6

RESISTANCE IN ZOONOTIC BACTERIA AND ANIMAL PATHOGENS

6. Resistance in zoonotic bacteria and animal pathogens

Highlights: In Denmark, antimicrobials are generally not recommended for treatment of self-limiting diarrhoea in humans including salmonellosis and campylobacteriosis. In prolonged or severe cases, treatment may be required and in these cases macrolides (azithromycin) and in hospital settings, ciprofloxacin are recommended.

Erythromycin (macrolide) resistance was present in 4% of *Campylobacter jejuni* isolates from humans with a known travel history. Erythromycin resistance was not observed in human isolates from domestic infections, broiler meat, broilers and cattle.

Resistance to quinolones remained common in *C. jejuni* isolates from humans, Danish broiler meat, broilers and cattle. The levels of ciprofloxacin resistance was 70% in human isolates and 45% and 65% in isolates from broilers and meat hereof, respectively. Resistance towards ciprofloxacin were often accompanied by tetracycline resistance and 51% of the human isolates, and 39% and 30% of the broiler and broiler meat isolates, respectively, were tetracycline resistant.

The level of azithromycin resistance in *Salmonella* Typhimurium isolates was 1% in human isolates and 3% in isolates from Danish pork. Among human cases, resistance to fluoroquinolones was observed in 14% of *S.* Typhimurium isolates from travel-related cases and in 5% of the isolates from domestically acquired cases. Fluoroquinolone resistance has not been recorded in *S.* Typhimurium from Danish pigs and pork since 2010 and 2007, respectively.

Resistance to 3rd generation cephalosporins and carbapenems was not observed in *S.* Typhimurium isolates from animals, food and domestically acquired human cases. Two percent of *S.* Typhimurium from travel-associated cases were resistant to 3rd generation cephalosporins. Carbapenem resistance was not observed.

Surveillance of resistance in animal pathogens was expanded to include pathogens from small animals and mastitis pathogens in addition to the usual porcine pathogens. In all populations and pathogens, the resistance levels remained fairly stable.

6.1 Introduction

Zoonoses are infectious diseases that can be transmitted between animals and humans, either through direct contact with animals or indirectly by contaminated food, water, vectors or the environment. A detailed description of the trends and sources of zoonoses in Denmark and of national surveillance and control programmes can be found in Annual Report on Zoonoses in Denmark 2019 [www.food.dtu.dk].

Campylobacter and *Salmonella* surveillance has been part of the DANMAP programme since 1995. It monitors AMR patterns in broilers, cattle and pigs; isolates from human cases and from fresh meat were included from 1997.

In Denmark, antimicrobials are not recommended for treatment of diarrhoea in patients unless there is prolonged duration or the patient is severely ill. If treatment is required, macrolides (azithromycin) are recommended for treatment of *Campylobacter* infections. There are no general recommendations for treatment of zoonotic *Salmonella* infections in the primary sector, but for infections treated in hospitals, azithromycin or ciprofloxacin is recommended [http://pro.medicin.dk]. The Register of Medicinal Product Statistics at the Danish Health Data Authority does not register the use of antimicrobials specifically for treatment of *Campylobacter* and zoonotic *Salmonella* infections.

Macrolides are used to treat infections in animals in Denmark, whereas flourquinolones and cephalosporins are not used in food-producing animals. In 2019, 12,820 kg of macrolide was prescribed for animals. The majority (93%) of these were used in pigs, whereas cattle and poultry used 190 kg and 228 kg, respectively (Table 4.1).

6.2 *Campylobacter*

For more than a decade, campylobacteriosis has been the most frequently reported bacterial zoonotic disease in Denmark and in the rest of Europe. In 2019, the number of registered cases in Denmark was 5,389 or 92.7 cases per 100,000 inhabitants. Previous studies have shown that the main route of transmission is food, in particular poultry meat, raw milk, contaminated vegetables and water. Other sources are contact with contaminated water during recreational activities and contact with animals [Annual Report on Zoonosis in Denmark 2019].

