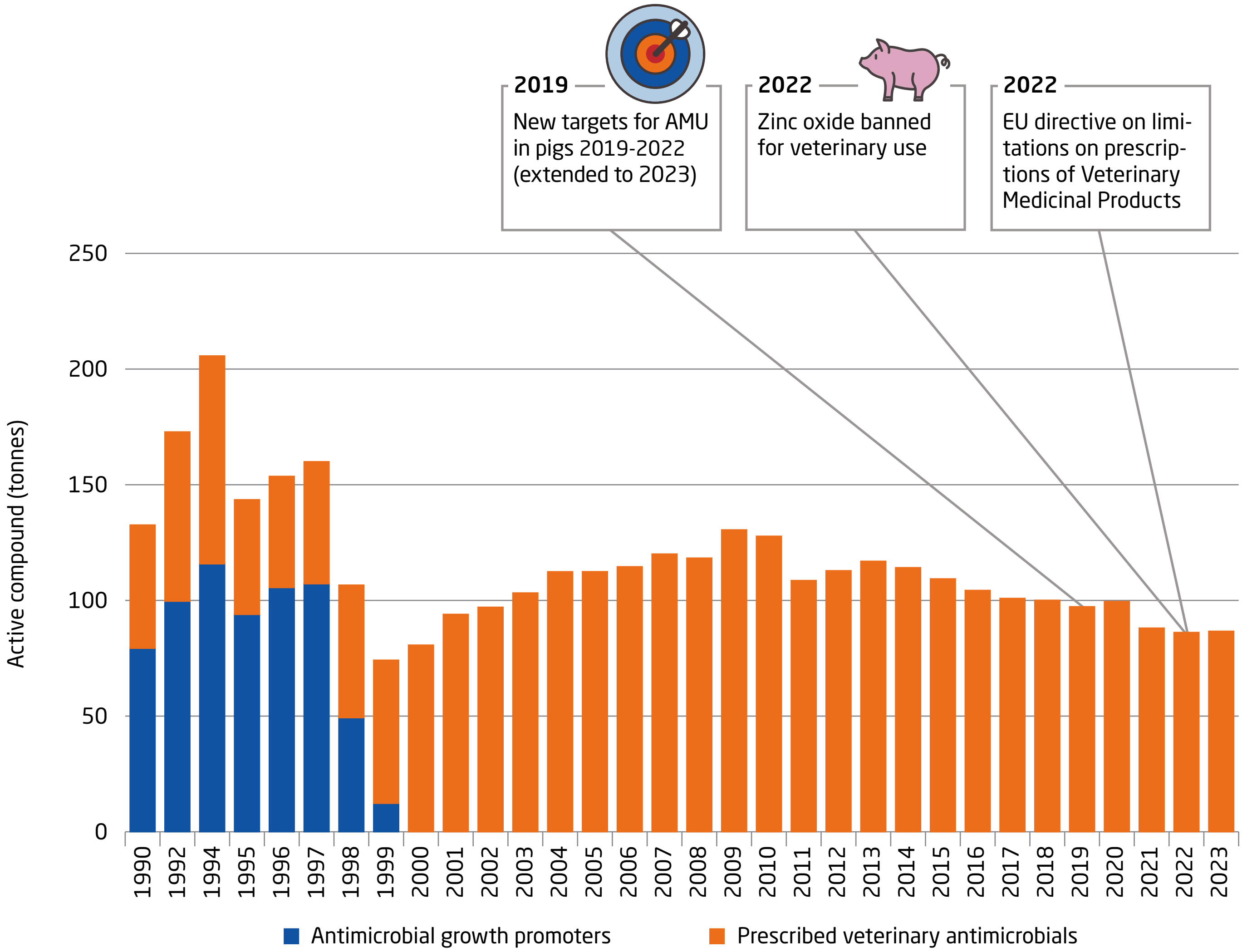


Since 2001, all medicines prescribed for use in animals have been recorded in the national database, VetStat, the database collecting consumption data on veterinary prescription medicines in Denmark.

Since the days when DANMAP was established, many initiatives have been taken to reduce antimicrobial consumption in both animals and humans, (Chapter 9. Timeline). These include discontinued use of antimicrobial agents for growth promotion, voluntary bans on the use of cephalosporins in pig and cattle production, regulatory legislation regarding therapeutic use and the Yellow Card Initiative. The initiatives have all had a marked effect on the antimicrobial consumption in animals, especially the consumption in pigs. Figure 2.2 shows the overall antimicrobial consumption in animals, along with recent important initiatives.

In addition, over time, antimicrobial consumption in animals has been affected, not only by the risk management measures established to reduce use, but also by changes in animal production, foremost increases in pig production.

Figure 2.2 Antimicrobial consumption in animals and recent important initiatives to reduce antimicrobial use in animals, Denmark, 1990-2023



The Danish One Health AMR Strategy
In 2017, the Ministry of Food, Agriculture and Fisheries and the Danish Ministry of Health launched a joint One Health strategy on tackling antimicrobial resistance. The aim of the One Health strategy was to provide a framework for continued strong and coordinated efforts across sectors to combat resistance. A former One Health AMR strategy from 2010 had established a cross-sectoral coordination mechanism, the National Antibiotic Council (discontinued in 2019), which was tasked to oversee a broad range of initiatives, e.g. improved microbiological diagnostics, extension of the surveillance programmes, establishment of digital tools and databases and, finally, development of guidelines for mitigation of spread of resistant bacteria as for treatment for both animal and human sectors. The aim of the new strategy was to strengthen the efforts and to focus on antimicrobial consumption by setting specific targets.

The Danish Veterinary and Food Administration's Action Plans against antimicrobial resistance
Together with the Danish One Health strategy, the Danish Veterinary and Food Administration launched a National Action Plan against antibiotic resistance in animals and food in 2017. The aim of the National Action Plan was to implement the One Health strategy within the veterinary and food producing sectors. It included measurable goals to reduce veterinary antibiotic consumption, including a 15% reduction target for the overall use of antibiotics for pigs by 2018 (later extended to 2019).

A second National Action Plan was launched in 2021, which continued the visions and goals of the first National Action Plan and included new reduction targets for pigs: a 2% incremental reduction of antibiotic use from 2019 to 2022 totaling an 8% reduction by 2022 (compared to the consumption level in 2018). As this 8% target was not met by the end of 2022, the target was initially extended to 2023 and, as of 2024, has been further extended to be achieved by 2027.

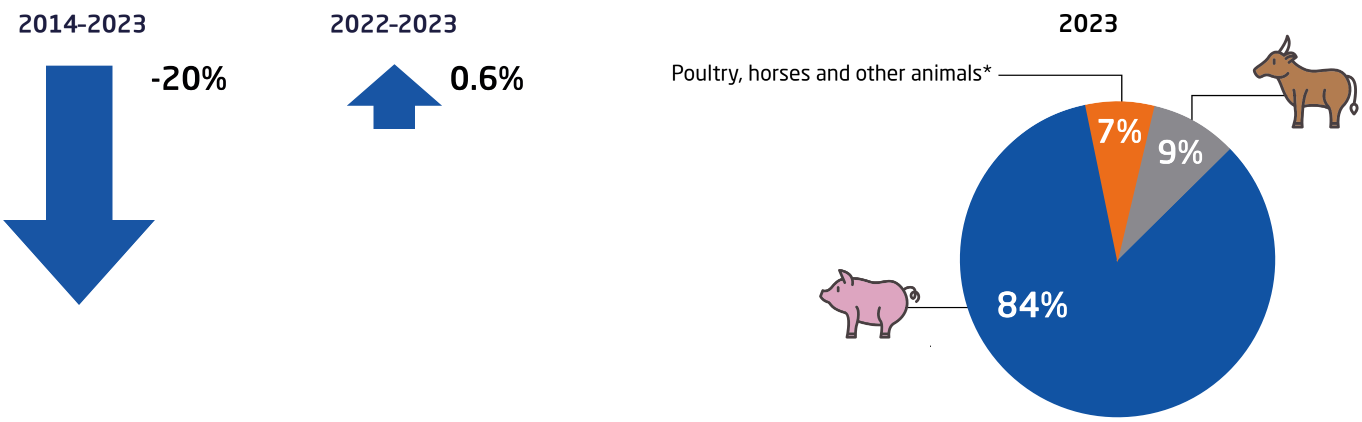
Overall antimicrobial consumption in animals

In 2023, the total consumption of antimicrobials in animals amounted to 86.7 tonnes of active compounds, (Figure 2.2). The consumption in animals accounted for approximately two thirds of all antimicrobials prescribed in the human and animal sectors in Denmark.

Since 2014, the overall usage of active compounds of antimicrobials in animals has decreased every year, except in 2020, (Figure 2.2). The total consumption was 20% (11.12 tonnes) lower in 2023 compared to 2014 and almost unchanged, <1% (523.18 kg) higher than in 2022, (Figure 2.3). In 2023, the consumption in cattle, and aquaculture was lower, while the consumption in pigs and companion animals was higher, (Figure 2.4).

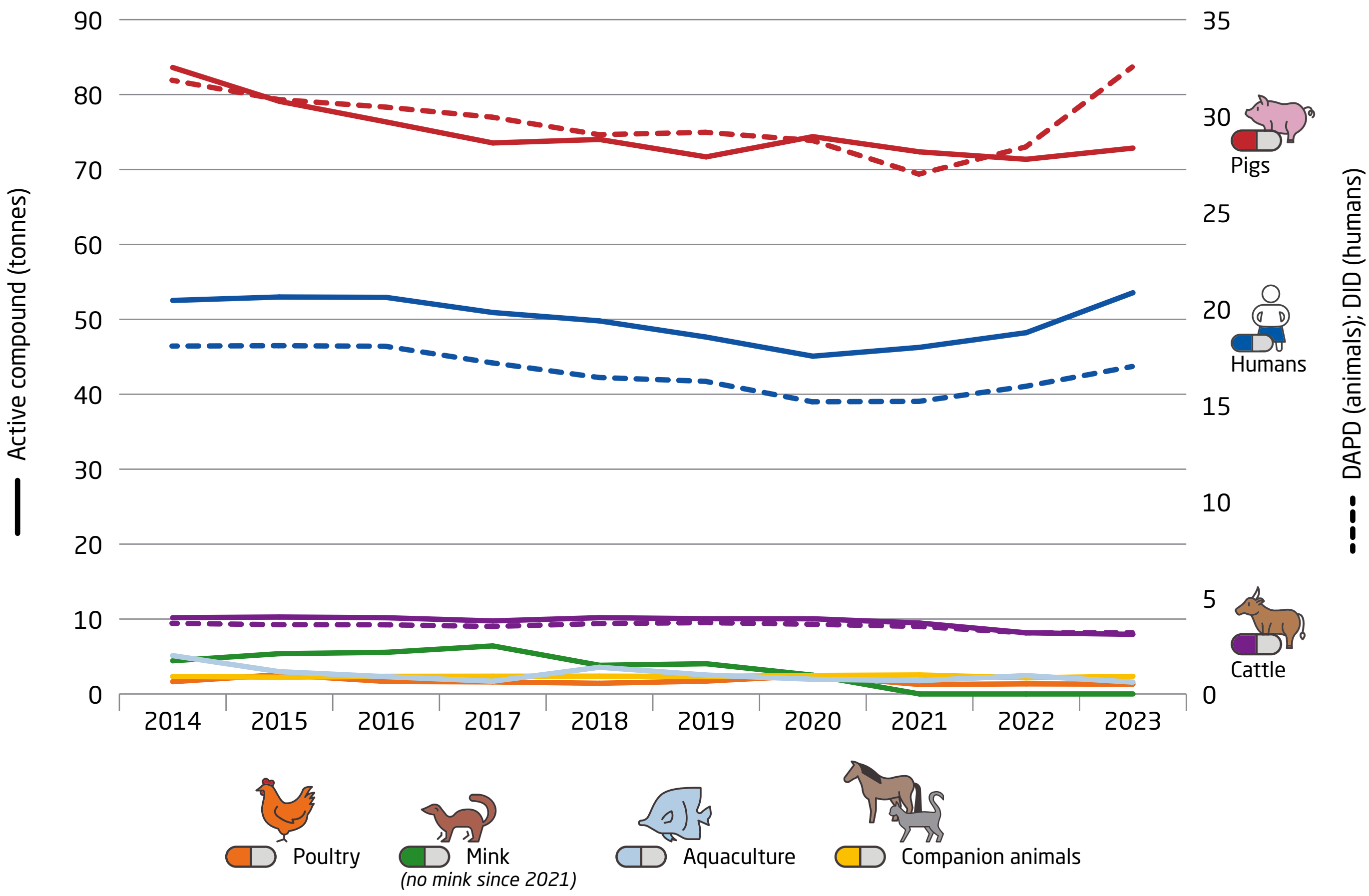
The pig sector is the main driver of veterinary antimicrobial consumption in Denmark. Therefore, any major changes in usage patterns in the pig sector will also have a major impact on the overall antimicrobial consumption in animals. In 2023, approximately 84% of veterinary prescribed antimicrobials were used for pigs, amounting to 72.85 tonnes of active compounds, (Figure 2.3).

Figure 2.3 Changes in overall antimicrobial consumption and its distribution (%) by main animal species, Denmark



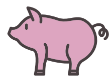
* Other animals include aquaculture and pets

Figure 2.4 Total antimicrobial consumption of active compounds (kg) by animal species and humans, Denmark, 2014-2023



Small amounts of kg active compound were used by unspecified animal species in 2023

Antimicrobial consumption in pigs



Animal definitions

Antimicrobial consumption varies between different age groups in the pig population, with weaners accounting for a high share. DANMAP reports both jointly and separately for three age groups: sows/piglets, weaners and finishers.

Sow: Any breeding female pig on a farm.

Piglet: A newborn pig is called a piglet from birth until it is permanently separated (weaned) from the sow at 3-4 weeks of age. The weight of a piglet at weaning is approximately 7 kg.

Weaner: A pig of 7-30 kg live weight after it has been weaned (dry diet and water only).

Finisher: A pig of 30-100 kg live weight, after the weaner stage until time of slaughter.

Changes in antimicrobial consumption for pigs driven by the Yellow Card initiatives

Several initiatives have aimed at reducing tetracycline usage, since this may select for livestock-associated methicillin resistant *Staphylococcus aureus* (LA-MRSA). Similarly, other initiatives have aimed at phasing out critically important antimicrobials such as fluoroquinolones, cephalosporins and colistin. For a more historical view of the changes in AMU in the different age groups of pigs, please refer to DANMAP 2023.

The overall consumption of antimicrobials in pigs increased from 2022 to 2023 by 1,495 kg of active compounds, (Figure 2.4 and 2.5). Although the consumption of antimicrobials has decreased during the period from 2019 to 2022, Goal 1: To achieve a reduction of 2% per year in the use of antimicrobials in pigs has not been fulfilled by 2023, (Figure 2.5).

The Yellow Card Initiative

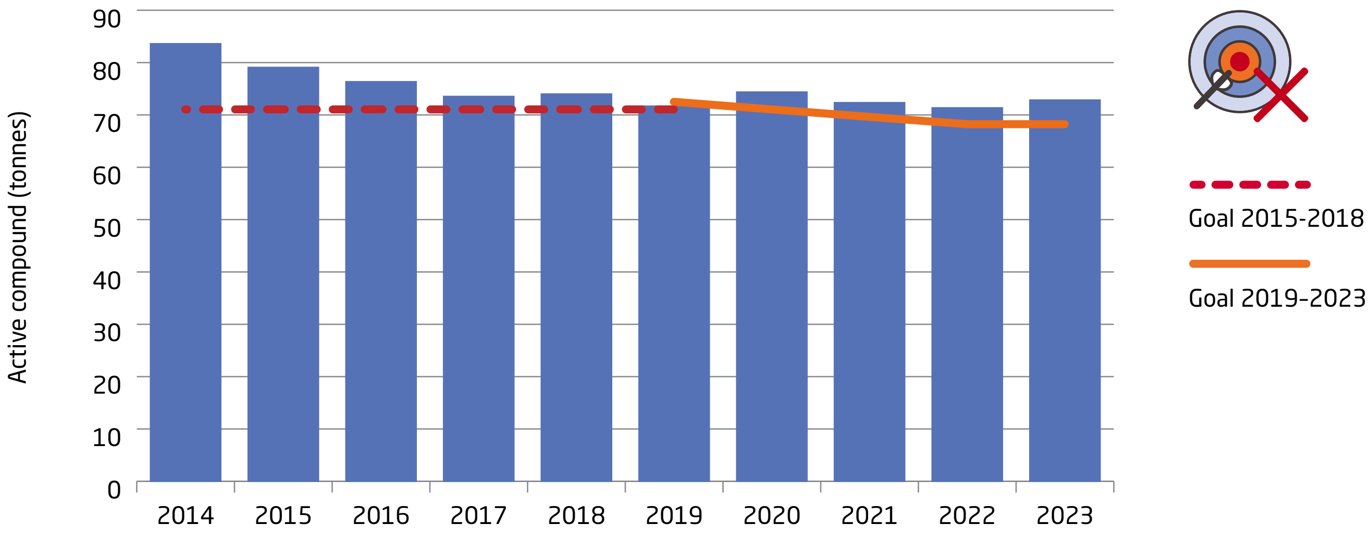
In 2010, the Danish Veterinary and Food Administration (DVFA) introduced the Yellow Card initiative to reduce the use of antimicrobials in Danish pigs. The initiative targets farms with high consumption of antimicrobials and works as an incentive for pig producers to contribute to the goal of reducing AMU.

The initiative is based on monitoring AMU on detailed levels on each farm. If the average antimicrobial consumption in a holding, within a nine-month period, exceeds the given threshold levels, (which is an individual threshold level based on the production size of the holding) the DVFA may issue an order or injunction (a yellow card) compelling the owner of the holding to reduce the antimicrobial consumption below the maximum limits within nine months of the issuance of the injunction.

In 2016, the initiative was developed further and multiplication factors were added to adjust the use of specific antimicrobial agents. Multiplication factors were determined by the DVFA and are used as risk mitigation tools for each class of antimicrobials. Fluoroquinolones and cephalosporins, which are classified as critically important for the treatment of humans, have been given the highest multiplication factor of ten. Tetracyclines have been given the multiplication factor of 1.5 to promote further reduction in tetracycline use for pigs. Furthermore, colistin has also been given a multiplication factor of ten as a precautionary measure.

The differentiated Yellow Card has proven to be an efficient tool to reduce overall antimicrobial use in pig herds and to discourage use of certain critically important antimicrobials. For more information see DANMAP 2010 or www.fvst.dk.

Figure 2.5 Antimicrobial consumption in pigs in relation to national goals for reducing use of antibiotics in pigs, Denmark, 2014-2023

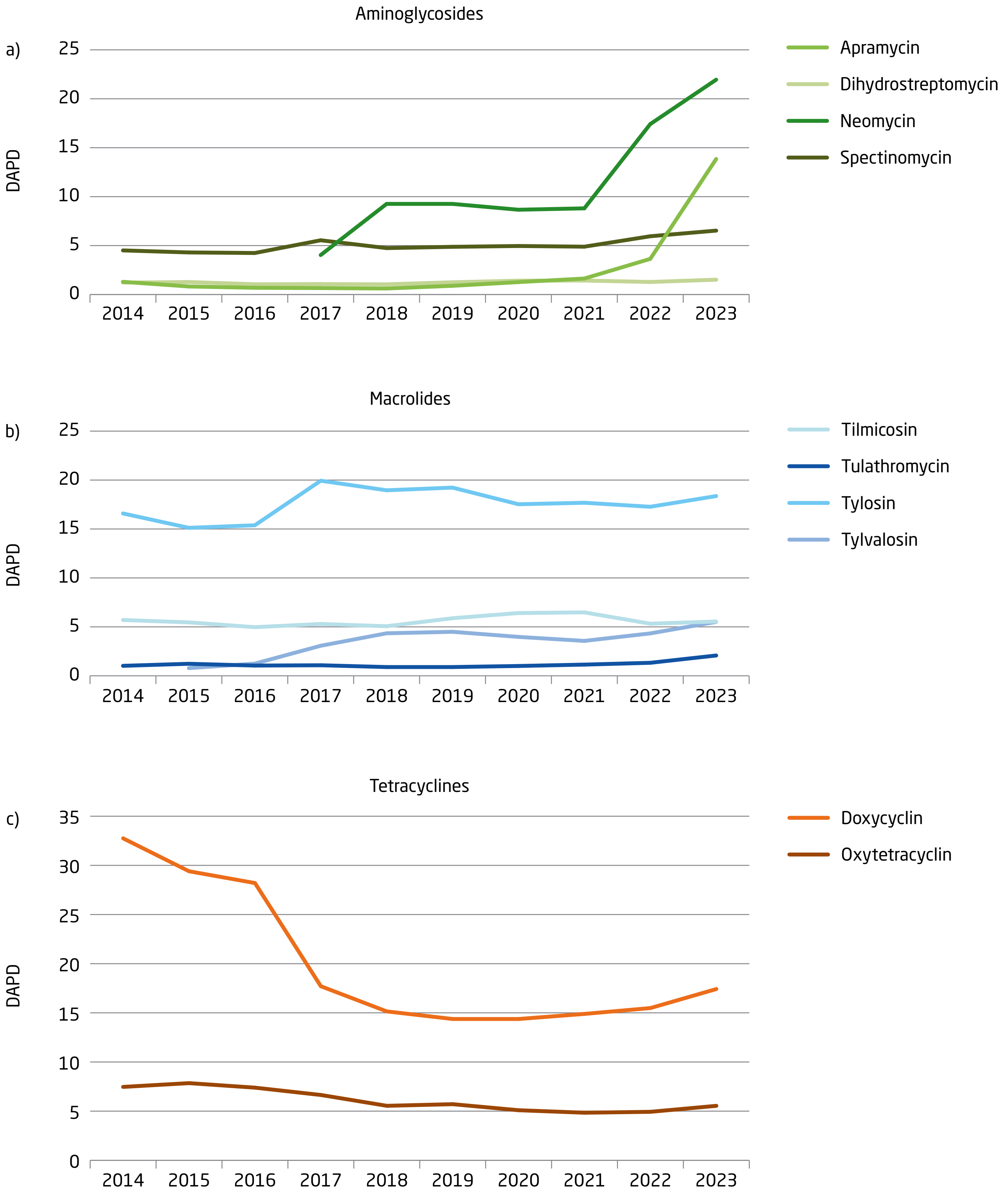


Measured in treatment proportion (DAPD), which includes adjustments for changes in production, an estimated 3.2% (32.1 DAPD) of all pigs received treatment with an antimicrobial per day in 2023. In 2010, when the Yellow Card initiative was introduced, approximately 3.6% of all pigs received treatment per day. The treatment proportion is much higher in weaners than in the other age groups. Thus, on a given day in 2023, approximately 1-2% of the sows and piglets and finishers and 11.9% of the weaners received treatment with an antimicrobial.