Several studies on *Campylobacter* sources and their relative impact were carried out in 2015-2017 [Annual Report on Zoonosis in Denmark 2017]. A source attribution study, where isolates from domestically acquired cases were compared to food, animal and environmental isolates, in conjunction with a case control study pointed at chicken meat and cattle/beef as the two major sources. Furthermore, recent analyses of whole-genome sequencing (WGS) data have revealed a large number of small clusters of human cases (comprising 47% of all cases) as well as genetic matching of 30% of the isolates from humans to isolates from food, primarily chicken meat [Joensen et al 2020. Emerg Infect Dis 26: 523].

In humans, campylobacteriosis is a notifiable disease. In order to monitor resistance, a selection of isolates from human *C. jejuni* cases are susceptibility tested. *Campylobacter* isolates were submitted to Statens Serum Institut (SSI) by three clinical microbiological laboratories. The isolates were geographically dispersed and represented both urban and rural areas of Denmark. Roughly, the same number of isolates was susceptibility tested each month and only one isolate per patient was tested. Travel histories of the patients were collected, when possible, and reported to the diagnostic laboratory with submission of patient samples. A human isolate was categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. The DANMAP sampling strategy has been the same in recent years.

The isolates were typed at SSI, and 284 *C. jejuni* isolates were susceptibility tested in accordance with the ECDC recommendations. Of 284 isolates, 278 were assigned a sequence type (ST, 7-locus MLST) [Dingle et al 2001. J Clin Microbiol 39:14]. A total of 86 different ST types was present among the tested isolates. With 44 isolates, ST122 was the most frequent ST type among the tested strains. ST50 (14 isolates), ST19 (13 isolates), ST42 (12 isolates), ST48 (11 isolates), and ST572 (10 isolates) were also frequent ST types. The high occurrence of ST122 isolates was due to a large nationwide outbreak caused by Danish chicken meat that continued throughout 2019, and the isolates selected for DANMAP included 43 of these outbreak isolates.

The animal *Campylobacter* isolates for DANMAP were obtained at slaughterhouse sampling of randomly selected broiler caeca (174 flocks), broiler leg-skin samples (1,248 slaughter batches) and cattle caeca (142 cattle <1 year of age). One isolate per farm or leg-skin sample was susceptibility tested. Sampling, isolation and susceptibility methods followed EFSA's recommendations for animal and food isolates.

MIC distributions for *C. jejuni* from broilers, cattle and humans are available in the web annex (Tables A6.1- A6.2). For further details on methodology, see chapter 9.

6.2.1 Resistance in *Campylobacter jejuni*

Among the domestically acquired human infections, 35% of the isolates were fully sensitive to all antimicrobials tested (Table 6.1). This is the lowest number of fully sensitive *C. jejuni* reported within the last five years, and mainly due to the increase in ciprofloxacin and tetracycline resistance (Figure 6.2). The number of fully sensitive isolates from patients with a known history of travel was 12% and significantly lower than the corresponding number from domestically acquired cases.

Among isolates of broiler origin, the level of fully sensitive isolates continued to decrease. This reflects the continued increase in isolates with resistance to ciprofloxacin and tetracycline from 28% in 2018 to 32% in 2019 in broilers and broiler meat combined (Figure 6.2).

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and human cases, Denmark DANMAP 2019

	Broilers	Broiler meat	Cattle	Human			
	Danish	Danish	Danish	Domestically acquired	Travel abroad reported	Unknown origin	Total
Antimicrobial agent	%	%	%	%	%	%	%
Ciprofloxacin	45	65	20	63	86	70	70
Erythromycin	0	0	0	0	4	2	1
Gentamicin	0	0	0	0	0	0	0
Nalidixic acid	43	65	20	63	86	70	70
Streptomycin	2	4	3	4	14	4	7
Tetracycline	39	30	11	43	67	52	51
Fully sensitive (%)	55	35	71	35	12	26	27
Number of isolates	56	209	114	155	83	46	284

Note: An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Total number of human cases includes infections of unknown origin

In cattle, the level of fully sensitive isolates remained the same as in previous years (67-72%, Figure 6.2). The level of resistance to ciprofloxacin dropped significantly in 2018 from 30% to 20%, and remained at the lower level in 2019 (Figure 6.1).

Macrolide resistance in *Campylobacter* was monitored using erythromycin, and no erythromycin resistance was observed in *C. jejuni* from cattle, broilers, broiler meat or domestically acquired human cases in 2019. Low levels of resistance were observed among isolates from travel-related cases, and from patients with unknown travel status (Table 6.1).

These low levels of erythromycin resistance were in line with the levels observed over the last decade (Figure 6.1). During the last ten years, macrolide resistance never exceeded 7% in human isolates in any year. Only few erythromycin-resistant *C. jejuni* isolates were identified in poultry and cattle in the last decade, varying between zero and two resistant isolates per year. This suggests that the actual prevalence of macrolide resistance in animal and food isolates remains very close to the limit of detection by the current sampling scheme and is only captured sporadically. Based on the available number of isolates, we are 95% confident that macrolide resistance in 2019 is not exhibited by more than 1.1% and 3% *C. jejuni* from broiler/broiler meat and cattle, respectively (see section 9.7).

A continued increase was observed for ciprofloxacin resistance in *C. jejuni* from broilers and broiler meat, and statistically significant increasing trends have been observed over the last 10 years (Figure 6.1). The increase in ciprofloxacin resistance in isolates from domestically acquired human infections was influenced by the large nationwide outbreak that was caused by a ciprofloxacin-, nalidixic acid- and tetracycline-resistant clone. The implementation of routine-based WGS typing for surveillance of human campylobacteriosis has improved the possibility of identifying epidemiological clusters, and this again has facilitated a more sophisticated analysis of the resistance data. However, clusters of outbreak-related strains have likely been around in the previous years, so it is hard to estimate the influence of outbreaks when establishing trends on resistance. However, high ciprofloxacin resistance was also observed internationally in broilers, broiler meat and humans [EFSA/ECDC 2019. EFSA journal 17(2):5598]. Ciprofloxacin resistance levels were similar in domestically acquired human infections and the main *Campylobacter* sources: broilers and broiler meat.

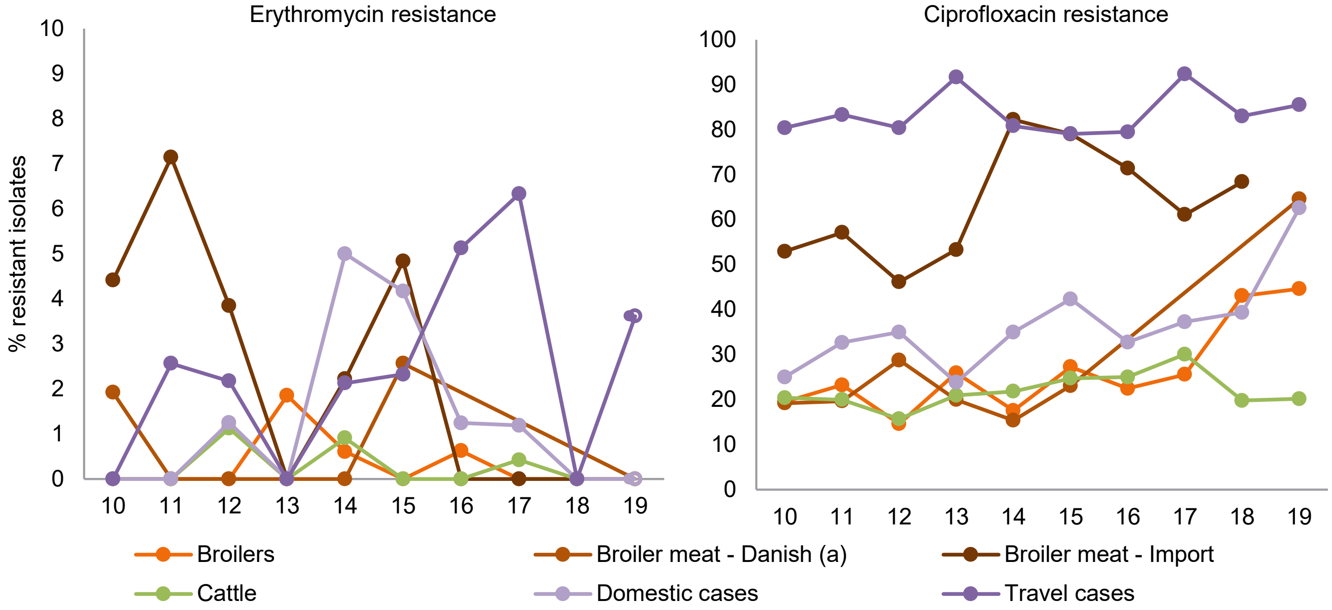
Previously, ciprofloxacin resistance was almost similar in broilers and cattle. However, in 2018 and 2019, the levels in cattle dropped and the levels in broilers increased, resulting in an approximately 20% difference in ciprofloxacin resistance between the two animal reservoirs. Flouroquinolones have not been used for production animals in Denmark for over a decade.