For weaners, the DAPD increased by 14.9% from 2022 to 2023, (Figure 2.6). The increase was primarily driven by increased consumption of aminoglycosides – specifically, apramycin and neomycin, (Figure 2.6 a.). The DAPD also increased for macrolides and tetracyclines, (Figure 2.6 b. and c.). This coincides with the EU-wide withdrawal of medical zinc oxide in pigs by the European Commission, effective from June 2022. Medical zinc oxide was commonly prescribed to newly weaned pigs to prevent or treat diarrhea.

In addition, the previous ceased use of colistin, and the implementation of Order 2019/6 on veterinary medicinal products applied since January 2022 could have affected antimicrobial use. Lastly, a change in the production of pigs, i.e. the increase in the number of 30 kg pigs exported and the decrease in the number of pigs slaughtered in Denmark may have affected the levels of antimicrobial use observed.

Figure 2.6 Consumption (DAPD) of aminoglycosides, macrolides and tetracyclines at active compound level in weaners, Denmark, 2014-2023

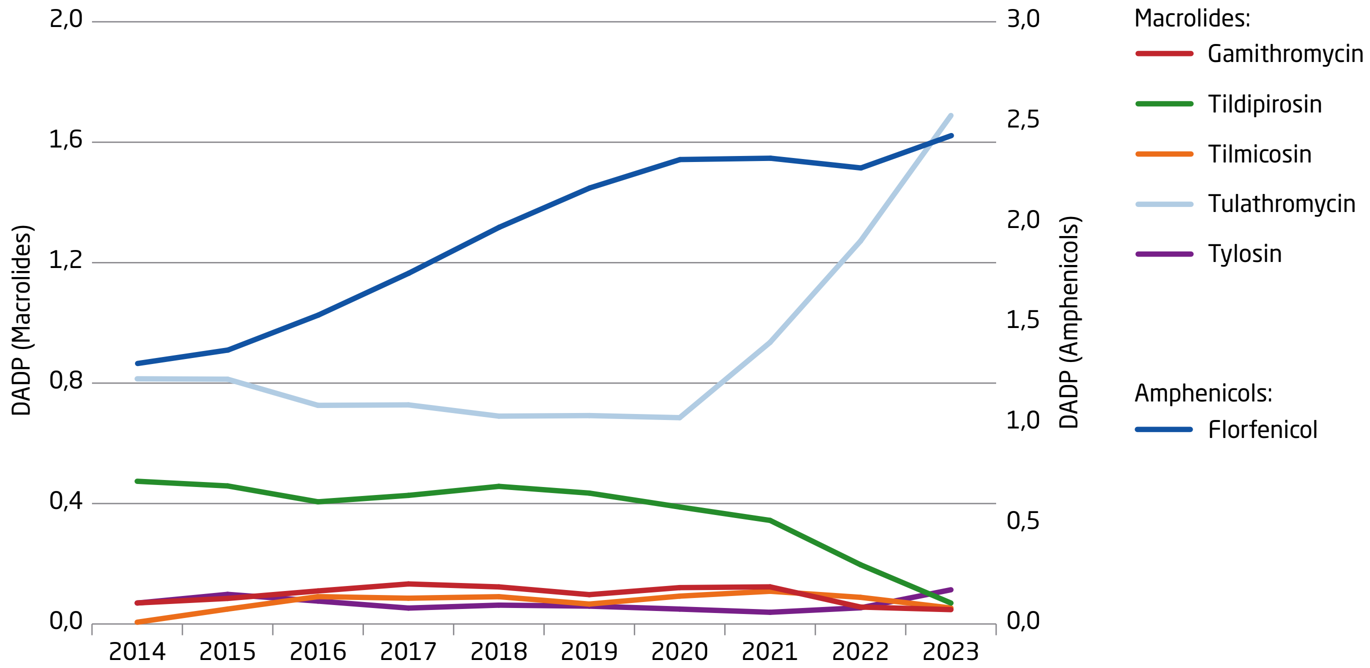


Antimicrobial consumption in cattle

In 2023, a total of 7,961.2 kg was prescribed for **cattle** (Figure 2.4), of which approximately 67.4% was used for treating cattle older than 12 months, including 457.6 kg used for intramammary treatment. An overall decreasing trend has been observed over the last decade and in 2023 the use in cattle was 21.8% lower than in 2014 and 2.5% lower than in 2022. No fluoroquinolones or 3rd or 4th generation cephalosporins were registered for use in cattle.

Measured in DAPD, consumption in cattle <1 year increased by 4.9%. The increase was mostly driven by increased consumption of amphenicols and macrolides, (Figure 2.7).

Figure 2.7 Consumption (DAPD) of amphenicols and macrolides at active compound level in cattle <1 year, Denmark, 2014-2023



Antimicrobial consumption in food animals

Antimicrobial consumption in Danish **poultry** is generally low. The statistics will be markedly affected by disease outbreaks in just a few farms. In 2023, usage increased by 3.0% compared to the usage in 2022, (Figure 2.4). For the past decade, cephalosporins have not been used in the poultry industry, and the use of fluoroquinolones has been close to zero.

Antimicrobial consumption in **aquaculture** varies considerably with water temperatures, because bacterial diseases are more likely to occur when temperatures are high. The AMU in 2023 was 1,581.9 kg, which was 35.5% lower than in 2022, (Figure 2.4). Mainly three compounds are used to treat bacterial infections in aquaculture: 58.9% of sulfonamides and trimethoprim, 33.2% of `other quinolones´ (oxolinic acid), and 7.9% of amphenicols (florfenicol).

Antimicrobial consumption in companion animals

Data on antimicrobial consumption in **companion animals** is less detailed than for the food-producing animals because registering of species is not mandatory. The consumption for companion animals was estimated to be 2,353.5 kg in 2023, which was the same as in 2014 and 9.0% higher than in 2022. More than half of all cephalosporins, all 3rd and 4th generation cephalosporins, as well as close to all fluoroquinolones prescribed for veterinary use, were prescribed for companion animals.

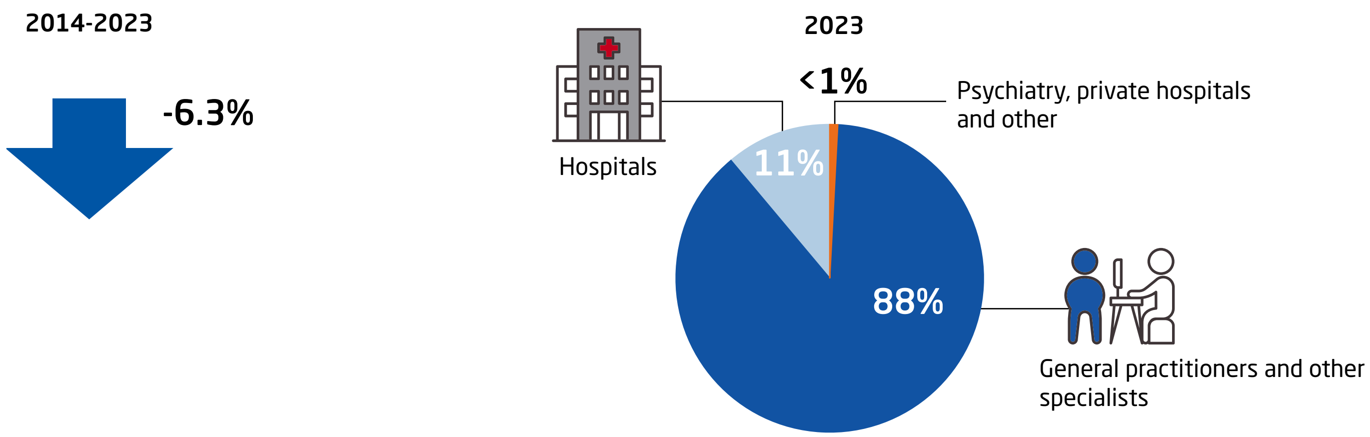
3. Antimicrobial consumption in humans

Surveillance of antimicrobial consumption in humans is based on sales data from all public and private healthcare providers in Denmark. In the following sections, antimicrobial consumption data are presented at national level as well as at health care sector level, i. e. primary health care and hospital care.

Antimicrobials in Denmark

Antimicrobial consumption in Denmark was 16.54 DID in 2023, which is -6.3% lower than consumption in 2014 (17.64 DID) and 6.6% higher than in 2022 (15.51 DID). Changes in the total consumption were driven by changes in antimicrobial prescribing in primary care, which accounts for the biggest share of antibiotics used in Denmark, Figure 3.1. In 2023, the consumption in primary care surpassed the prepandemic year 2019.

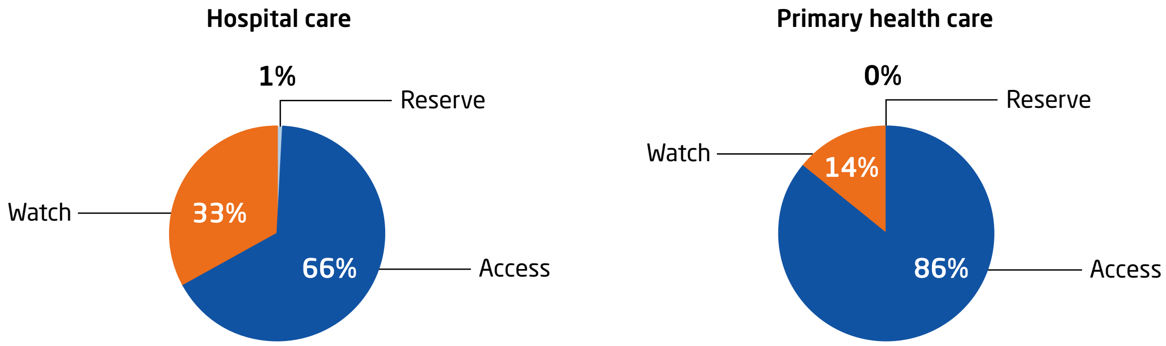
Figure 3.1 Change in total antimicrobial consumption and distribution by healthcare providers, Denmark, 2014-2023



AWaRe classification of antimicrobials in Denmark, 2023

The World Health Organization (WHO) has developed the AWaRe classification system as a tool to assist antibiotic stewardship and to reduce antimicrobial resistance. Antibiotics are classified into three groups to emphasise the importance of their appropriate use:

- **Access:** Antibiotics used to treat common susceptible pathogens with lower resistance potential than antibiotics in the other groups. 60% of total antimicrobial consumption should consist of Access agents.
- **Watch:** Antibiotics that have higher resistance potential, including most of the highest priority agents. These antibiotics should be prioritised as key targets of stewardship programs and monitoring.
- **Reserve:** Antibiotics reserved for treatment of confirmed or suspected infections due to multidrug resistant organisms. These antibiotics should be considered as "last resort" options.

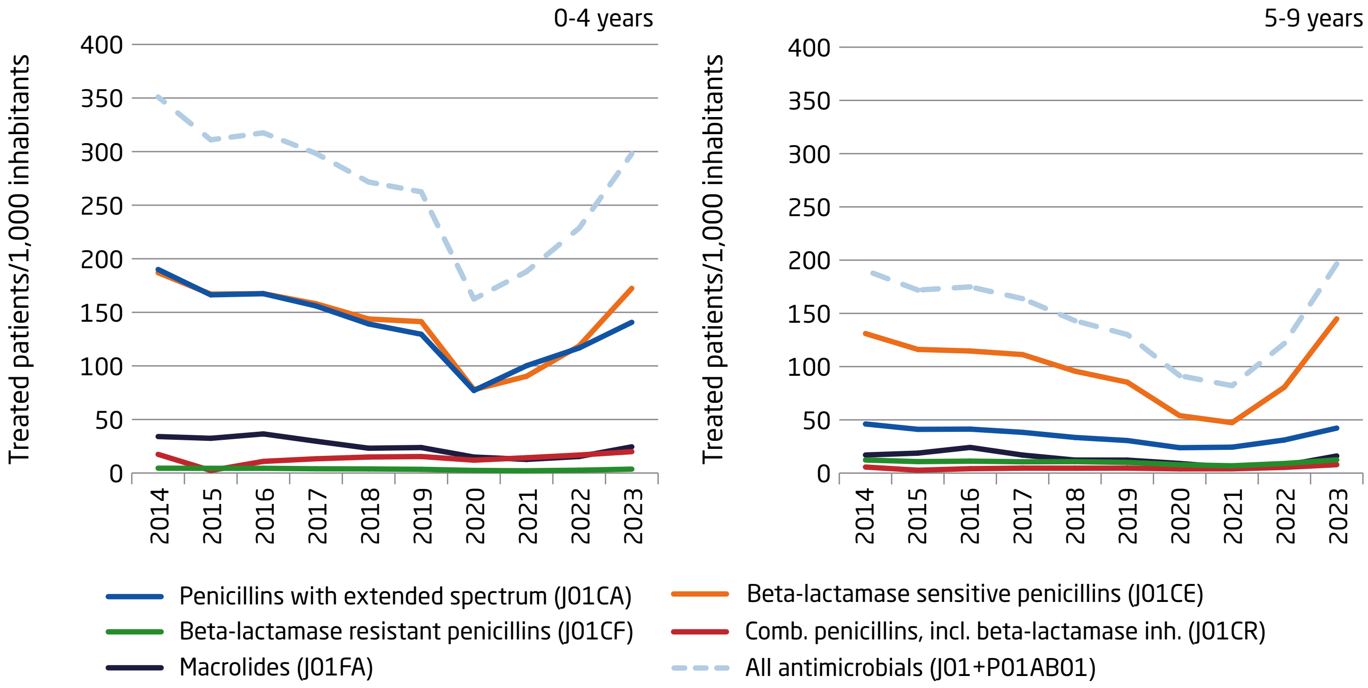


WHO Access, Watch, Reserve (AWaRe) classification of antibiotics for evaluation and monitoring of use, 2017. Geneva: World Health Organization; updated 2023 (WHO-MHP-HPS-EML-2023.04)

Antimicrobials for children

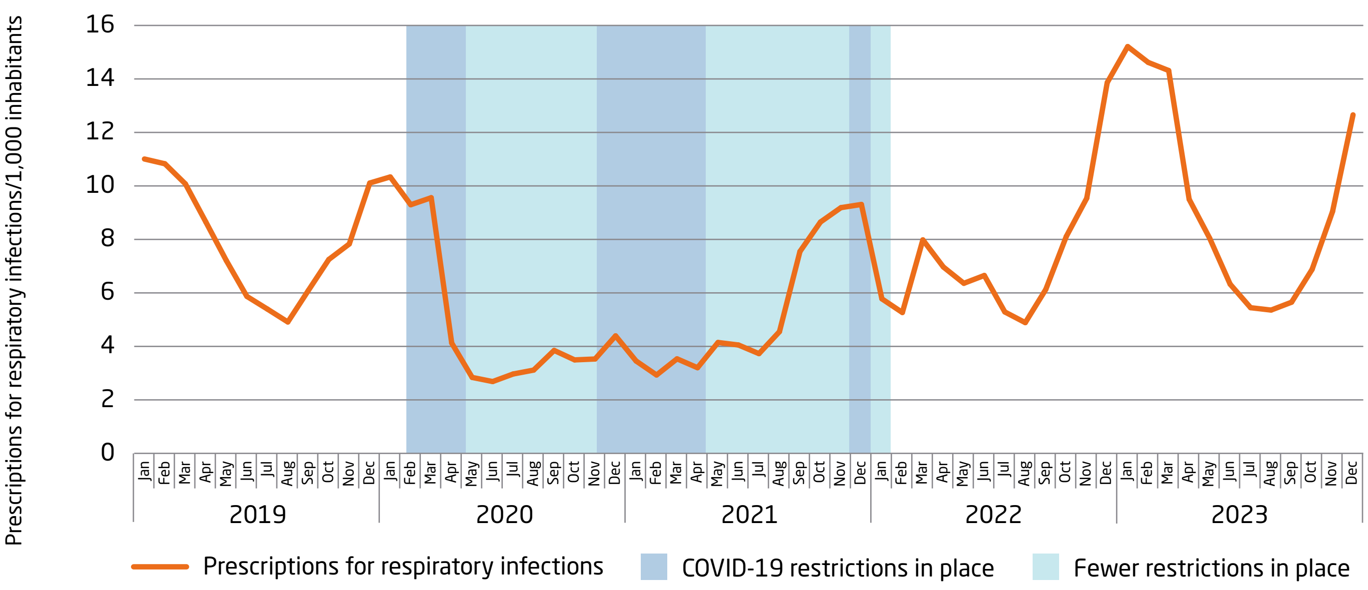
Antimicrobials prescribed to treat children in primary health care increased markedly in the recent two years post COVID-19 pandemic, (Figure 3.2). Among the 0-4 year olds, 298 per 1,000 inhabitants were treated with antimicrobials in 2023, compared to 229 per 1,000 inhabitants in 2022. Among the 5-9 year olds, 197 per 1,000 inhabitants were treated in 2023 compared to 122 per 1,000 inhabitants in 2022.

Figure 3.2 Consumption of main antimicrobial agents by children, treated patients/1,000 inhabitants, primary health care, Denmark, 2014-2023



The tendency was driven by increased prescribing of beta-lactamase sensitive penicillins to treat respiratory infections, compared to not only the pandemic years of 2020-2021 but also to the winter seasons two years before, (Figure 3.3).

Figure 3.3 Monthly antimicrobial prescriptions indicated for treatment of respiratory tract infections in primary health care, prescriptions per 1,000 inhabitants, Denmark, 2019-2023



Antimicrobials for elderly inhabitants

Antimicrobial consumption for elderly inhabitants (65 years and above) living at care homes received 90% more antimicrobials than elderly inhabitants living in their own homes in 2023, (Figure 3.4). Higher numbers in the treatment of urinary tract infections is the main cause of the difference observed in the two populations of elderly inhabitants, (Figure 3.5). However, a continuous decrease in the consumption for elderly inhabitants living at long term care facilities was observed from 2017-2023 due to several initiatives promoting more prudent prescribing of antimicrobials.

Figure 3.4 Consumption of antimicrobials (J01 and P01AB01) in primary health care for inhabitants ≥65 years, Denmark, 2017-2023

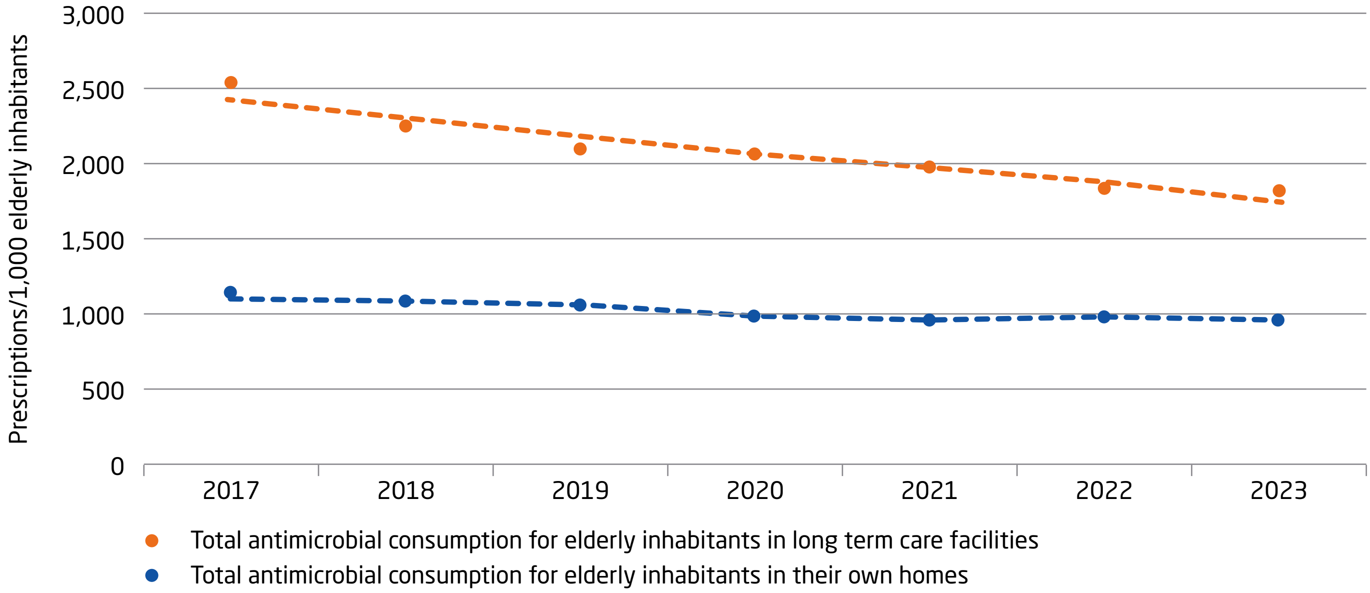
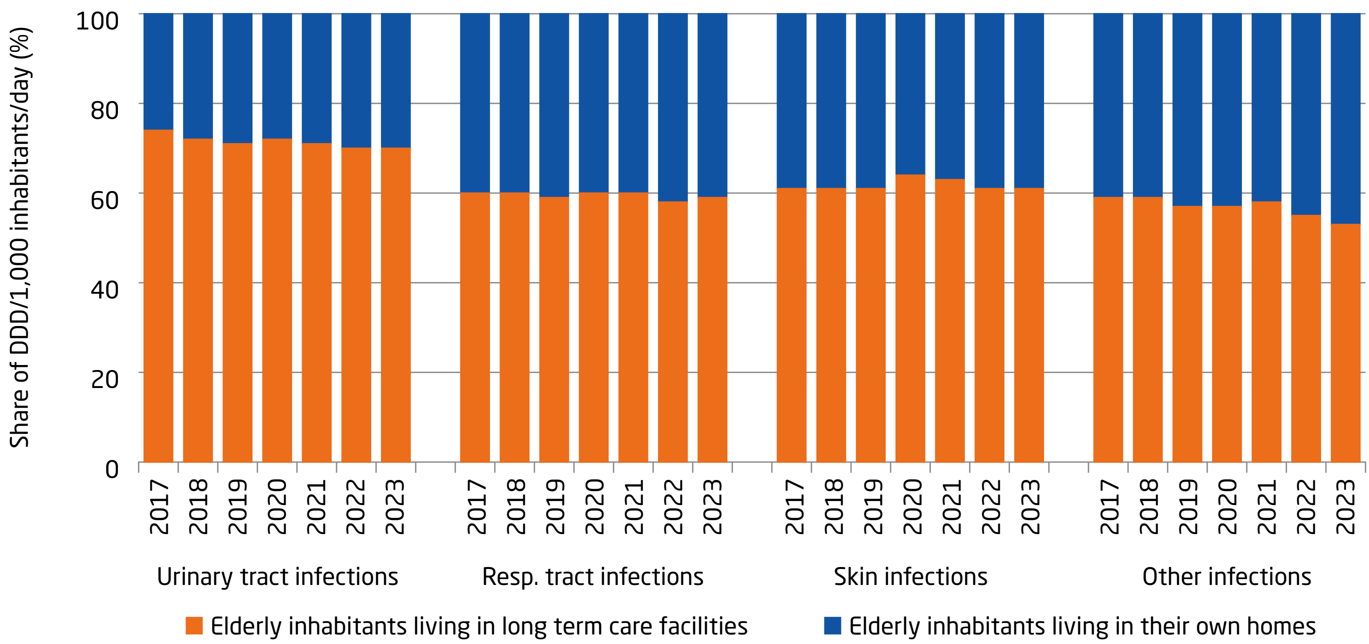


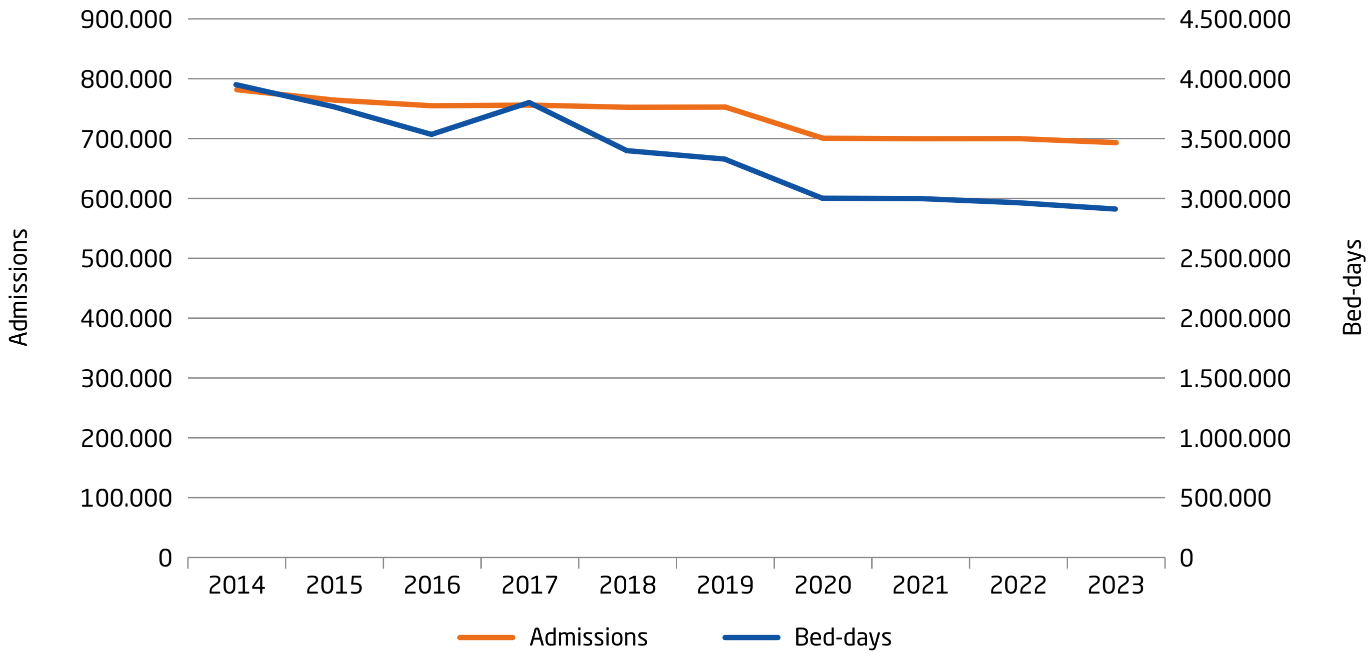
Figure 3.5 Treatment of infections in primary health care for inhabitants ≥65 years, Denmark, 2017-2023



Antimicrobials in hospital care

Trends in antimcrobial consumption at hospitals are highly dependent on the applied unit of measure. Figure 3.6 shows the activity at Danish hospitals measured in bed-days and admissions from 2014-2023.

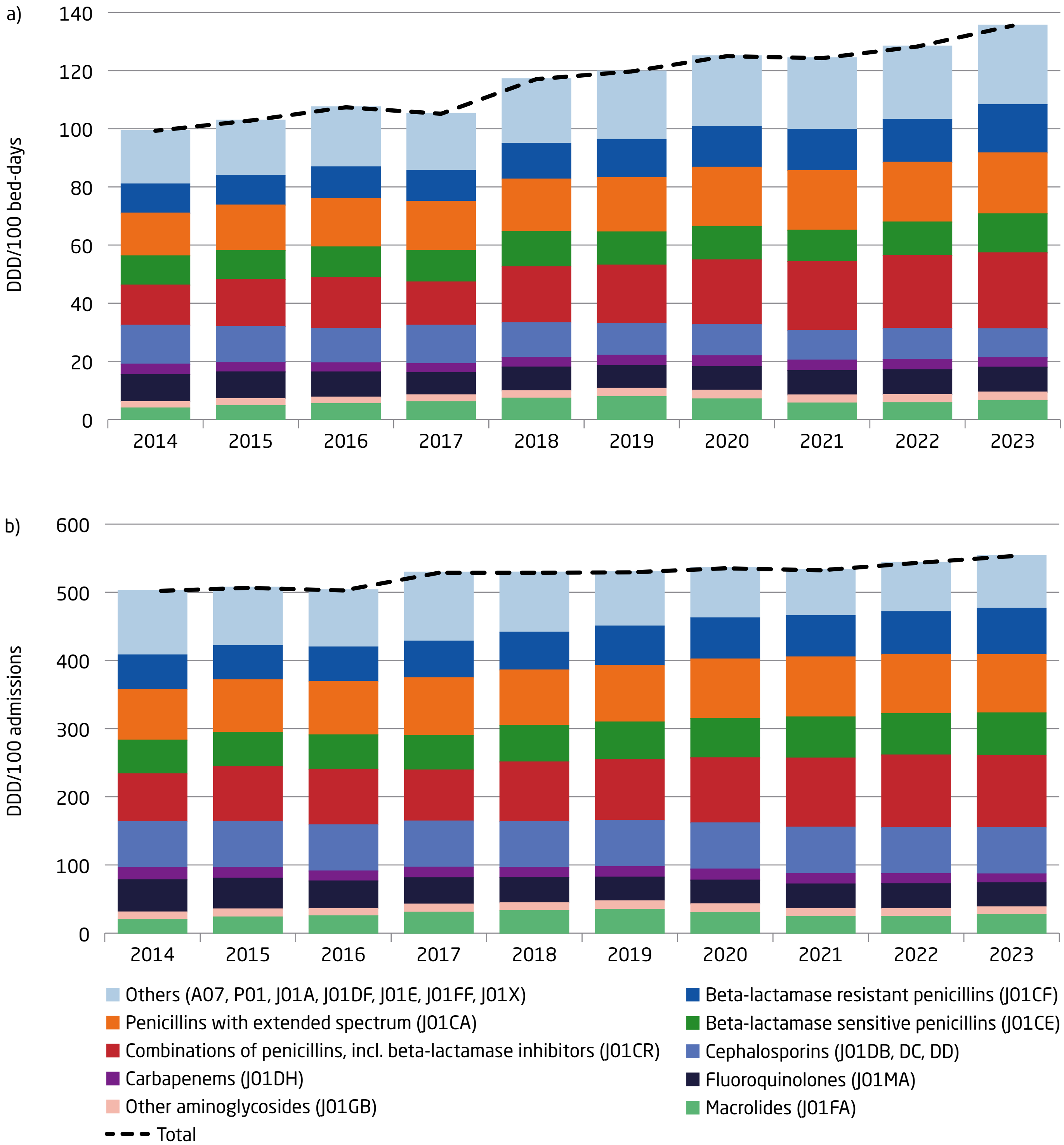
Figure 3.6 Activity at Danish hospitals measured in bed-days and admissions, Denmark, 2014-2023



Over the past decade the consumption of antimicrobials at Danish hospitals changed from 99.40 to 135.66 DDD/100 bed-days and from 502.6 to 569.52 DDD/100 admissions. However, when measured in DDD/1,000 inhibitants/day the consumption showed no marked changes in 2023 compared to a decade ago.

The observed changes measured in DDD/100 bed-days were driven by increased use of combination of penicillins including beta-lactamase inhibitors (90% increase from 2014 to 2023) and of beta-lactamase resistant penicillins, (Figure 3.7). At the same time, consumption of antimicrobials of special critical interest (cephalosporins, fluoroquinolones and carbapenems) decreased notably by 17%.

Figure 3.7 Antimicrobial consumption at somatic hospitals by antimicrobial group, a) DDD per 100 bed-days and b) DDD per 100 admissions, Denmark, 2014-2023

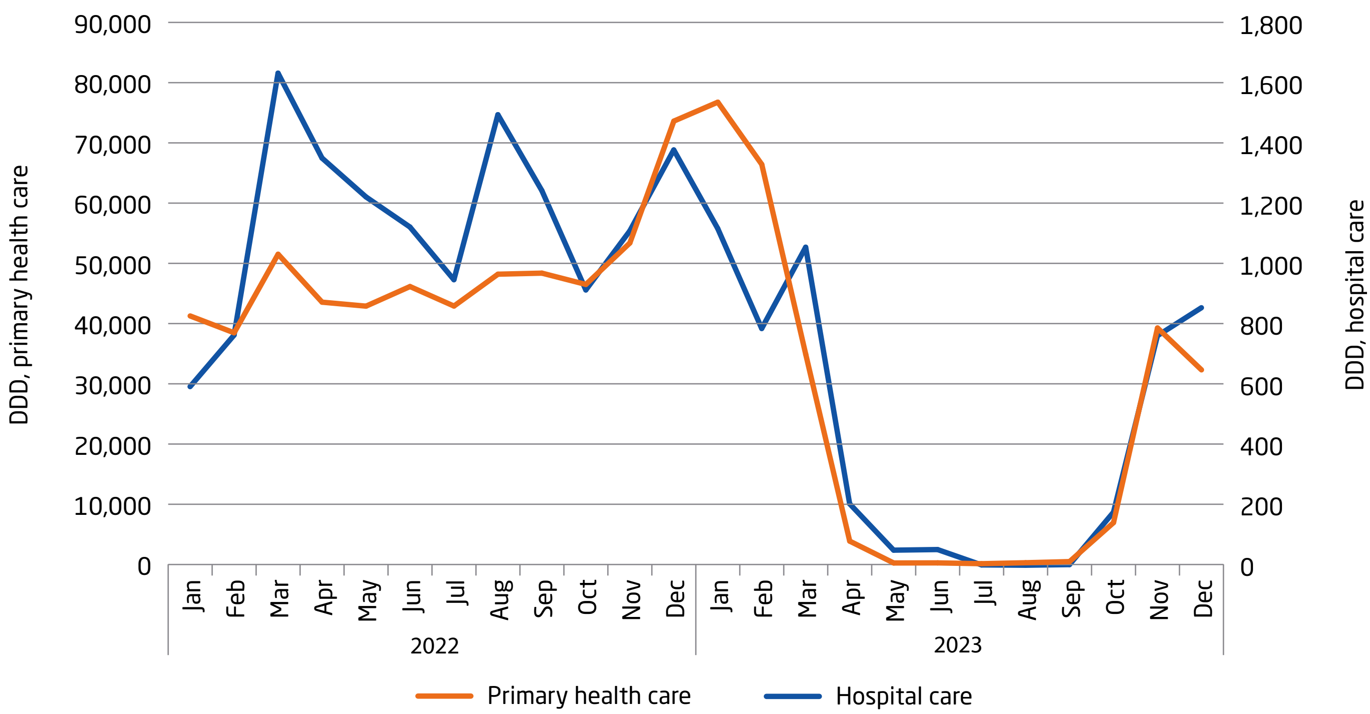


Product shortages

Increasing issues with supply chains of antimicrobials can lead to product shortages of variable duration. Analysis of monthly consumption data can reveal short product shortages like in 2022, where penicillin/beta-lactamase inhibitor combinations decreased sharply in July and August due to product shortages. However, approximately 70,000 DDD penicillin/beta-lactamase inhibitor combinations were purchased through special delivery issued by Danish Medicines Agency to cover the shortage (in comparison to 4,000 DDD in 2019-2021).

In 2023, nitrofurantoin supply was hit by a long shortage and even with increased special deliveries, it was not possible to cover the need of it, (Figure 3.8). Thus, consumption of other antimicrobials for urinary tract infection treatment increased in the same period.

Figure 3.8 Monthly consumption of nitrofurantoin by health care sector during shortage, DDD, Denmark, 2022-2023



4. Resistance in zoonotic bacteria

Monitoring results of the occurrence of antimicrobial resistance (AMR) in *Campylobacter* and *Salmonella* can be accessed and interactively visualized in the DANMAP explorer, available at www.danmap.org.

Campylobacter spp. is commonly associated with intestinal disease in humans in Denmark. *C. jejuni* is the dominating human pathogen followed by *C. coli*. Both species are ubiquitous in food-producing animals. However, *C. jejuni* is most commonly found in broilers and cattle, while *C. coli* in pigs.

Salmonella spp. is also commonly associated with intestinal disease in humans in Denmark and is distributed across many serotypes. There is a correlation between *Salmonella* serotypes and resistance levels, making it difficult to compare these across serotypes. Thus, DANMAP focuses on *S.* Typhimurium and monophasic *S.* Typhimurium as these serotypes are present in clinical human isolates and isolates from food-producing animals, especially in pigs. In Denmark, antimicrobials are generally not recommended to treat diarrhea in human patients unless there is prolonged disease, or the patient is severely ill. If necessary, macrolides (azithromycin) are recommended to treat campylobacteriosis, whereas azithromycin, fluoroquinolones, and 3rd generation cephalosporins are used to treat salmonellosis in hospital patients.

In animal production, macrolides are often used to treat infections in Danish pigs, while the use of antimicrobials in Danish poultry is low and primarily limited to tetracyclines.

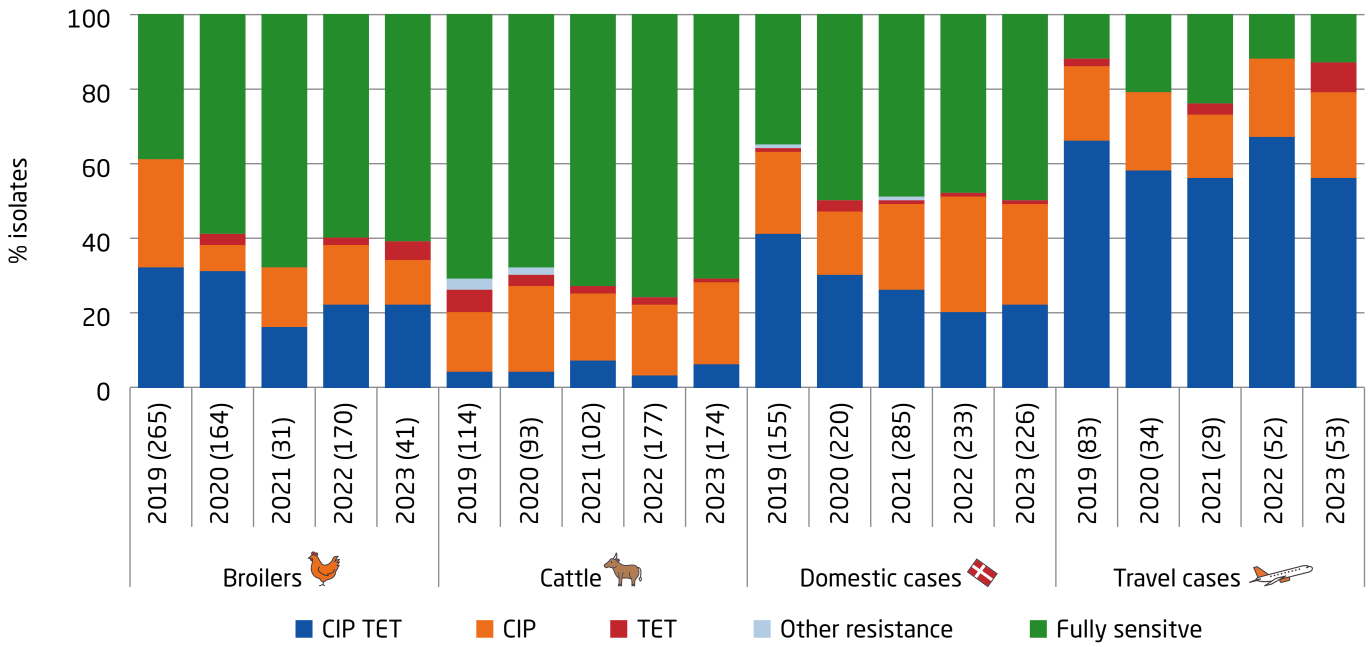
Resistance in *Campylobacter jejuni*

Resistance levels in human isolates were comparable with those observed in the last years, with travel-associated cases showing higher resistance levels than dosmestically acquired cases, (Figure 4.1). As in previous years, resistance levels in *C. jejuni* isolated from humans were higher than in isolates from broilers and cattle, (Figure 4.1).

Increased percentage of fully sensitive *C. jejuni* isolates from broilers

Compared to 2022, the percentage of fully sensitive *C. jejuni* increased in isolates from broilers (from 59% to 61%) and decreased in isolates from cattle, (from 76% to 70%; Figure 4.1).

Figure 4.1 Distribution (%) of AMR profiles among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark, 2019-2023



A human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease
CIP: all isolates with only ciprofloxacin resistance (i.e. without tetracycline resistance), TET: all isolates with tetracycline resistance (i.e. without ciprofloxacin resistance), CIP TET: all isolates with both ciprofloxacin and tetracycline resistance, Other resistance: all isolates without both ciprofloxacin and tetracycline resistance, Fully sensitive: all isolates susceptible to all antimicrobial agents included in the test panel

Resistance to ciprofloxacin and tetracycline remained common and increased in cattle
In 2023, resistance to ciprofloxacin but not tetracycline in *C. jejuni* isolates from broilers decreased when compared to the previous year (from 16% to 12%) and combined resistance to ciprofloxacin and tetracycline remained the same, (22%; Figure 4.1).

C. jejuni isolates from cattle in 2023 showed an increase in ciprofloxacin resistance (from 19% in 2022 to 22%) and combined resistance to ciprofloxacin and tetracycline (from 3% in 2022 to 6%; Figure 4.1), but not tetracycline resistance alone.

Fluoroquinolones are not used in food-producing animals in Denmark, and their use is not allowed in broiler production across the EU. This suggests that the development and spread of ciprofloxacin resistance in *C. jejuni* isolates from broilers and cattle is driven by mechanisms other than the direct use of fluoroquinolones.