Despite the increase in ciprofloxacin and tetracycline resistance in domestic human *C. jejuni* isolates, the occurrence of resistance to ciprofloxacin and tetracycline was still higher in travel-associated isolates (86% and 67%, respectively) than in isolates from domestically acquired infections (63% and 43%, respectively).

Resistance to ciprofloxacin or ciprofloxacin in combination with tetracycline was the most frequently observed resistance in the human isolates from 2019. Ciprofloxacin resistance was prevalent in 22% and 20% of the domestic and travel related isolates, respectively, and ciprofloxacin in combination with tetracycline was prevalent in 41% and 65% of the domestic and travel-related isolates, respectively (Figure 6.2).

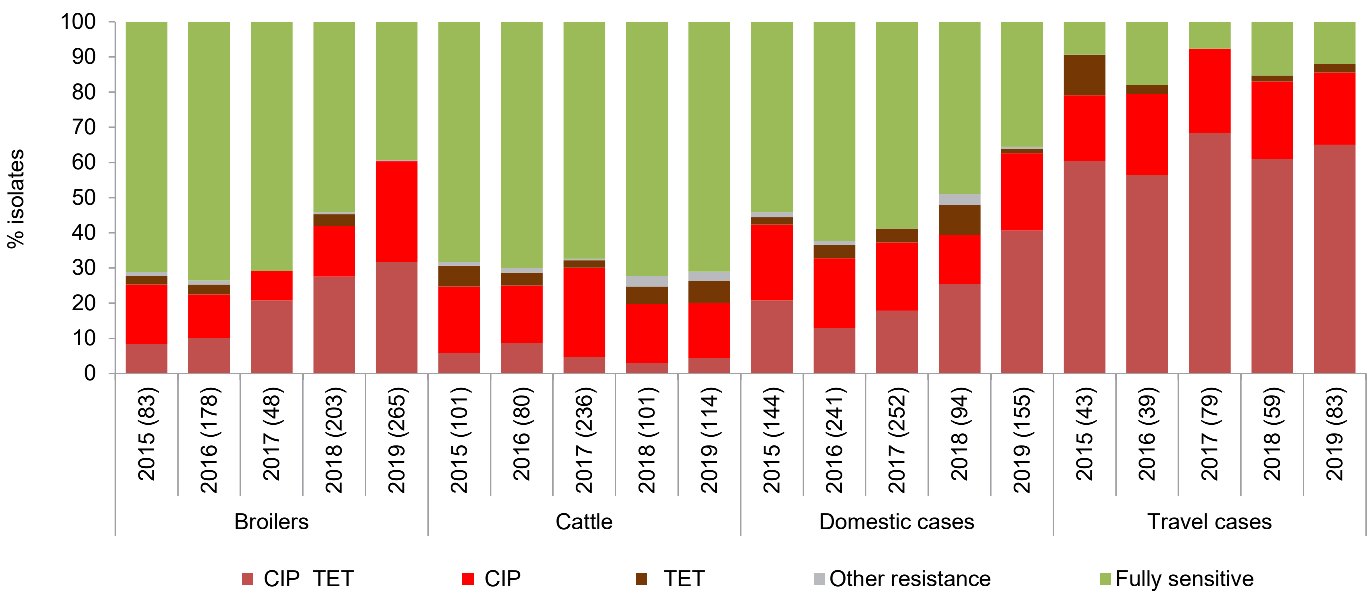
Also among the resistant isolates from animal and meat from 2019, resistance to quinolones only (94/194) or quinolones in combination with tetracycline only (81/194) was most frequent in 2019 (Figure 6.2). The distribution of AMR profiles is presented in the web annex (Table A6.3).