In humans, resistance levels to ciprofloxacin and tetracycline were higher in infections from travel-related cases (57%) compared to domestically acquired cases (22%) with levels showing a decreasing trend in domestically acquired cases, (Figure 4.1).

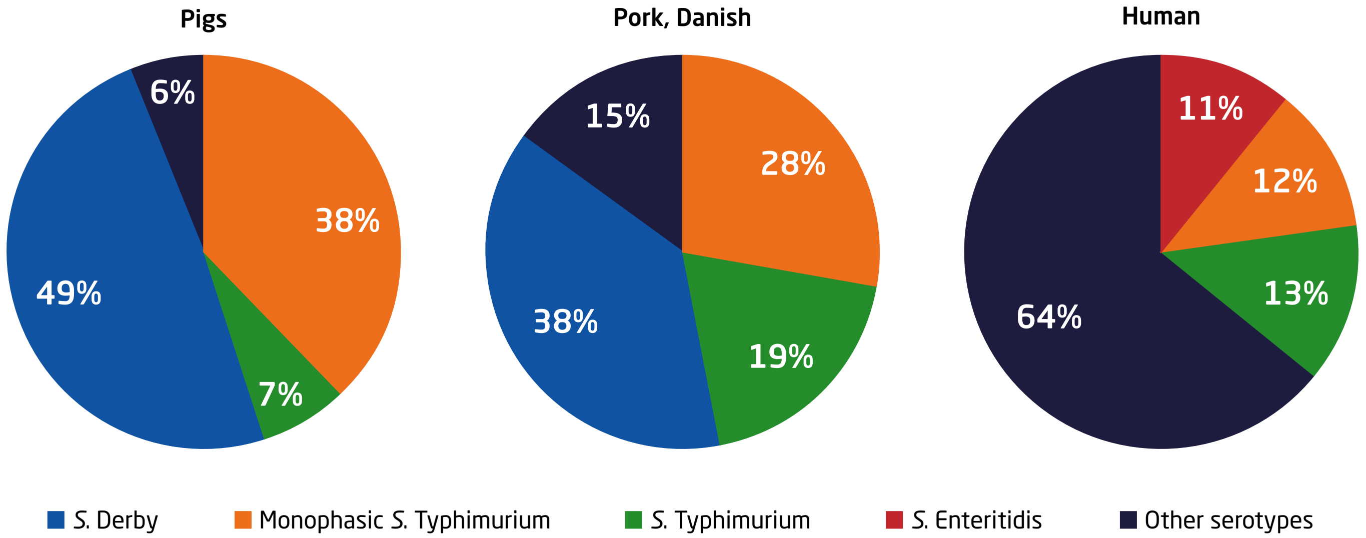
Resistance to macrolides and carbapenems remained low
Macrolide (erythromycin) resistance remained rare in *C. jejuni* from humans (1%) and resistance was not observed in isolates from broilers or cattle. Carbapenem resistance (ertapenem) was comparable to the levels found in 2022. It was observed at low levels in humans (6%), broilers (2%) and cattle (1%).

Resistance in *Campylobacter coli*
In 2023, *C. coli* was monitored in pigs, and for the first time, data on clinical human isolates was reported.

Resistance to ciprofloxacin and tetracycline in *C. coli* from pigs increased compared to the levels observed in 2021 (from 20% to 25% and from 26% to 35%, respectively), while resistance to ertapenem was not observed. *C. coli* isolates from humans exhibited higher resistance levels than isolates from pigs, indicating that the human isolates likely originated from sources other than Danish pigs. Erythromycin resistance was seen in 11% of the tested human *C. coli* isolates and 6% in isolates from pigs. Erythromycin resistance levels in human clinical cases will be followed in the coming years.

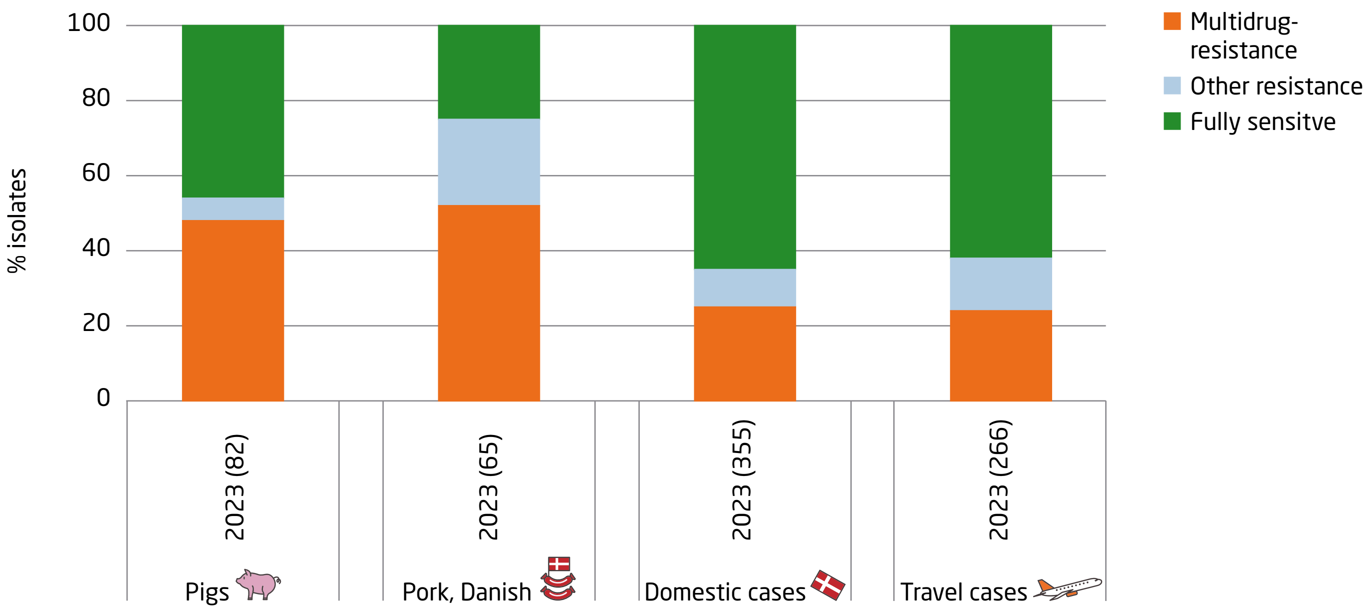
Resistance in *Salmonella* spp.
In 2023, a total of 697 *Salmonella* spp. isolates from human clinical cases, 82 from pigs and 65 from domestic pork were tested for antimicrobial susceptibility. The serotype distribution for the three reservoirs is presented in Figure 4.2. The most common serotypes reported in humans were *S.* Typhimurium, monophasic *S.* Typhimurium and *S.* Enteritidis, and the most common serotypes in pigs and domestic pork were *S.* Derby, monophasic *S.* Typhimurium, and *S.* Typhimurium, (Figure 4.2).

Figure 4.2 Distribution (%) of *Salmonella* serotypes from pigs (N = 82), Danish pork (N = 65) and human cases (N = 697) included in DANMAP, 2023



Most *Salmonella* spp. isolates from humans were fully sensitive (64%), while isolates from pigs showed a lower level of full sensitivity (46%). Domestic pork showed a marked difference, with only 25% of the *Salmonella* spp. isolates sensitive to all antimicrobials tested, (Figure 4.3). In 2023, 22% of human isolates were multidrug-resistant (MDR), with comparable levels observed in domestic and travel-associated cases, (Figure 4.3). Higher MDR levels were reported in pigs (48%) and domestic pork, (52%; Figure 4.3).

Figure 4.3 Relative distribution (%) of multidrug-resistance, full sensitivity and other resistance profiles among *Salmonella* spp. from pigs, Danish pork and human cases, Denmark, 2023



Low levels of macrolide, fluoroquinolone and aminoglycoside resistance
Macrolide (azithromycin) resistance was low in human travel-associated (3%) and domestic cases (1%), and pig isolates (1%), while no resistance was reported in isolates from domestic pork.

Resistance to fluoroquinolones (ciprofloxacin) was observed in 15% of the isolates from humans, while resistance levels in pigs and domestic pork were markedly lower (1% and 0%, respectively).

For amynoglicosides, gentamicin resistance was found in 2% of the human isolates. Similar levels were observed in pigs (2%), while a higher level was recorded in domestic pork (8%). Similar amikacin resistance levels were found in human isolates (2%) and Danish pork (2%).

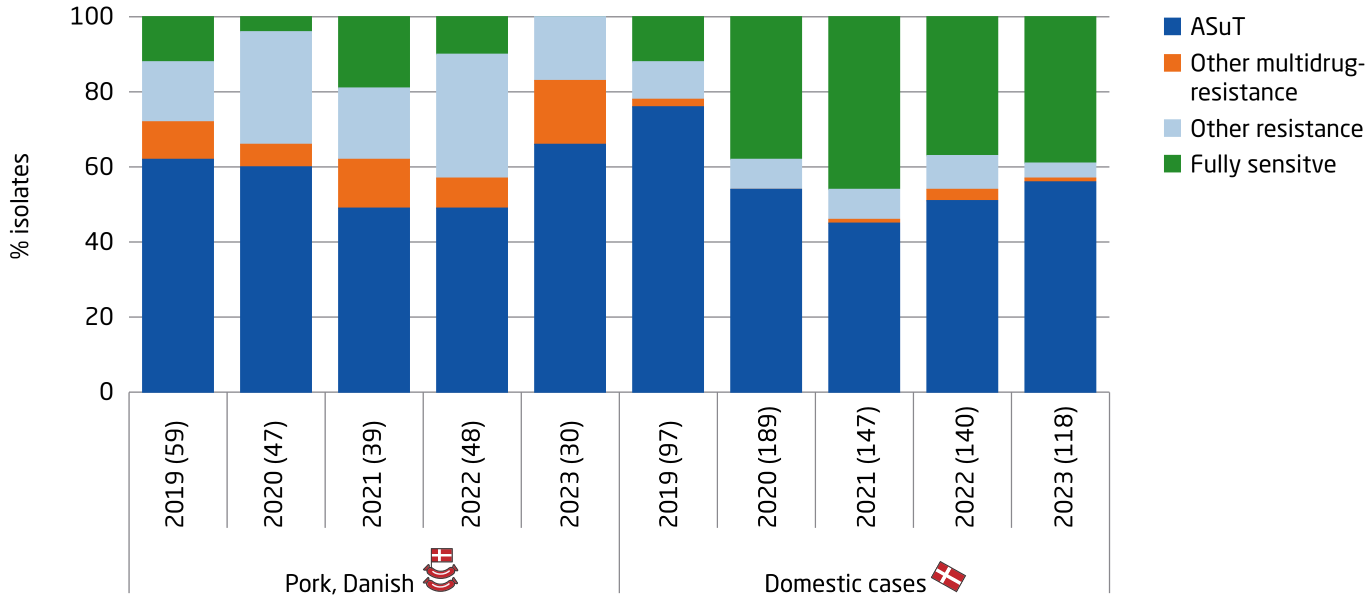
Resistance in *S.* Typhimurium and monophasic *S.* Typhimurium

Clonal dissemination plays an important role in the occurrence of antimicrobial resistance among *S.* Typhimurium. The global dissemination of genomic islands conferring resistance to ampicillin, sulfamethoxazole and tetracycline (the ASuT multidrug-resistance profile) among *S.* Typhimurium, and particularly monophasic *S.* Typhimurium variants, continues to contribute to a high level of multidrug-resistance among isolates from animals and humans.

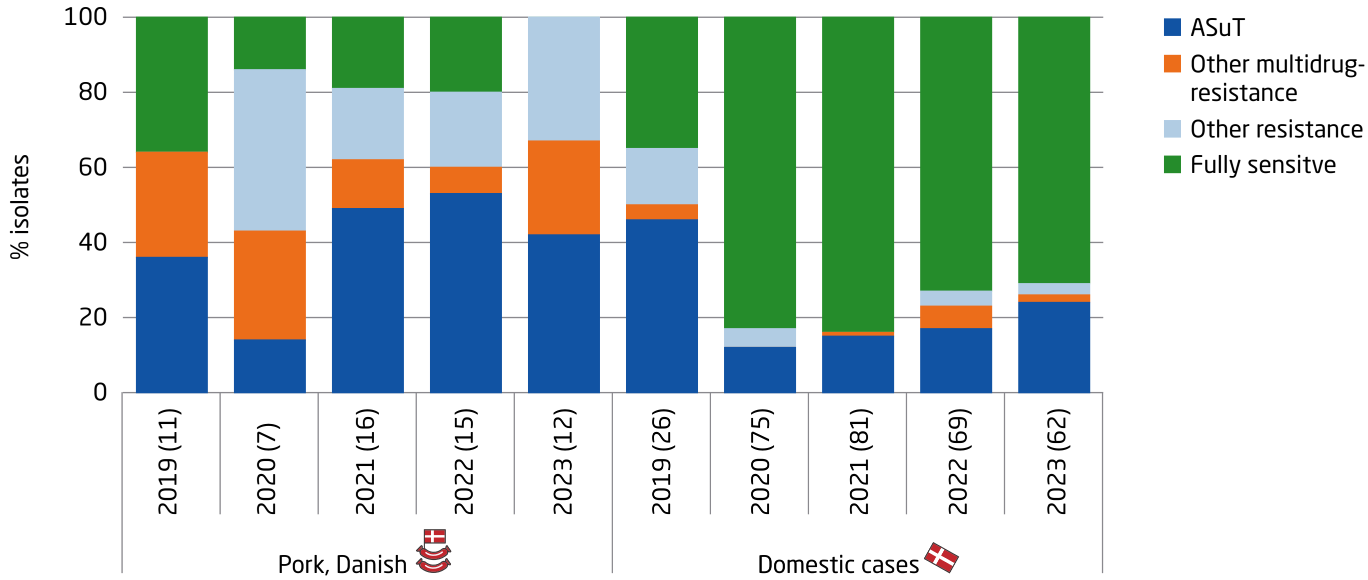
Figure 4.4 a-c shows the distribution (%) of AMR profiles for *S.* Typhimurium and monophasic *S.* Typhimurium. Overall in 2023, MDR in combined *S.* Typhimurium and monophasic *S.* Typhimurium from domestic pork showed a 27% increase compared to 2022, with an increase in the ratio between the prevalence of other MDR profiles and ASuT. This finding is opposed to the decrease in the occurrence of MDR observed between 2019 and 2022, (Figure 4.4a).

Figure 4.4 Relative distributions (%) of AMR profiles among *Salmonella* Typhimurium and its monophasic variants combined (a), *S.* Typhimurium (b) and monophasic *S.* Typhimurium (c) from pork and human domestic cases, Denmark, 2019-2023

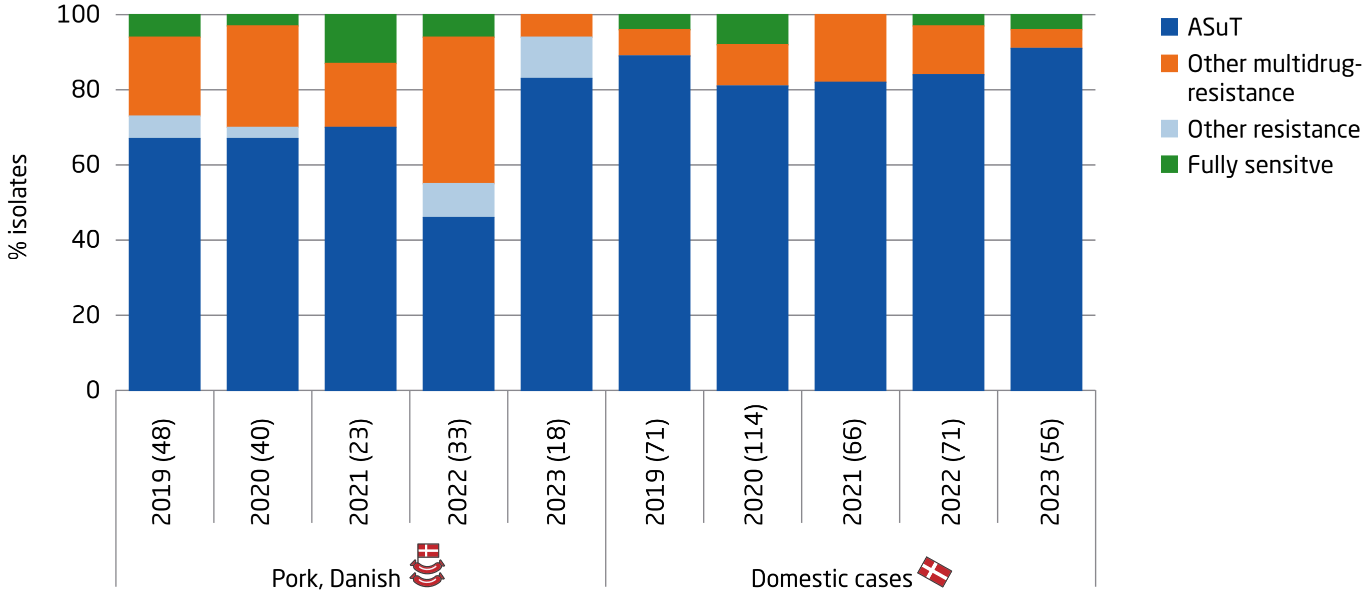
a) *S.* Typhimurium and monophasic combined



b) *S.* Typhimurium



c) Monophasic *S.* Typhimurium



Caution in data interpretation should be taken in years when a small number (n <15) of isolates were recovered

Most *S.* Typhimurium isolates from human domestic cases were fully sensitive, (Figure 4.4b). However, in monophasic *S.* Typhimurium isolates from human domestic cases markedly higher MDR levels were registered (91%) with most isolates showing the ASuT resistance profile, (Figure 4.4c). Likewise, MDR was also found at markedly higher levels in domestic pork (94%) and ASuT was the most found MDR profile (83%). The new approach of analysing *S.* Typhimurium and monophasic *S.* Typhimurium separately reveals marked differences in their levels of MDR and especially ASuT. These are most evident in human isolates.

Changes in fluoroquinolone resistance in human clinical isolates

Ciprofloxacin resistance levels in *S.* Typhimurium and monophasic *S.* Typhimurium from humans were 12% and 11%, respectively compared to 3% and 8% in 2022. Historically, ciprofloxacin resistance has predominantly been observed in *S.* Typhimurium and monophasic *S.* Typhimurium isolates from travel-associated cases, but in 2023 the resistance levels in isolates from domestically acquired and travel-related cases were comparable.

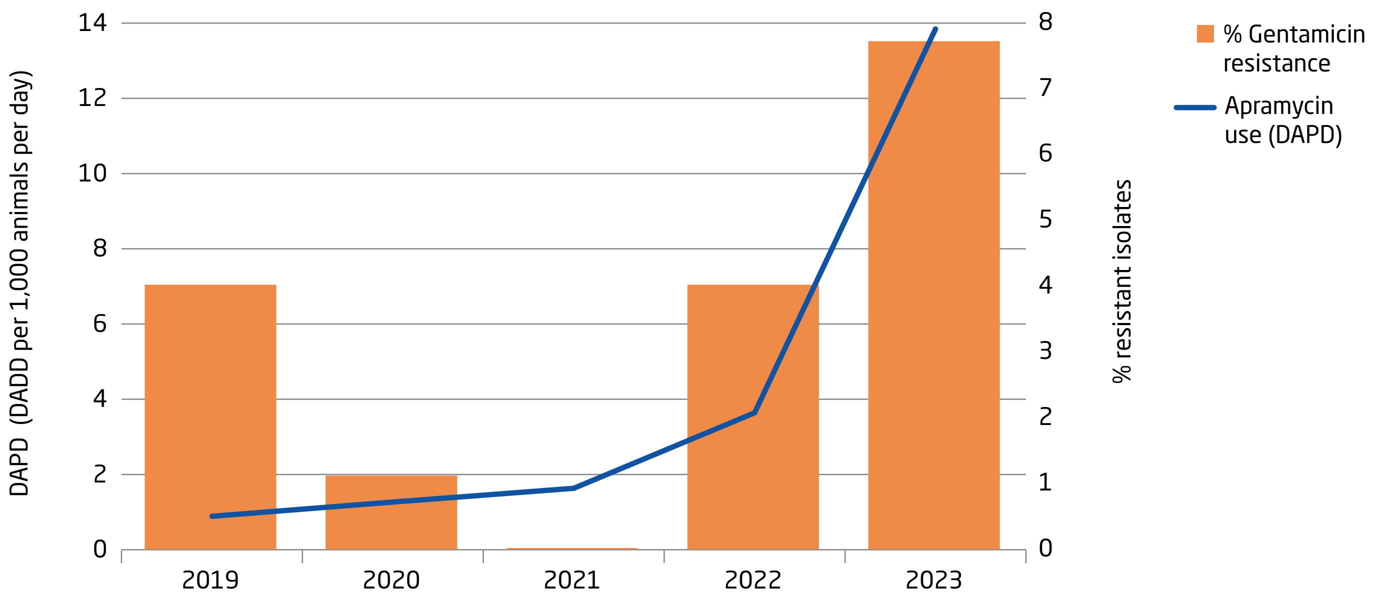
Resistance in *Salmonella* Enteritidis in human clinical isolates

S. Enteritidis isolates from humans (N = 76) showed low resistance levels in isolates from both domestic and travel-related cases. However, resistance to ciprofloxacin was observed in 39% of the isolates, with levels in domestic cases showing higher resistance levels than in travel-associated cases (47% and 32%, respectively).

Resistance levels in *Salmonella* spp. from Danish pork and antimicrobial consumption

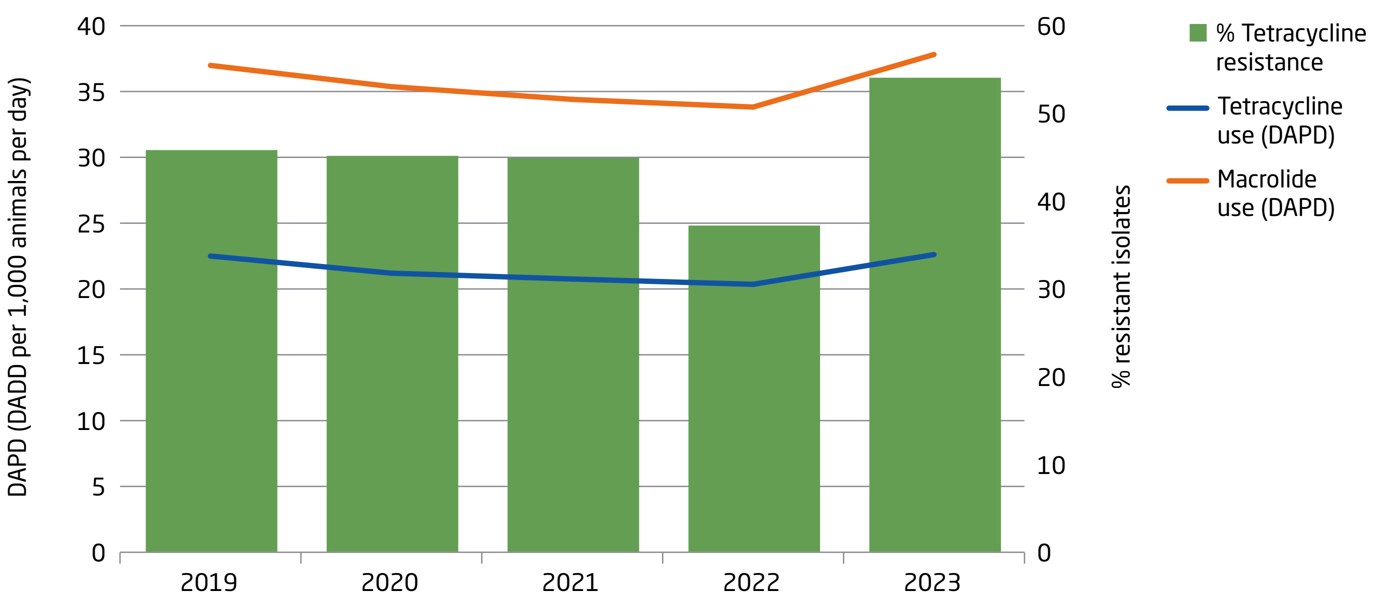
The usage of aminoglycosides has increased in 2022 and 2023 possibly due to the ban on prescribed zinc oxide in pig production and the Order 2019/6 on veterinary medicinal products implemented in June and January 2022, respectively. The increase observed in 2022 and 2023 (Chapter 2, Figure 2.6) was mostly due to the increased use of apramycin and neomycin to treat post-weaning diarrhea. The increased usage of apramycin in weaners in 2022 and 2023 follows a concomitant increase in the prevalence of gentamicin-resistant *Salmonella* spp. from Danish pork, (Figure 4.5).

Figure 4.5 Gentamicin resistance in *Salmonella* spp. isolates from Danish pork and apramycin consumption (DAPD) in weaners, Denmark, 2019-2023



After a decreasing trend in the usage of macrolides and tetracyclines in weaners and finishers from 2019 to 2021, an increase was registered in 2023, (Figure 4.6). Tetracyclines and macrolides are mostly used to treat gastrointestinal diseases in these age groups, and their increased usage may also be related to the reasons mentioned above. Also in 2023, an increase in tetracycline resistance (from 37% to 54%) in *Salmonella* spp. from Danish pork was observed, which can probably be explained by the direct selective pressure due to the registered increased use of tetracyclines and due to co-selection due to the increased use of macrolides, (Figure 4.6).

Figure 4.6 Tetracycline resistance in *Salmonella* spp. isolates from Danish pork and macrolide and tetracycline consumption (DAPD) in weaners and finishers, Denmark, 2019-2023



5. Resistance in indicator bacteria

Monitoring results for occurrence of resistance in indicator *E. coli*, indicator Enterococci and β-lactamase-producing *E. coli* can be accessed and interactively visualized in DANMAP *explorer*, available at www.danmap.org.

Escherichia coli and Enterococci (*E. faecalis* and *E. faecium*) are included in the DANMAP programme as indicators to monitor trends in the occurrence of antimicrobial resistance in different production animals. In 2023, resistance was monitored in indicator *E. coli* from broilers, pigs and calves at slaughter, and in *E. faecalis* from pigs at slaughter.

E. coli exhibiting resistance to 3rd generation cephalosporins via production of beta-lactamases, including extended-spectrum β-lactamases (ESBLs) and cephalosporinases (AmpC), as well as carbapenemase (CP)-producing *E. coli* are monitored in DANMAP, both via antimicrobial susceptibility testing and whole genome sequencing. In 2023, ESBL-/AmpC-producing *E. coli* was recovered from pigs and cattle at slaughter and from pork and beef meat at retail, while CP-producing *E. coli* was not detected, similarly to previous years.

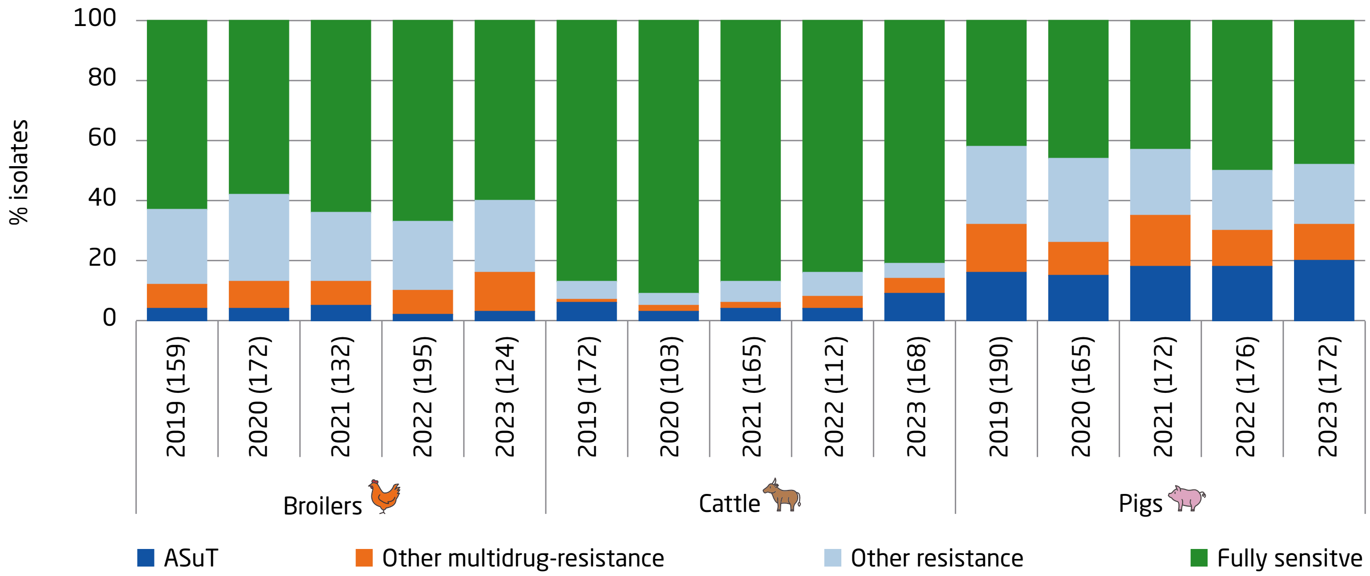
Resistance in indicator *E. coli*

Significant decrease in full sensitivity and increase in multidrug-resistance in *E. coli* from cattle

Although no significant trend has been detected in the prevalence of fully sensitive indicator *E. coli* from Danish broilers or pigs, over the last five monitoring years, the occurrence of fully sensitive *E. coli* has significantly decreased in cattle (from 87% in 2019 to 82% in 2023), (Figure 5.1).

Combined resistance to ampicillin, sulfamethoxazole and tetracycline (ASuT) continued to be the most common multidrug-resistance profile in isolates from cattle and pigs, however the occurrence of other multidrug-resistance profiles clearly increased in isolates from broilers in 2023. A significant increasing trend in the occurrence of multidrug-resistant *E. coli* over the past five years was detected for cattle, with a clear increase in occurrence of the ASuT profile in 2023, (Figure 5.1). Additionally, among cattle isolates, the occurrence of resistance to ampicillin increased by 7%, and the occurrence of resistance to chloramphenicol increased by 3% from 2022 to 2023.

Figure 5.1 Distribution (%) of fully sensitive, resistant and multidrug-resistant *Escherichia coli* isolates from broilers, cattle and pigs, Denmark, 2019-2023



The number of isolates included each year is shown in parentheses. An isolate is considered fully sensitive if susceptible to all antimicrobial agents tested, and multidrug-resistant if resistant to three or more antimicrobial classes included in the test panel. ASuT are the multidrug-resistant isolates resistant to ampicillin, sulfamethoxazole and tetracycline, which may also be resistant to other antimicrobials

Indicator *E. coli* has low or absent resistance towards critically important antimicrobials
Regarding resistance to critically important antimicrobials, as in previous years, no colistin, meropenem or tigecycline resistance were detected in indicator *E. coli*. Resistance to ciprofloxacin continued to be low among isolates from cattle and pigs, and after the increase observed in 2022 in isolates from broilers, it decreased back to low levels (3%) in 2023. Azithromycin resistance was again detected in few isolates from pigs (3%), and additionally in 2% of broiler isolates. Resistance to 3rd generation cephalosporins was not detected or detected at very low levels (up to 1%) in indicator *E. coli* using non-selective procedures.

Occurrence of β-lactamase-producing *E. coli*

Overall decrease in occurrence of ESBL- /AmpC-producing *E. coli*
Following selective enrichment, *E. coli* resistant to 3rd generation cephalosporins were obtained from pigs (18%), Danish pork (2%), imported pork (8%) , cattle (4%), Danish beef (1%) and imported beef (4%). The occurrence of *E. coli* producing beta-lactamases continued the decreasing trend observed since 2019 in Danish cattle and pigs, and in imported pork meat. It was also lower in cattle meat (domestic and imported) in 2023, compared to 2021, (Figure 5.2). Antimicrobial susceptibility testing of ESBL/AmpC-producing *E. coli* showed, like in previous years, a very high occurrence in resistance to 4th generation cephalosporins (cefepime) in isolates from imported beef (100%) and pork (60% in domestic and 78% in imported). However, compared to 2021, there was a decrease in the prevalence of cefepime resistance among pig- and pork ESBL/AmpC-producing *E. coli*.

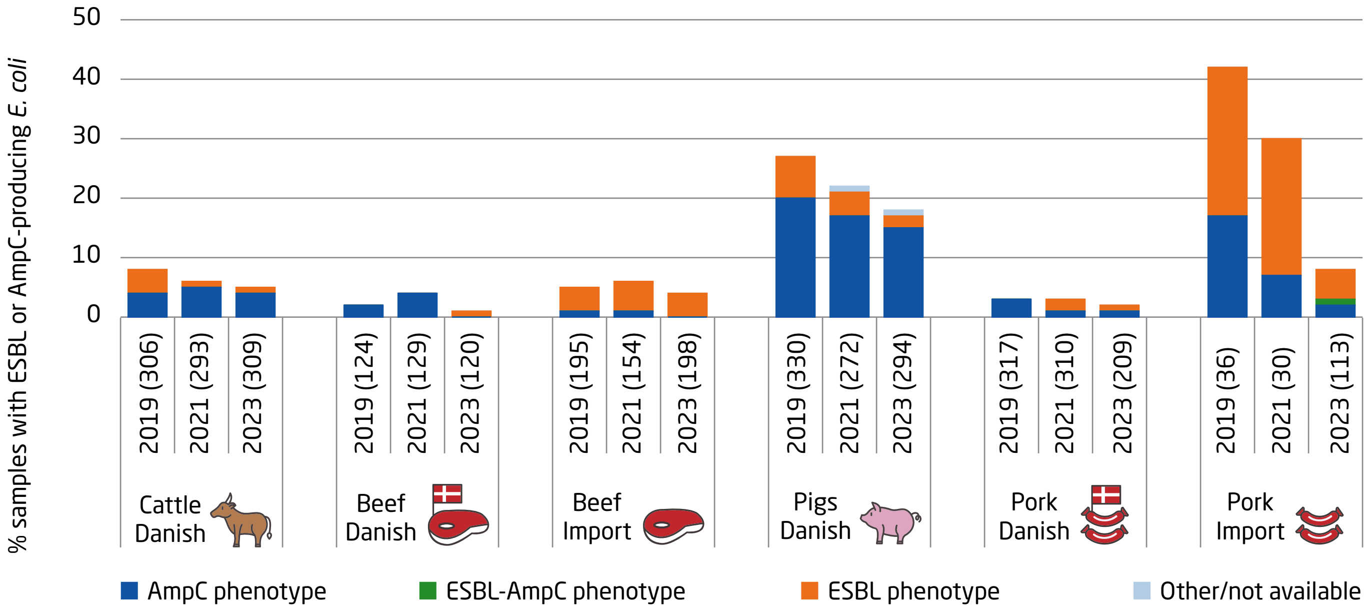
For all but one AmpC genotype, resistance was conferred by upregulated AmpC promotor C-42T mutations. A single isolate from pigs showed a T-32A mutation instead. Among the ESBL genotypes, 11 different ESBL-encoding genes were detected. Overall, the most commonly observed ESBL encoding genes were *CTX-M-1* and *CTX-M-15*, with the latter being mostly observed in isolates from imported beef, (Table 5.1).

Table 5.1 Number of ESBL and/or AmpC enzymes detected in β-lactamase-producing *E. coli* isolates from animals and meat recovered by selective enrichment, Denmark, 2023

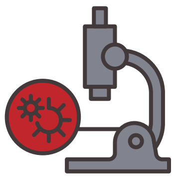
	Cattle	Beef		Pigs	Pork	
Enzymes	Danish	Danish	Import	Danish	Danish	Import
blaCTX-M-1		1	1	5	3	2
blaCTX-M-15	1		3			1
blaCTX-M-27						1
blaCTX-M-32			2			
blaCTX-M-55				2		3
blaDHA-1	1			2		
blaOXA-1			1	1		
blaOXA-10	1					
blaSHV-12			1			
blaTEM-15	1					
blaTEM-52B	1					
Chromossomal AmpC (T-32A)				1		
Chromossomal AmpC (C-42T)	10		1	44	2	2
Number of AmpC genotypes	9	0	1	44	2	2
Number of ESBL genotypes	3	1	7	9	3	7
Number of AmpC+ESBL genotypes	1	0	0	1	0	0
Number (%) positive samples	13 (4%)	1 (1%)	8 (4%)	54 (18%)	5 (2%)	9 (8%)
Number of tested samples	309	120	198	294	209	113

Number (%) positive samples are isolates recovered by selective enrichment methods for specific monitoring of ESBL/AmpC producing *E. coli*. ESBL/AmpC enzymes were determined by whole genome sequencing of the recovered isolates

Figure 5.2 Occurrence (%) of samples with phenotypic ESBL- or AmpC-producing *E. coli* from animals and meat recovered by selective enrichment, Denmark, 2019-2023



Number of samples tested per year is presented in parentheses. Classification of ESBL and/or AmpC phenotypes based on antimicrobial susceptibility testing



Extended-spectrum beta-lactamases (ESBL) and Cephalosporinases (AmpC)

Gram-negative bacteria, such as *E. coli*, resistant to β-lactam antibiotic(s) most commonly produce one or more β-lactamase enzymes, which hydrolyze the given antibiotic(s).

β-lactamases can be classified according to different structural and functional classification systems. The functional classification has the advantage to relate the enzymes to their ability to hydrolyze specific β-lactam classes, and to identify the possibility of their inactivation with β-lactamase inhibitor substances, thus supporting antimicrobial therapy choices in clinical practice.

According to their functional classification, β-lactamases can be classified in three major groups, and several subgroups. In DANMAP, *E. coli* is monitored for ESBL and AmpC enzymes.

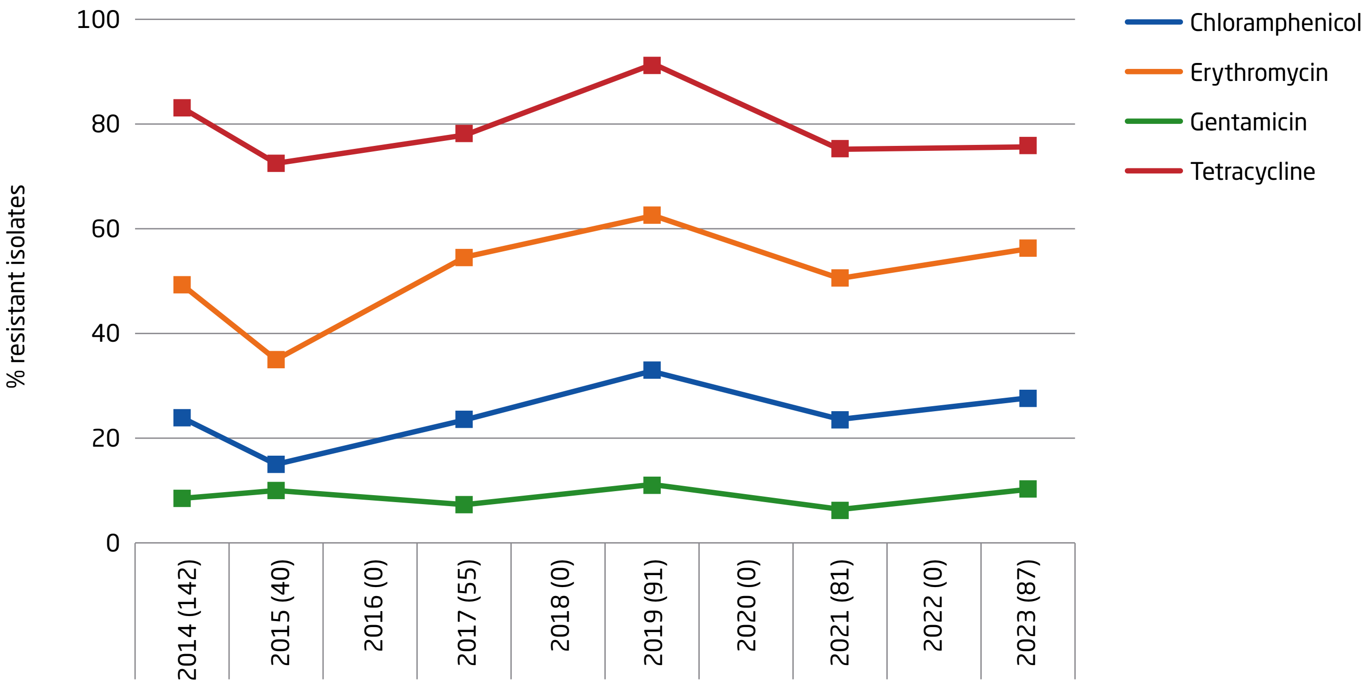
AmpC enzymes
AmpCs are active on cephalosporins and cephamycins, and usually resistant to inhibition by clavulanic acid. In large amounts, especially against low amounts of β-lactams, AmpCs can also provide resistance to carbapenems. These enzymes are encoded on the chromosomes of many *Enterobacteriaceae*, however, plasmid-mediated AmpCs also occur for example in the families CMY or DHA, although less commonly than plasmid-mediated ESBLs. Some AmpC variants have greater activity against ceftazidime (a 3rd generation cephalosporin), and are therefore called extended-spectrum AmpCs.

ESBL enzymes
The largest functional group of β-lactamases includes 12 subgroups. Subgroup 2be comprises the ESBLs. ESBLs hydrolyze penicillins and early-generation cephalosporins, and additionally hydrolyze one or more 3rd generation cephalosporins. They include mostly enzymes of the families TEM, SHV, and the more rapidly proliferating CTX-M enzymes.

Resistance in *Enterococcus faecalis* from pigs

In 2023, 87 *Enterococcus faecalis* isolates were obtained from pigs and tested for antimicrobial susceptibility. Overall, 22% of the isolates were fully sensitive, as observed in 2021. None of the enterococci isolates showed resistance to ampicillin, ciprofloxacin, linezolid, teicoplanin, tigecycline or vancomycin. Resistance to tetracycline (76%) and erythromycin (56%) were the most common, followed by resistance to chloramphenicol (28%) and gentamicin (10%). Compared to 2021, the prevalence of resistance to these four substances increased in 2023 from 1 to 5%.