Figure 6.1 Erythromycin and ciprofloxacin resistance (%) among *Campylobacter jejuni* from broilers, broiler meat, cattle and human cases, Denmark DANMAP 2019



Please note the 10-fold difference in the y-axis in the two graphs. Isolates originate from broiler meat ready for retail (2010 to 2015) and leg-skin samples collected after slaughter (2019). No data from 2016-2018

Figure 6.2 Distribution (%) of AMR profiles in *Campylobacter jejuni* from broilers, cattle and human cases, Denmark DANMAP 2019



Note: The number of isolates included each year is shown in parentheses, where broilers include isolates from Danish broiler meat. CIP: all isolates with ciprofloxacin resistance but not tetracycline resistance, TET: all isolates with tetracycline resistance but not ciprofloxacin resistance, CIP TET: all isolates with both ciprofloxacin and tetracycline resistance, Other resistance: all isolates without both ciprofloxacin and tetracycline resistance. CIP TET, CIP and TET isolates may be resistant to erythromycin, nalidixic acid or streptomycin

Fluoroquinolones are not used in food production animals in Denmark, suggesting that the continued increase in ciprofloxacin resistance in broilers is driven by something other than the direct usage of fluoroquinolones. In general, the Danish poultry sector uses only a few antimicrobials, but tetracyclines is the most common antimicrobial used in poultry. The high level of *C. jejuni* isolates with both ciprofloxacin and tetracycline resistance suggests the potential for co-selection of ciprofloxacin resistance by the use of tetracycline in poultry. Whether that is what happens in reality warrants further investigation.

As in 2018, gentamicin resistance was not observed in any of the human isolates, and the level of streptomycin resistance was also in line with the reported levels in the previous years with a total of 7% resistant isolates, and resistance levels of 4% and 14%, respectively, for isolates from domestic and travel-related cases.

Similar to previous years, no resistance to gentamicin was observed in broiler isolates in 2019 (Table 6.1), providing 95% confidence that resistance to the antimicrobial is only present in 1.1% or less of the *C. jejuni* isolates from broilers and broiler meat from Denmark.

Campylobacter jejuni from organic and free-range broilers

During 2019, *Campylobacter* isolates were collected from organic and free-range broiler flocks. At slaughterhouses, leg-skin samples were randomly collected from 123 slaughter batches. One isolate per sample was sequenced using WGS. ResFinder 4.0 was used for detection of antimicrobial resistance genes and the corresponding phenotypic resistance (see section 9.6).

Campylobacter jejuni was recovered from 68% of the samples, and WGS data was available from 83 isolates. No genes conveying resistance to erythromycin or gentamycin was detected, and point mutations in the *gyrA* genes were found to cause quinolone resistance (Table 1). Genotypic resistance to ciprofloxacin and nalidixic acid (51%) was lower than observed in isolates from conventionally produced broiler meat (65%, based om MIC testing). In organic broiler meat, the occurrence of isolates with tetracycline resistance genes was higher than in isolates from conventionally produced broiler meat with phenotypic tetracycline resistance (Tables 1 and 6.1).

This is the first report on resistance in a nationally representative sample of organic broilers in Denmark, and it is uncertain whether the high levels are persistent or just a sporadic finding. Different methods for resistance detection in isolates were used in isolates from conventional and organic broilers, which may contribute to the observed differences.

Table 1 Occurence of genotypic resistance in *Campylobacter jejuni* isolates from organic and free-range broilers, Denmark
DANMAP 2019

Antimicrobial agent	Number (%) of isolates	Resistance genes (ResFinder 4.0)
Ampicillin	1 (1%)	*bla*OXA-61 (n = 1)
Ciprofloxacin	42 (51%)	*gyrA* (p.T86I) (n = 42)
Erythromycin	None	
Gentamicin	None	
Nalidixic acid	42 (51%)	*gyrA* (p.T86I) (n = 42)
Streptomycin	9 (11%)	*ant*(6)-Ia (n = 4), *aad*E-Cc (n = 5)
Tetracycline	48 (58%)	*tet*(O/32/O) (n = 47), *tet*(O) (n = 16)
Number of isolates	83	

Note: ResFinder 4.0 identified resistance genes in 48 of the 83 isolates with available WGS data

6.3 *Salmonella*

Salmonella is the second most frequent zoonotic bacterial pathogen in humans in Denmark as well as in the EU and can have a severe impact on both animal and human health [Annual Report on Zoonoses in Denmark 2019; ECDC/EFSA 2018. EFSA journal 16(12):5500].