Figure 5.3 Resistance (%) among *Enterococcus faecalis* isolates from pigs, Denmark, 2014-2023



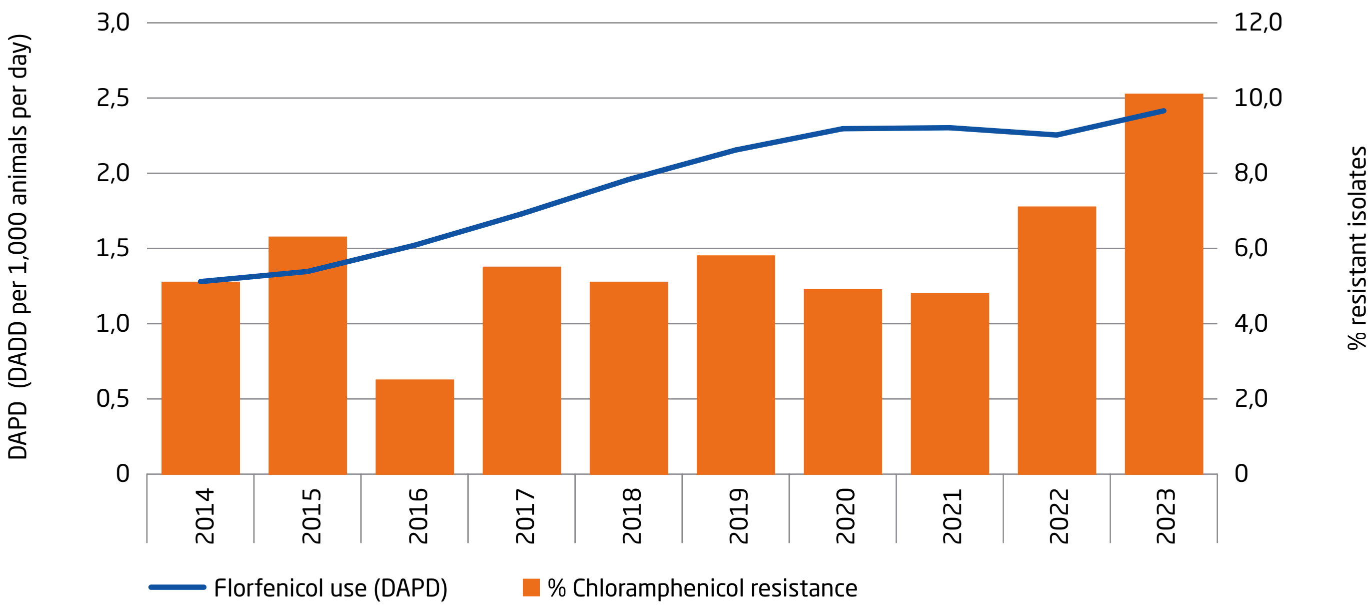
Number of isolates included each year is presented in parentheses

Trends of antimicrobial consumption and resistance in calves

Selection and co-selection of antimicrobial resistant indicator *E. coli*

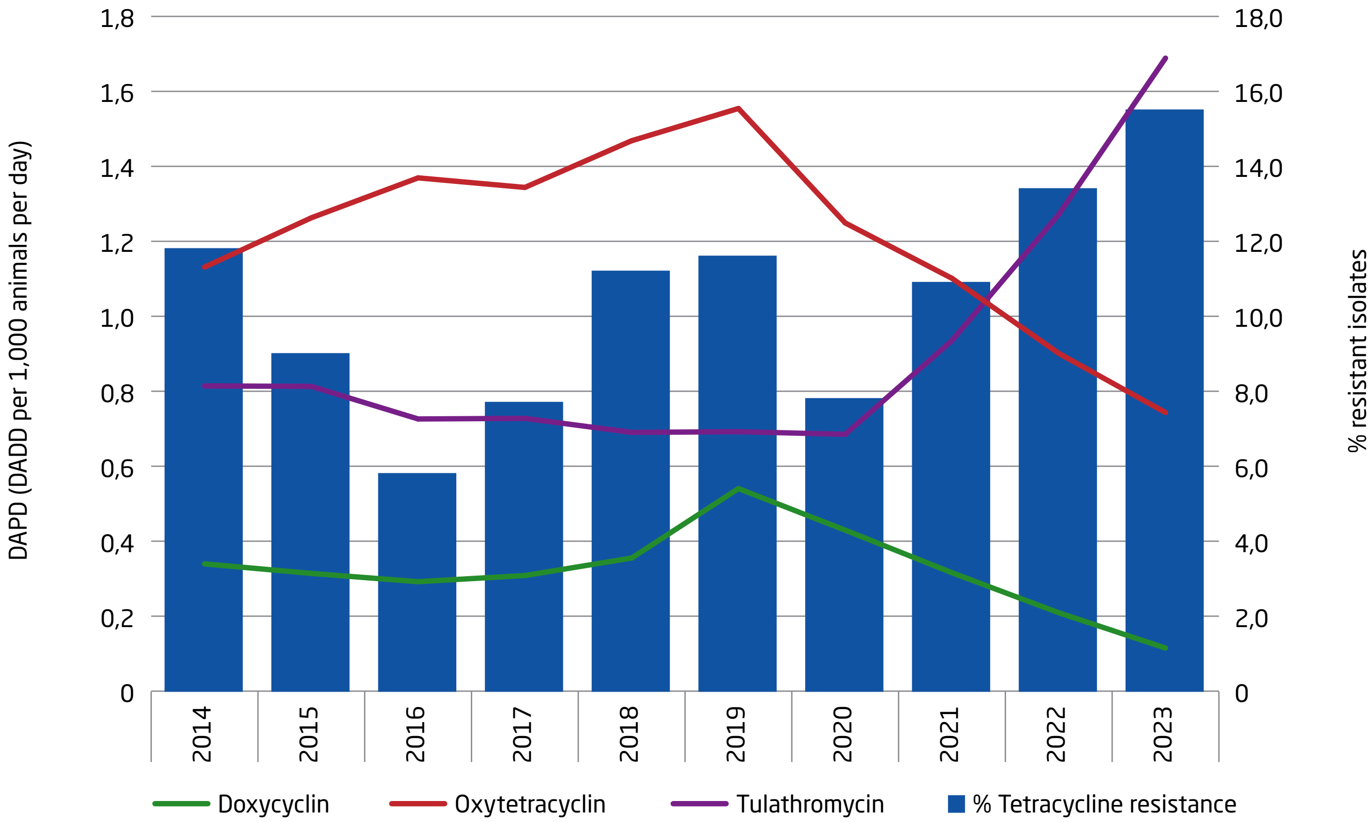
Over the last decade, there has been an increase in the consumption of macrolides and amphenicols in calves (cattle <1 year) in Denmark (see Chapter 2, Figure 2.7). That increase has been accompanied by an increase in the occurrence of resistance to chloramphenicol and tetracycline among indicator *E. coli* isolates from calves, (Figures 5.4, 5.5).

Figure 5.4 Amphenicol resistance (%) in indicator *E. coli* and florfenicol consumption (DAPD) in calves, Denmark, 2014-2023



The increased use of florfenicol has represented a direct selective pressure for chloramphenicol-resistant *E. coli*, as shown in Figure 5.4. The explanation for the observed increase in tetracycline-resistant *E. coli* is however more complex. While the increase observed between 2016 and 2019 may be the result of the increased use of tetracyclines, that observed after 2020 could result from co-selection due to the increased use of macrolides (tulathromycin), (Figure 5.5).

Figure 5.5 Tetracycline resistance (%) in indicator *E. coli* and antimicrobial consumption (DAPD) in calves, Denmark, 2014-2023



6. Resistance in human pathogens

DANMAP's surveillance of antimicrobial resistance in bacteria from human infections is based on information on clinical isolates and covers all antimicrobial susceptibility testing performed in Denmark. Data include phenotypic results from all Departments of Clinical Microbiology (DCM) and phenotypic and/or genotypic results from isolates submitted to national reference laboratories (NRLs) at Statens Serum Institut (SSI). Data for DANMAP is either extracted from the Danish Microbiological database (MiBa) or collected from the registers at the NRL.

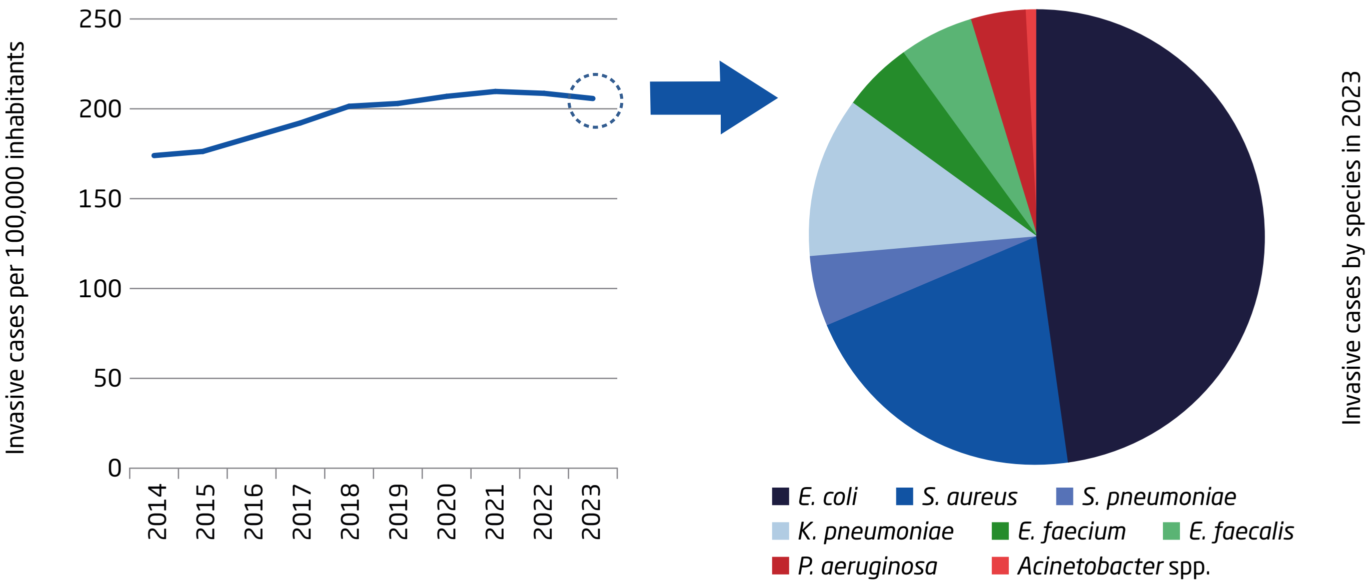
The number of invasive bacterial species monitored in Denmark stabilised in 2023

DANMAP collects data on the eight most common bacterial species causing invasive infections in humans. The total number of unique cases with these species has been continuously increasing since 2014 (+25%), but seems to be stabilized with 12,205 cases in 2023 (12,169 cases in 2022). The incidence of the monitored species from 2014 to 2023 is presented in Figure 6.1.

Escherichia coli is – and has been - the most frequent cause of invasive infections, accounting for an approximate share of 50%. In 2023, there were 5,835 invasive cases of *E. coli*, which corresponds to a 30% increase over the last decade. *Staphylococcus aureus* bacteraemia accounted for 2,559 patient cases (1,874 in 2014, 37% increase), followed by *Klebsiella pneumonia* with 1,399 patient cases (943 in 2014, 48% increase).

The increasing numbers of invasive infections mirror an increase in hospitalised patients and at-risk groups (elderly and immunocompromised/chronically ill patients) and may in addition be associated with a higher use of invasive medical and surgical procedures. In addition, the number of microbiological samples received for analysis at the DCM has increased considerably during the past decade. In 2023, a total of 3,313 patients per 100,000 inhabitants had at least one blood culture taken compared to 2,549 patients per 100,000 inhabitants in 2014, an increase of 30%.

Figure 6.1 Incidence of monitored invasive cases, Denmark, 2014-2023



Resistance in monitored bacterial species in Denmark

Resistance in *E. coli* has remained below the 10% percentile for most antimicrobials for the past decade. Since 2014 decreasing trends in resistance in invasive isolates were noted particularly for ciprofloxacin (- 14%), 3rd generation cephalosporins (- 17%) and gentamicin (- 39%) and increasing trends for piperacillin-tazobactam (31%), Figure 6.2. This trend is mirrored in isolates from urinary tract infections. The COVID-19 pandemic enforced many of these trends. However, from 2022, increases in resistance towards most of the monitored antibiotics were observed. For *K. pneumoniae* similar trends were observed, the decreases in resistance to cephalosporins, fluoroquinolones and gentamicin being even more notable, (Figure 6.2).

Table 6.1 shows resistance levels in both species for the past five years span, 2018 and 2023.

Figure 6.2 Resistance trends in invasive isolates of *E. coli* and *K. pneumoniae*, 2014-2023

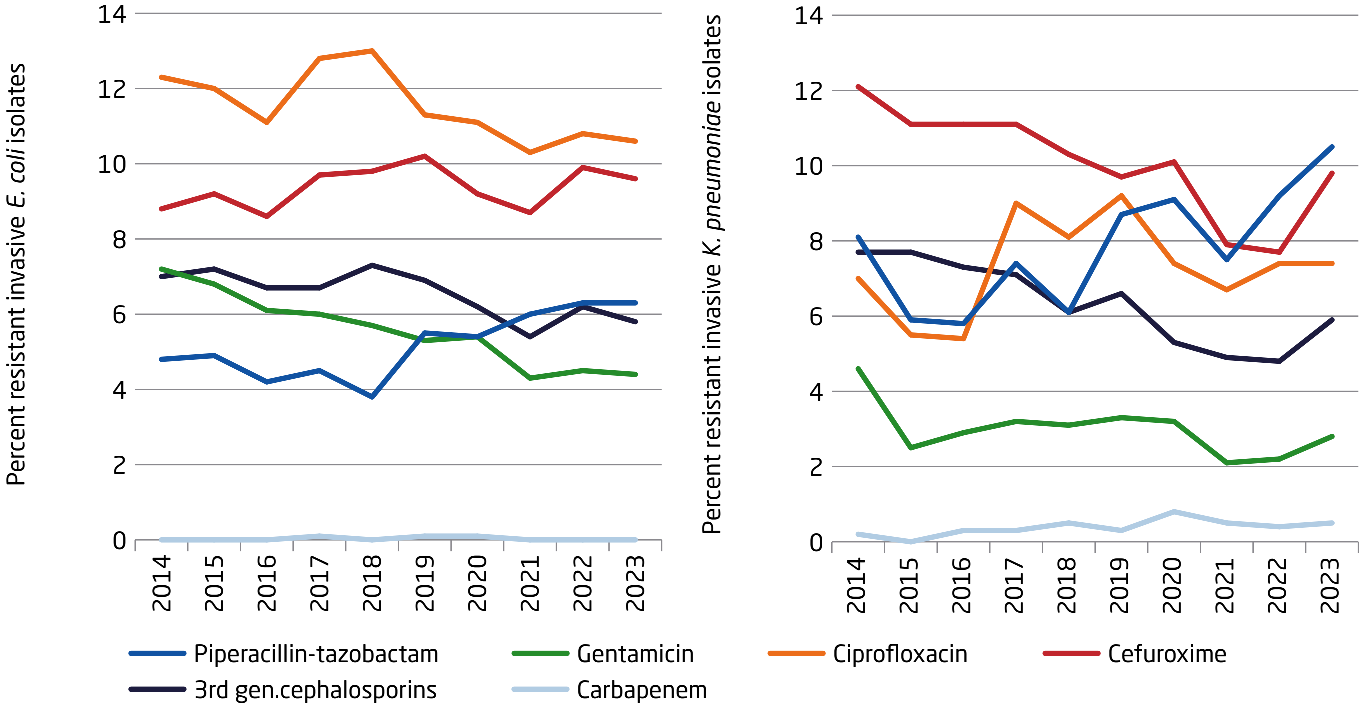


Table 6.1 Resistance (%) in *E. coli* and *K. pneumoniae* from blood cultures and urine, 2018 and 2023

	E. coli				*K. pneumoniae*			
	2018 (%)		2023 (%)		2018 (%)		2023 (%)	
	Invasive	Urines from PHC*	Invasive	Urines from PHC	Invasive	Urines from PHC	Invasive	Urines from PHC
Ampicillin	45.5	37.4	40.8	34.2	NA	NA	NA	NA
Mecillinam	-	5.1	-	4.0	-	15.9	-	7.2
Amox-clav	-	-	-	-	-	-	-	-
Sulfonamides		28.3		25.5		24.6		13.4
Ciprofloxacin	13	8.1	10.6	7.5	8.1	6.4	7.4	5.2
Pip-tazo	3.8	-	6.3	-	6.1	-	10.5	-
Cefuroxim	9.8	-	9.6	-	10.3	-	9.8	-
3rd. gen ceph	7.3	4.9	5.8	5.3	6.1	5.3	5.9	4.6
Gentamicin	5.7	-	4.4		3.1		2.8	
Carbapenem	0	-	0	-	0.5	-	0.5	-
Samples tested	5,398	80,851	5,835	106,236	1,28	9,227	1,399	4,246

* Primary Health Care

Carbapenemase-producing organisms (CPO) and Enterobacterales (CPE)

Carbapenemase-producing organisms (CPO) are of national and international concern as they are resistant to beta-lactam antimicrobials including carbapenems, which are used to treat serious infections, caused by multi-resistant bacteria. CPO infections are associated with high mortality and healthcare costs due to longer treatment and length of hospital stay. Importantly, CPO have the potential for transmission of resistance to other bacteria via mobile genetic elements and cause increasingly outbreaks in healthcare settings.

Detection of CPO was made notifiable in Denmark in September 2018. Acting in accordance with the CPO guidelines including infection prevention control (IPC) is of utmost importance in order to prevent further spread of CPO.

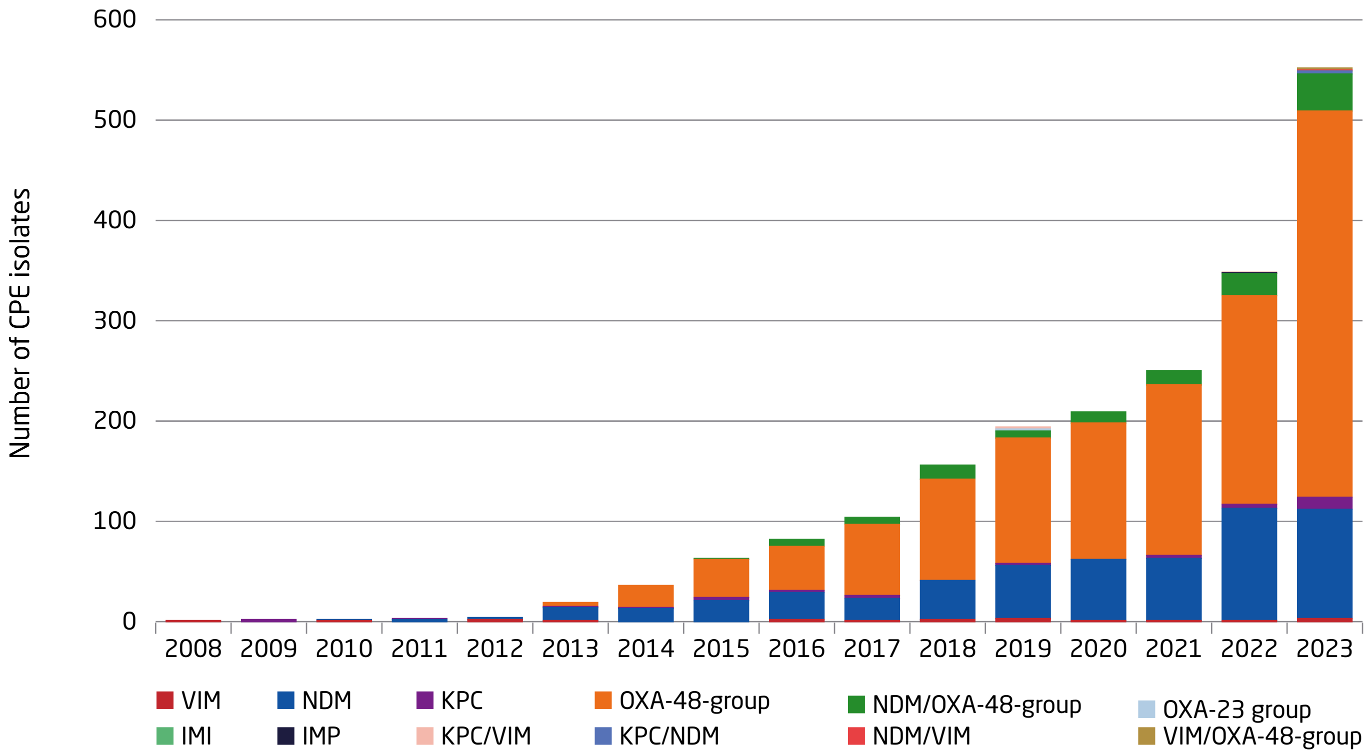
CPO comprise of two main groups:

- Intestinal bacteria (Carbapenemase-producing Enterobacterales [CPE]) e.g. *Escherichia coli, Klebsiella pneumoniae*
- Environmental bacteria e.g. *Pseudomonas aeruginosa, Acinetobacter baumannii*

Increasing numbers of CPO isolates associated with domestic outbreaks

Carbapenemase-producing organisms (CPO) have been notifiable since 2018, which includes reporting both laboratory and relevant epidemiological findings. In Denmark, the majority of cases reported and isolates received are Carbapenemase-producing Enterobacterales, CPE. All isolates received at SSI are analysed by whole genome sequencing (WGS). In 2023, a total of 552 CPE isolates were reported from 436 patients compared with 350 CPE from 304 patients in 2022, resulting in a 58% increase of CPE isolates and a 43% increase in numbers of patients. The numbers of CPE and distribution of different carbapenemase genes is presented in Figure 6.3.

Figure 6.3 Distribution of carbapenemase genes in CPE isolates, 2008-2023

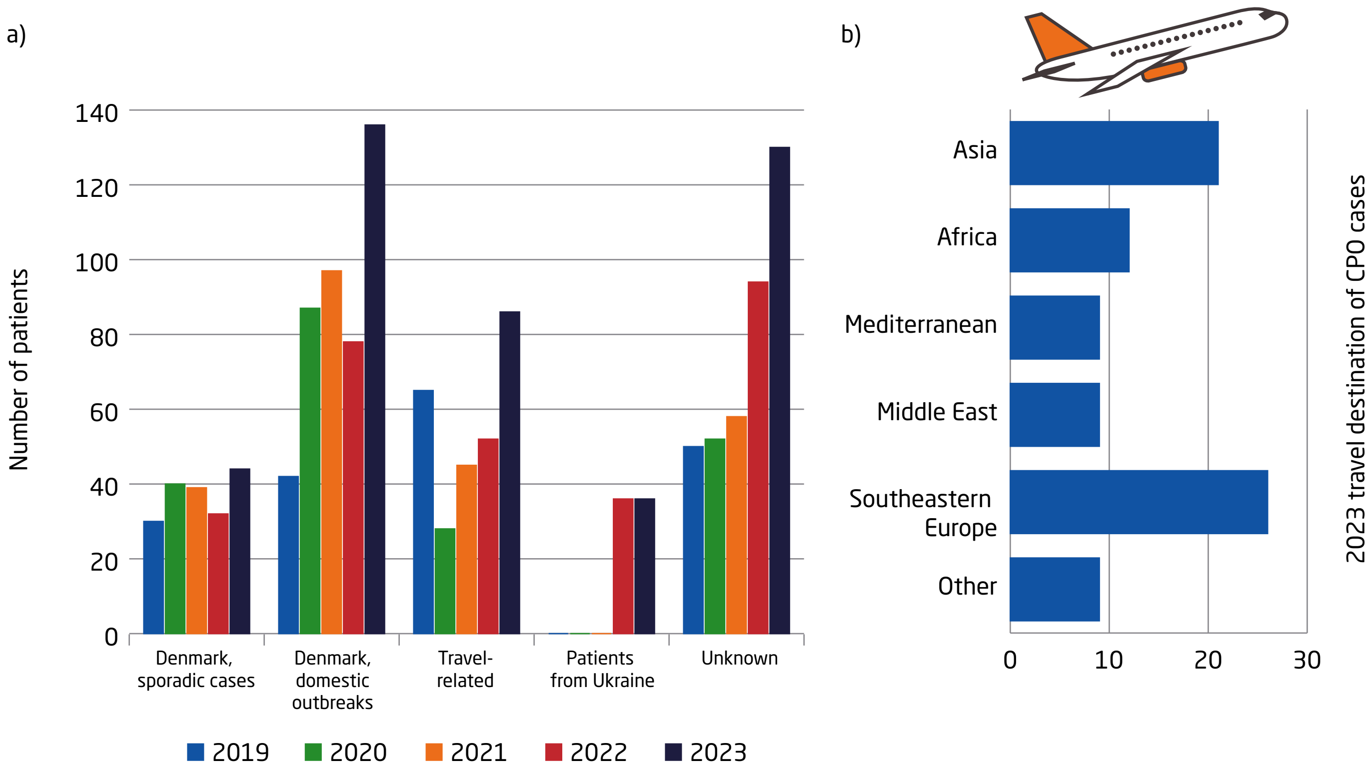


More than one isolate from the same patient was included if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases

Many of the new CPO cases were associated with outbreaks, the majority at hospitals: 26 registered outbreaks in 2023 (138 new patients) compared to 17 outbreaks in 2022 (78 new patients), (Figure 6.4a).

In 2023, for 86 persons the acquisition of CPO was associated with travel outside the Nordic countries in the period of six months prior to positive detection of CPO, (52 persons in 2022 and 45 in 2021). The destination of travel can be seen in Figure 6.4b. Due to the still ongoing conflict in Ukraine, Denmark receives patients as part of medical evacuations, (101 patients in 2023) of which 36 patients carried altogether 58 CPOs. As in former years for a relatively high number of patients no information regarding travel or probable place of origin could be obtained; 130 patients in 2023.

Figure 6.4 a) Classification of CPO cases, 2019-2023 b) World regions where patients with travel related cases have travelled



The number of vancomycin-resistant enterococci (VRE) decreased for the first time since 2014

In 2023, 722 VRE isolates were detected in Denmark; a 17% decrease compared to the 845 isolates found in 2022. This is a marked change after increases observed for most years during the past decade and particularly from 2020 to 2022. Although most VRE are detected in urinary samples, many of these may not be associated with a clinical infection but rather interpreted as an expression for colonization in patients at risk. The decreasing numbers should not lead to neglect of the importance of infection prevention and control teams to control outbreaks in healthcare facilities and implement prevention strategies.

The numbers of linezolid-resistant enterococci (LRE) and linezolid-vancomycin-resistant enterococci (LVRE) remained small in 2023 with nine *E. faecium* harboring both the G2576T and *VanB* gene. Treatment options for LVRE are very limited, which emphasizes again the importance of infection prevention and control measures but also of antimicrobial stewardship.

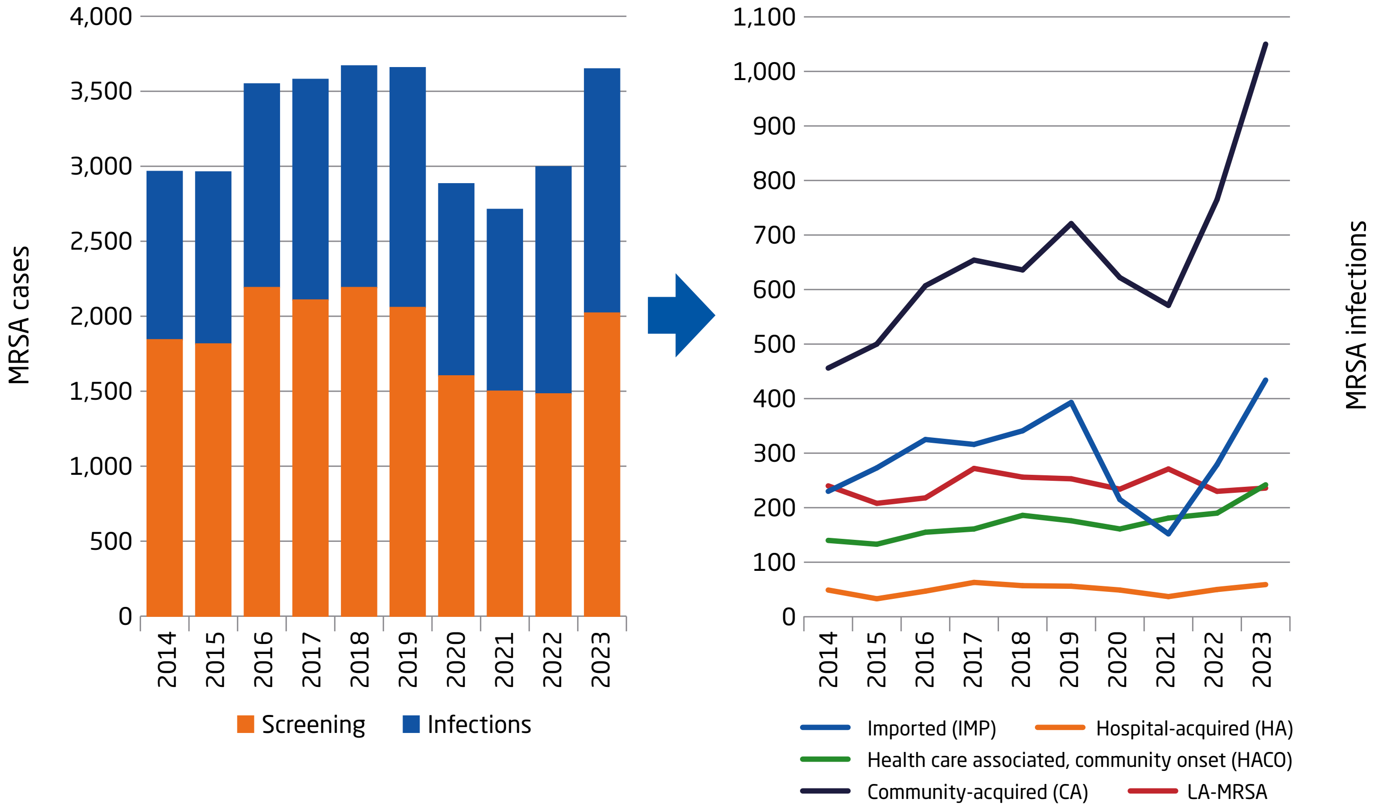
Staphylococcus aureus – MRSA cases back to prepandemic levels in 2023

In 2023, the number of invasive *S. aureus* isolates remained stable with 2,571 cases compared to 2,578 cases in 2022. Resistance levels did not change notably and remained for most cases well below the 10% percentile; noteworthy are decreasing trends for resistance to penicillin, 68% in 2023, (77% in 2014 and around 86% in the mid-90ies). Thirty-nine (1.5%) of the bacteraemia cases were caused by MRSA out of which nine were livestock-associated MRSA (LA-MRSA).

Surveillance of all MRSA cases, e.g. infected and colonized persons, showed an increase of 20% in 2023 (3,649 cases) compared to 2022 (2,982 cases) and a 23% increase compared to 2014 (2,965 cases), Figure 6.5. LA-MRSA constituted 23% of all new MRSA cases and primarily affected persons working with pigs and their households. Trends in MRSA in infected persons based on their epidemiological classification is presented in Figure 6.5.

The number of MRSA outbreaks in hospitals, nursing homes and other institutions increased in 2023 compared to 2022 and 2021, with 41 outbreaks involving 199 patients (39 outbreaks in 2022 with 143 patients; 30 outbreaks in 2021 with 109 patients).

Figure 6.5 Number and epidemiological trends of MRSA under surveillance (screening and infections), Denmark, 2014-2023



Neisseria gonorrhoeae – resistance to antimicrobials used for treatment of gonorrhoeae is currently not of concern in Denmark

Gonorrhoea, the second most common sexually transmitted bacterial infection in Denmark, is caused by *N. gonorrhoeae* (gonococci) and the DCMs have submitted isolates to SSI since 1962. Over the past decade the number of received isolates and of reported cases increased significantly. In 2023, the reference laboratory at SSI received 2,653 isolates from 2,152 unique cases. Ciprofloxacin resistance was at 45%. Azithromycin-resistance was found in 6% of tested isolates in 2023 compared to 2.9% of tested isolates in 2022.

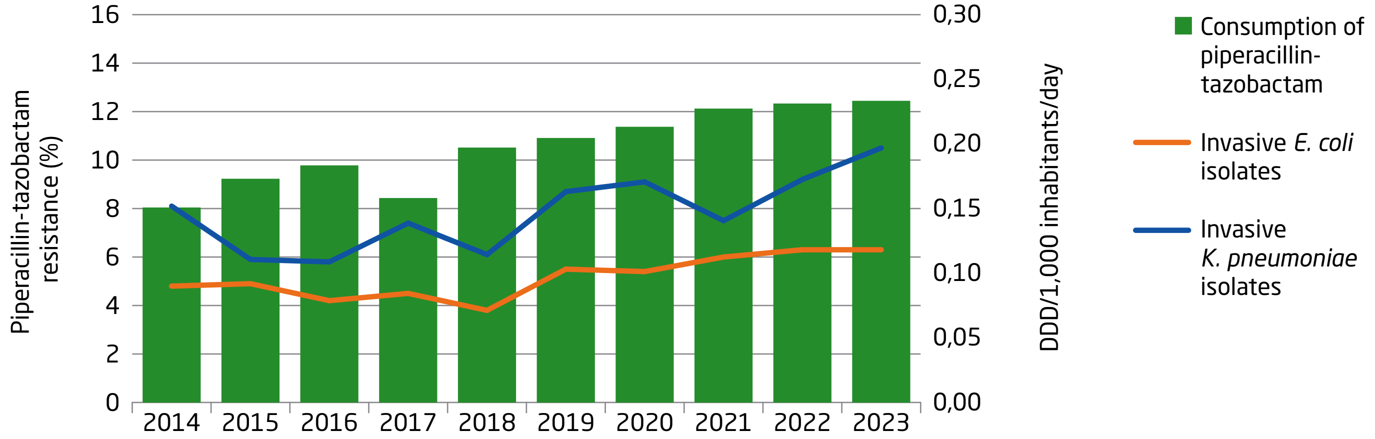
Although the occurrence of resistance among gonococci is currently not of concern in Denmark, the frequent emergence of resistance mechanisms in *N. gonorrhoeae* globally compromises the management, prevention and control of the infection in many countries. This highlights that surveillance of resistance trends is vital to ensure that treatment for gonorrhoea remains effective.

Trends of antimicrobial consumption and resistance in humans

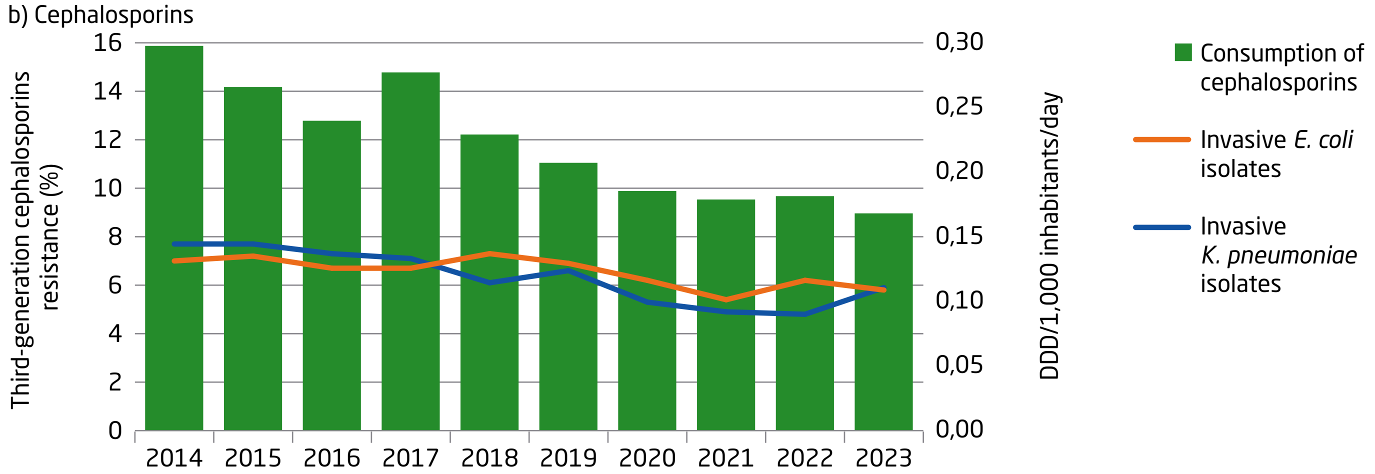
Over the last decade, there has been several changes in consumption of antimicrobials for humans, followed by changes in resistance trends. A steep increase was observed for combinations of penicillins and beta-lactamase inhibitors in hospital care. This was followed by an increase in the occurrence of resistance towards piperacillin-tazobactam in invasive *Escherichia coli* and *Klebsiella pneumoniae* from human cases, (Figure 6.6 a). On the other hand, reduced consumption of cephalosporins and fluoroquinolones (ciprofloxacin) was followed by reduced trends in resistance towards these antibiotics also in invasive *Escherichia coli* and *Klebsiella pneumoniae,* (Figure 6.6 b and c).

Figure 6.6 Resistance (%) in invasive *Escherichia coli* and *Klebsiella pneumoniae* combined with antimicrobial consumption (DID), Denmark, 2014-2023

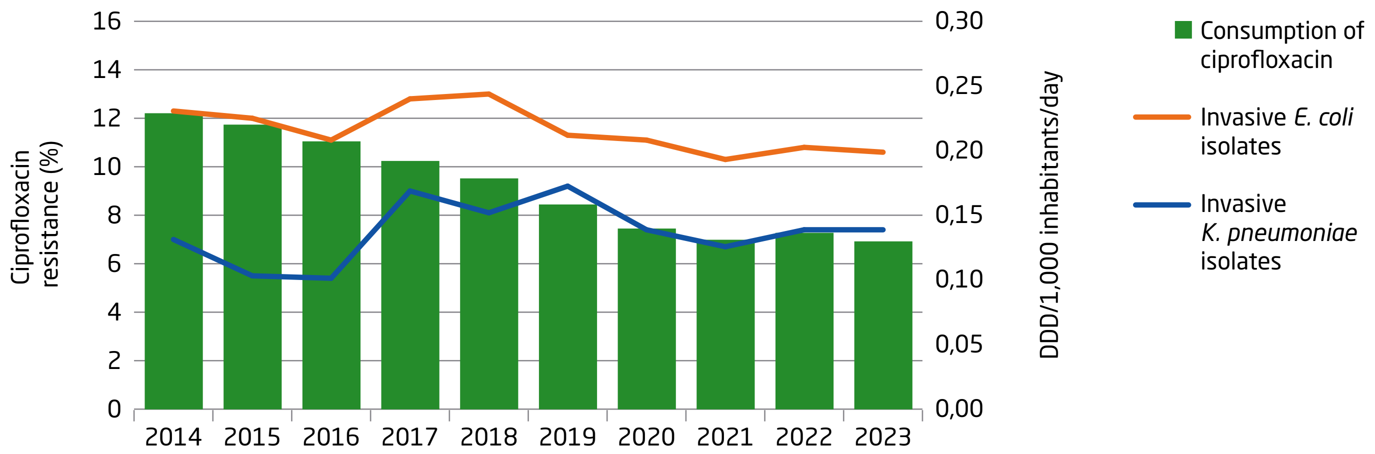
a) Piperacillin-tazobactam



b) Cephalosporins



c) Ciprofloxacin



7. Resistance in animal pathogens

Surveillance of antimicrobial resistance in 2023 focused on pathogenic bacteria from pigs and included results obtained through antimicrobial susceptibility testing (AST) and/or whole genome sequencing (WGS) of isolates belonging to *Actinobacillus pleuropneumoniae* (AST and WGS), *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *Escherichia coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *Streptococcus suis* (AST and WGS).

Most pathogenic bacteria isolated from pigs in 2023 displayed similar frequencies of phenotypic resistance as in 2022 (1-year period) and 2018 (5-year period). However, nine pathogen-drug combinations were associated with significantly increased resistance, whereas one was associated with a significantly decreased frequency.

The increased frequency of neomycin resistance in haemolytic *E. coli* (52.3%) is concerning because it is one of only a few drugs recommended in Denmark as first choice for treating *E. coli*-associated post-weaning diarrhoea. The rapid increase in neomycin resistance coincided with increased use of neomycin in weaners, (Chapter 2, Figure 2.6).

The increased frequency of gentamicin resistance in haemolytic *E. coli* (35.2%) is also concerning because it is considered critically important for human medicine by the World Health Organization.

WGS-based detection of resistance mechanisms (genes and point mutations) in pathogenic bacteria isolated from pigs in 2023 showed that 22 pathogen-resistance mechanism combinations were associated with significantly increased frequencies when compared to 2022 (1-year period) and 2021 (2-year period), whereas one was associated with a significantly decreased frequency.

Resistance towards carbapenems, 3rd, 4th and 5th generation cephalosporins, oxazolidinones and polymyxins remained at a low level.

The observed concordance between AST results and WGS-based detection of resistance mechanisms was 99.6% for *A. pleuropneumoniae*, 79.6% for *B. bronchiseptica*, 94.2% for haemolytic *E. coli*, 94.3% for non-haemolytic *E. coli*, 76.0% for *K. pneumoniae*, 97.3% for *S. enterica*, 91.5% for *S. hyicus* and 94.5% for *S. suis*.

8. One Health AMR

The zoonotic transmission of extended spectrum beta-lactamase-producing *E. coli* (here abbreviated to ESBL Ec), has been indicated as a possibility in many countries, underlining the importance of monitoring the occurrence of these bacteria in animals and humans in an integrated approach.

Here we map the frequency of multi-locus sequence types (MLST) and resistance genes and mutations in ESBL Ec recovered from livestock animals and meat and from humans. Furthermore, we study the genomic context of selected resistance genes which may have been horizontally transferred between reservoirs.

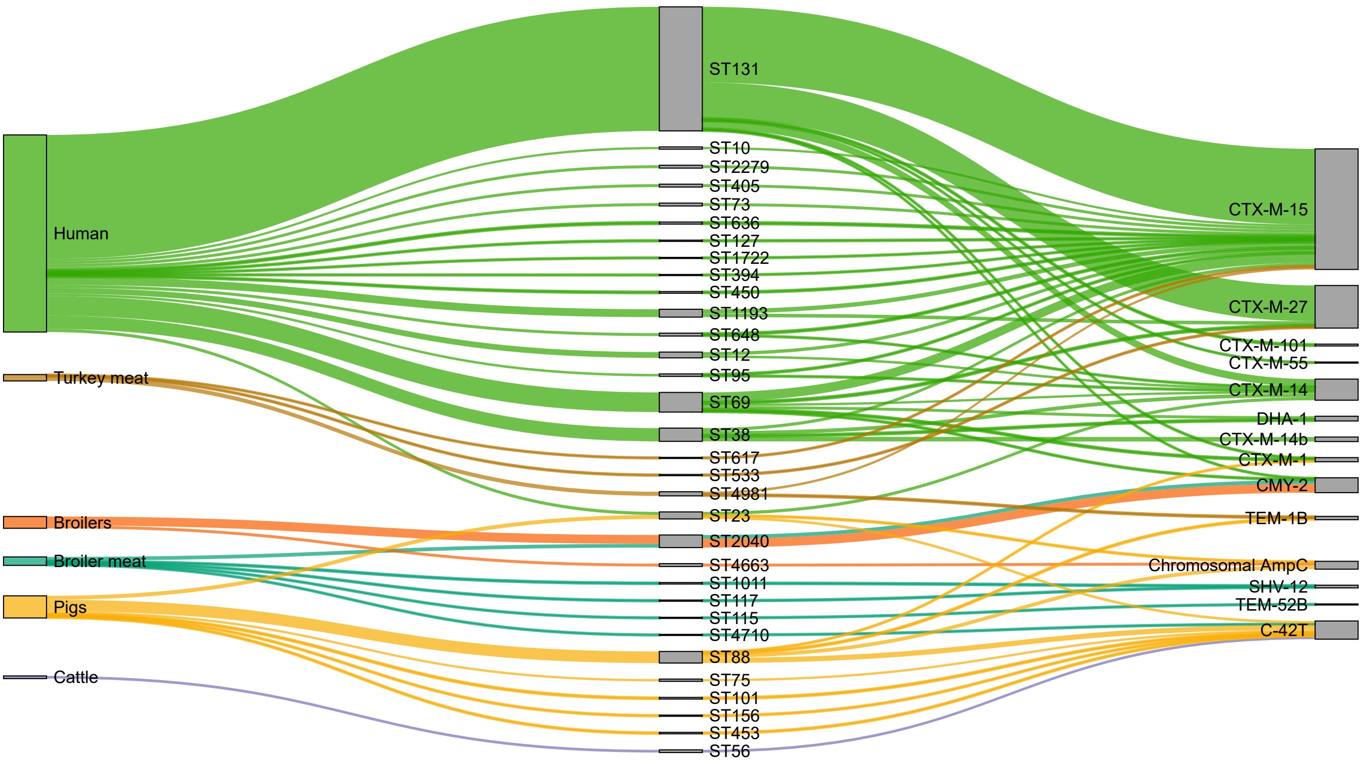
The analysis involved ESBL/AmpC *E. coli* isolates from human blood stream infections, food-producing animals and meat. A total of 1,964 isolates from 2018 through 2023 were included with 1,282 from humans, 317 from meat and 365 from animals.

Abundance distribution of MLSTs and ESBL/AmpC genotypes

Sequence types (ST) and ESBL/AmpC genes and mutations were compared using a Sankey diagram, (Figure 8.1). As in the previous years, limited overlap was found in the combination of STs and resistance genotype in isolates from humans vs. animals and meat.

Only the AmpC plasmid-mediated gene *CMY-2*, and the ESBL genes *CTX-M-1*, *CTX-M-15* and *CTX-M-27* were found in both humans and food-producing animals or meat. Interestingly, the ESBL-genes found in turkey meat were of the *CTX-M-15* and *CTX-M-27-kind*, the two most common ESBL genes among human isolates. The *CMY-2* AmpC gene was almost exclusively found among isolates from broilers and broiler meat, but also in human isolates. The *CTX-M-1* gene was mostly found among human isolates, but also in pig isolates. All isolates from cattle harboured a C-42T mutation, which was not detected among the selected human isolates.

Figure 8.1 Sankey diagram showing the host, MLST and ESBL/AmpC gene or mutation of the included isolates



Flows of a minimum of five isolates are shown

Genomic context of ESLB/AmpC genes

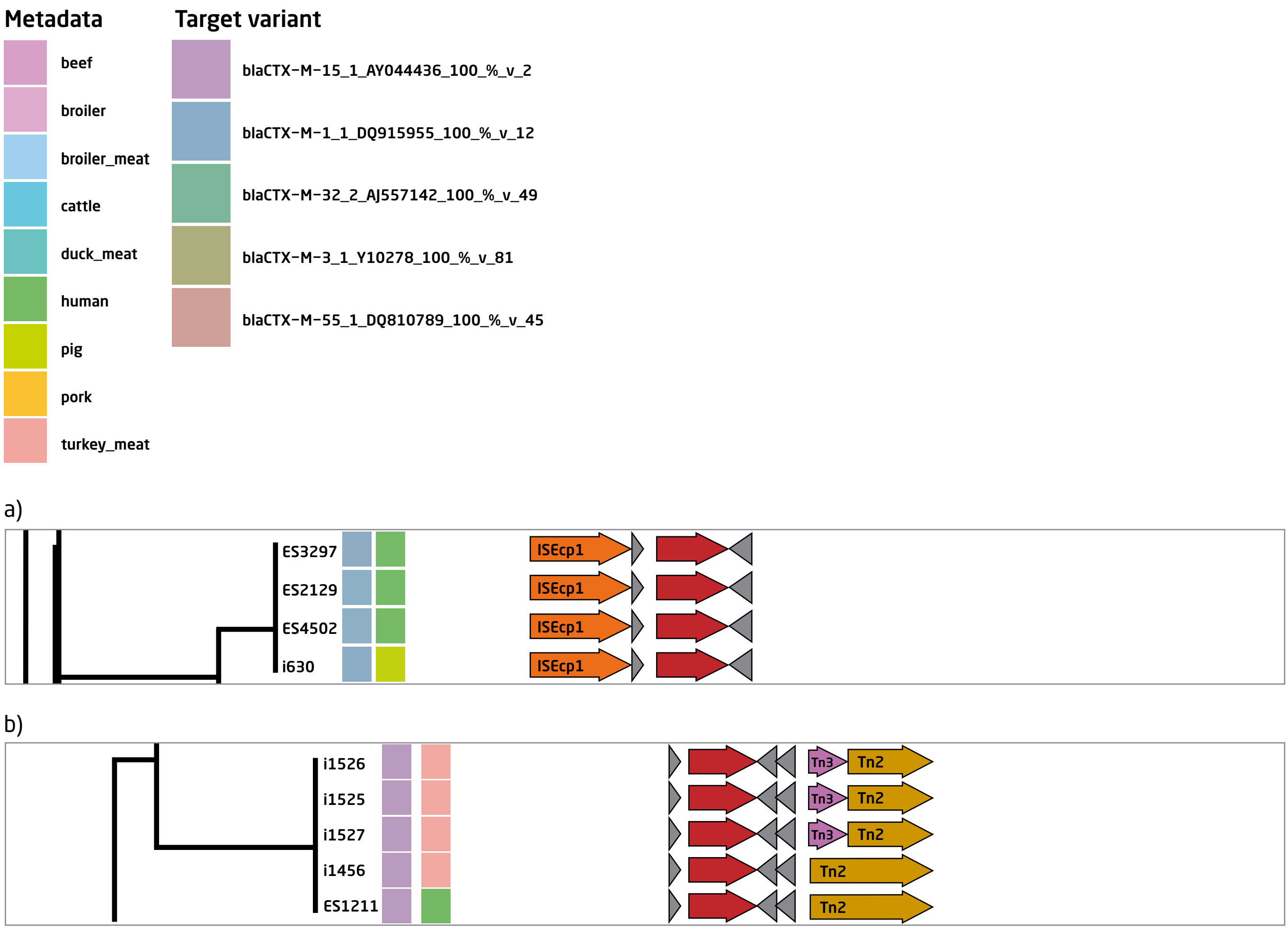
When the same acquired AMR gene is found in different reservoirs, a direct transmission link can be difficult to establish, especially if it is found in different sequence types of the same bacterium. The analysis of the DNA sequence of the gene's flanking region, i.e. the genetic code that comes before and after a gene, can assist in the determination of a probable common source, and thus indicate the possibility of occurrence of horizontal gene transfer between bacterial strains.

We further applied the bioinformatics tool Flankophile to a selection of the ESBL Ec isolates from humans, animals and meat, for analysis of flanking regions of selected ESBL/AmpC genes. The analysis focused on the ESBL genes *CTX-M-1*, *CTX-M-14*, *CTX-M-15*, *CTX-M-27*, *CTX-M-55*, *TEM-52B*, and the AmpC genes *DHA-1* and *CMY-2*.

The analysis showed some examples where the gene variant and its flanking region were identical in isolates from humans and from one or more animal hosts, (Figure 8.2).

The results of both analyses suggest that *CTX-M-1* could be shared between isolates from pigs and humans, (Figure 8.2a), as well as *CTX-M-15* could be shared between humans and turkey meat, (Figure 8.2b). The flanking region analysis clarified that the *CTX-M-27* present in turkey meat isolates, as well as the *CMY-2* present in isolates from broilers and broiler meat do not overlap with the variant present in human isolates for the isolate collection considered. Further research with larger collections of isolates, gathered over longer time spans, and including in depth genomic analyses, can be of interest to continue the investigation of the zoonotic link in ESBL Ec transmission.

Figure 8.2 ESBL genes *CTX-M-1* (a) and *CTX-M-15* (b) identical in both gene and flanking region sequences between *E. coli* isolates of animal origin and human origin



Details from Flankophile plots, showing a – clustering of *CTX-M-1* genes of human- and pig origin; b - clustering of *CTX-M-15* genes of human- and turkey meat origin. From left to right: distance tree of the gene's flanking regions (straight vertical lines indicate that the flanking regions are 95% identical); color annotation columns representing the target variant (left) and the host (right); arrows depicting the gene synteny, with the target sequence in red

9. Timeline

Early 1980's

Clinical microbiology and principles of rational antibiotic use introduced as part of medical training at universities

1986

Antimicrobial prescription guidelines for doctors in "The little green" published by Danish Medical Association

1993

Cascade rules for veterinary medicines

1994

MedStat Database with human use of medicinal products

1995

DANMAP Surveillance in food animals, food and humans

1995-1996

- VASC* contracts with farmers
- Veterinarians non profit on sale

1995-1999

Industry stop for antimicrobial growth promotors

1996

Guidelines for antimicrobial use in pigs and cattle

1997

1998

1999

2000

VetStat data collection

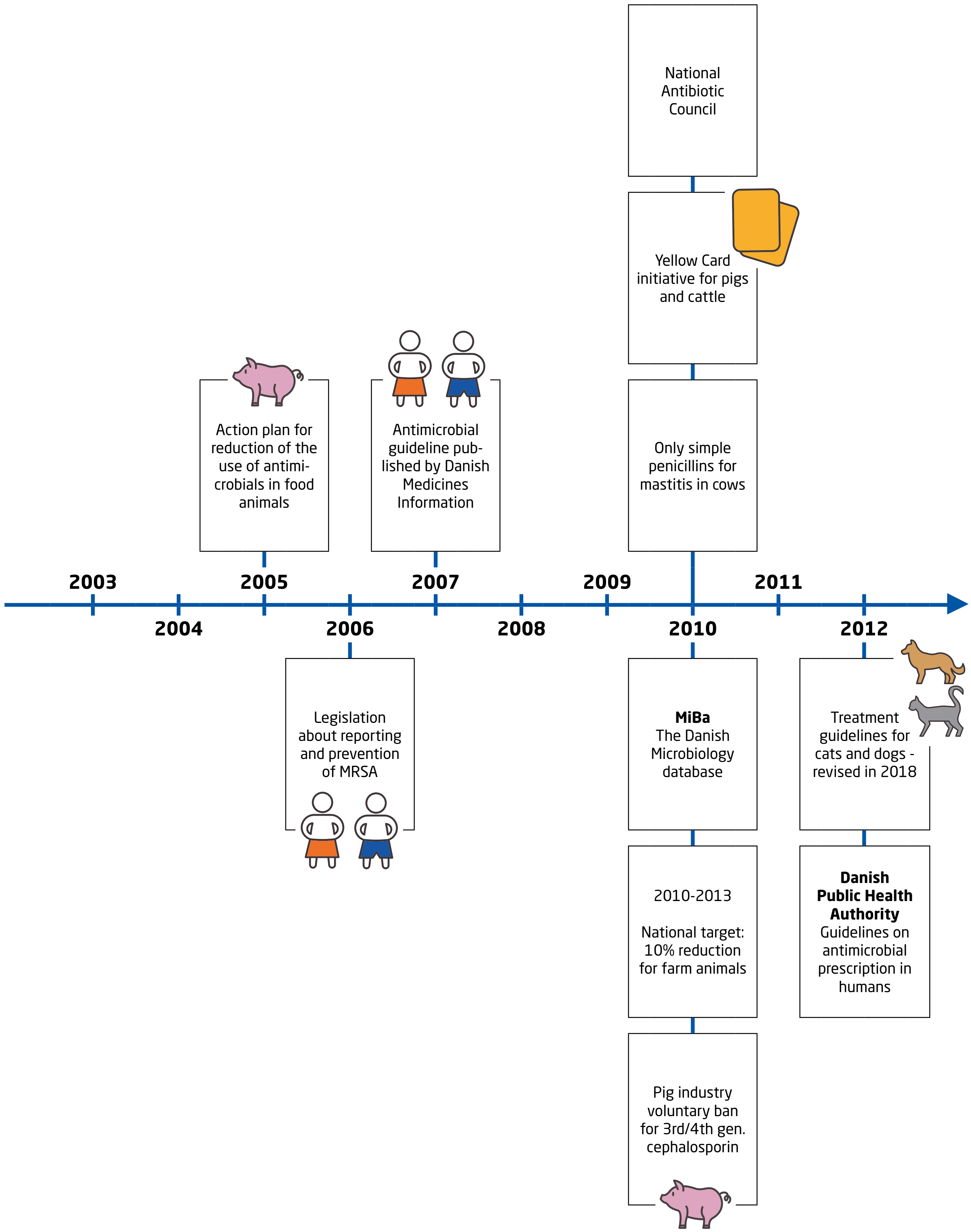
Regulation on stop for antimicrobial growth promotors

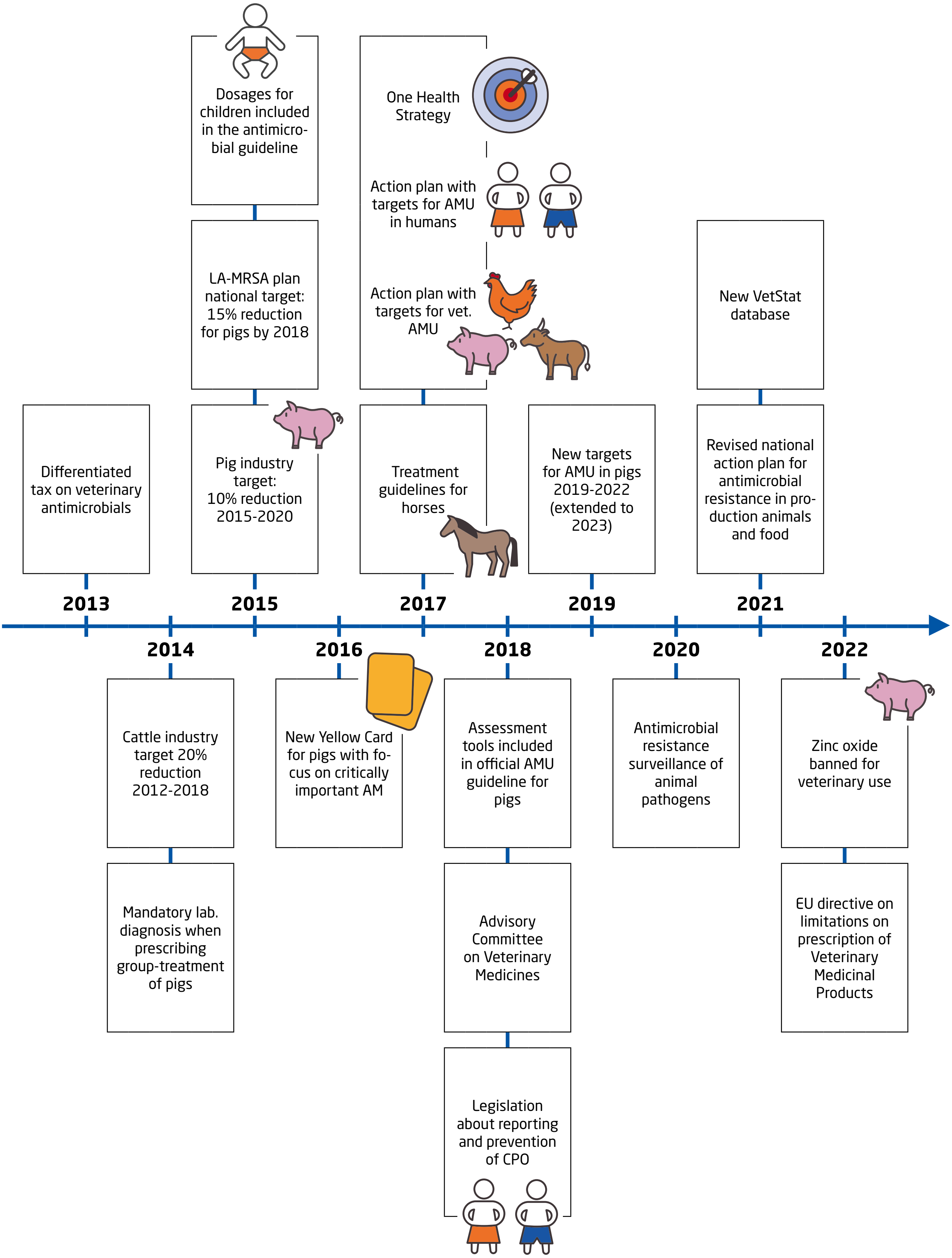
2001

2002

Restrictions on fluoroquinolones for food animals

* Veterinary Advisory Service contracts


National Antibiotic Council
Yellow Card initiative for pigs and cattle
Action plan for reduction of the use of antimi-crobials in food animals
Antimicrobial guideline pub-lished by Danish Medicines Information
Only simple penicillins for mastitis in cows
2003
2004
2005
2006
2007
2008
2009
2010
2011
2012
Legislation about reporting and prevention of MRSA
MiBa The Danish Microbiology database
Treatment guidelines for cats and dogs - revised in 2018
2010-2013
National target: 10% reduction for farm animals
Danish Public Health Authority Guidelines on antimicrobial prescription in humans
Pig industry voluntary ban for 3rd/4th gen. cephalosporin


2013
Differentiated tax on veterinary antimicrobials
2014
Cattle industry target 20% reduction 2012-2018
Mandatory lab. diagnosis when prescribing group-treatment of pigs
2015
Dosages for children included in the antimicrobial guideline
LA-MRSA plan national target: 15% reduction for pigs by 2018
Pig industry target: 10% reduction 2015-2020
2016
New Yellow Card for pigs with focus on critically important AM
2017
One Health Strategy
Action plan with targets for AMU in humans
Action plan with targets for vet. AMU
Treatment guidelines for horses
2018
Assessment tools included in official AMU guideline for pigs
Advisory Committee on Veterinary Medicines
Legislation about reporting and prevention of CPO
2019
New targets for AMU in pigs 2019-2022 (extended to 2023)
2020
Antimicrobial resistance surveillance of animal pathogens
2021
New VetStat database
Revised national action plan for antimicrobial resistance in production animals and food
2022
Zinc oxide banned for veterinary use
EU directive on limitations on prescription of Veterinary Medicinal Products

10. List of abbreviations

AGP	Antimicrobial growth promoter
AMU	Antimicrobial use
AMR	Antimicrobial resistance
CA	Community-acquired
CPE	Carbapenemase-producing Enterobacterales/Enterobacteriaceae
CPO	Carbapenemase-producing organisms
DAD	Defined Daily Doses per 100 admissions
DADD	Defined Animal Daily Dose
DAPD	Defined Animal Daily Dose per 1,000 animals per day
DBD	Defined Daily Doses per 100 occupied bed-days
DCM	Department of clinical microbiology
DDD	Defined Daily Dose
DID	Defined Daily Doses per 1,000 inhabitants per day (DDD/1,000 inhabitants/day)
DTU	Technical University of Denmark
DVFA	Danish Veterinary and Food Administration
ESC	Extended Spectrum cephalosporinase
HAI	Hospital-acquired infections
HCAI	Health care associated infections
HACO	Health care associated community onset
MiBa	The Danish Microbiology Database
MDR	Multidrug-resistant
MRSA	Methicillin-resistant Staphylococcus aureus
PHC	Primary health care
SSI	Statens Serum Institut
VASC	Veterinary advisory service contracts
VMP	Veterinary medicinal products
VetStat	Danish Register of Veterinary Medicines
VRE	Vancomycin-resistant enterococci
WGS	Whole-genome sequencing
WHO	World Health Organization

Read more - the DANMAP 2023 report

Further data and analyses can be found in the full DANMAP 2023 (www.danmap.org), including textboxes on topics of special interest of this year.

List of Textboxes

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Textbox 4.2	Veterinary medicines and antibiotic resistance
Textbox 5.1	International approach to improve supply of antibiotics
Textbox 5.2	HALT 4 – An audit on infections and consumption of antibiotics among residents in Danish nursing homes
Textbox 5.3	Infection Prevention and Control and prevention of Antimicrobial Resistance goes hand in hand
Textbox 5.4	Consumption of antimicrobials in the Faroe Islands
Textbox 6.1	Trends in phenotypic- and genotypic fluoroquinolone resistance in *Campylobacter jejuni* from broilers and broiler meat in Denmark
Textbox 7.1	Ecogenomics of Danish cattle *E. coli* between 2001 and 2019
Textbox 8.1	Danish surveillance of azole resistant *Aspergillus fumigatus* from clinical samples – a 4-year update
Textbox 8.2	Increasing rates of drug resistance in *Mycobacterium tuberculosis* isolates in Denmark
Textbox 8.3	First results from antimicrobial resistance monitoring in *Shigella* spp. in Denmark

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