In 2019, a total of 1,120 human laboratory-confirmed cases of salmonellosis were reported (19.3 cases per 100,000 inhabitants). The most common serotypes were *S.* Enteritidis and *S.* Typhimurium (including the monophasic variants) with 5.3 and 4.7 cases per 100,000 inhabitants, respectively [Annual report on Zoonoses in Denmark 2019].

In Denmark, human *S.* Typhimurium cases are often associated with contaminated pork, whereas cases caused by *S.* Enteritidis frequently are associated with travel. *S.* Typhimurium often displays a broad spectrum of resistance. Clonal dissemination seems to play an important role for the occurrence of antimicrobial resistance among *S.* Typhimurium [Lucarelli et al. 2010. J Clin Microbiol 48:2103-2109], and the rapid, global dissemination of genomic islands conferring resistance to ampicillin, streptomycin, sulfonamide and tetracycline (the ASSuT profile) among *S.* Typhimurium and its monophasic variants has increased the occurrence of multi-resistant *Salmonella* in Europe [EFSA/ECDC 2019. EFSA journal 17(2):5598].

The Danish *S.* Typhimurium isolates from production animals and humans are often resistant to ampicillin, sulfonamides and tetracycline (ASuT). However, these antimicrobials are not used for treatment of salmonellosis, and thus the public health impact of ASuT resistance is of less direct importance than resistance to critically important antibiotics such as macrolides and fluoroquinioloes that are used for treatment of humans.

Salmonellosis is a notifiable disease in humans, and all clinical isolates were submitted to SSI by the departments of clinical microbiology. The travel history of the patient was collected, when possible, and registered with the patient samples. With exception of S. Entertidtis, all incoming isolates were tested for susceptibility. For *S.* Enteritidis, a selection of strains were tested. Routinely the isolates were analysed by whole-genome sequencing, and the 7-locus MLST (sequence types) were derived (Kidgell et al. 2002, Infect Genet Evol. 2: 39-45.). The isolates were susceptibility tested phenotypically in accordance with the ECDC recommendations. Only one isolate per patient was tested.

Salmonella isolates from pigs were obtained from national surveillance and control programmes at slaughterhouses by sampling randomly selected pig caeca (798 animals) and carcase swabs (10,743 animals). One isolate per farm or pool of swabs was susceptibility tested. The occurrence of *Salmonella* in broilers, layers and cattle is monitored in Denmark, but only few isolates were found in 2019. Susceptibility testing of all *Salmonella* isolates was carried out in compliance with an updated Danish regulation on critically important antimicrobial resistance in *Salmonella* from poultry, cattle and pigs, which came into force 1st of January 2019 (order No. 1424/2018). For animal and food isolates, sampling, isolation, and susceptibility testing followed the methods recommened by EFSA.

In DANMAP, *S.* Typhimurium includes the monophasic variants with antigenic formulas S. 4, [5],12:i:-, unless otherwise stated. MIC distributions and occurrence of resistance among isolates from pigs, pork and humans are presented in the web annex (Tables A6.3 - A6.6).

6.3.1 Resistance in *S.* Typhimurium

S. Typhimurium, including the monophasic variants, was isolated from 45 pig cacae samples and from 59 pork samples, where 80 of the 104 isolates were monophasic variants.

A total of 271 human *S.* Typhimurium isolates, consisting of 86 diphasic and 185 monophasic variants, were susceptibility tested. ST types were derived for all human isolates except one. The monophasic isolates were dominated by ST34 (166 isolates) and the diphasic variants were dominated by ST19

Table 6.2 Resistance (%) in *Salmonella* Typhimurium isolates from pigs, pork and humans, Denmark DANMAP 2019

	Pigs	Pork	Human		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%
Ampicillin	76	78	77	63	73
Azithromycin	0	3	2	0	<1
Cefotaxime	0	0	0	2	<1
Ceftazidime	0	0	0	2	<1
Chloram-phenicol	11	12	8	12	7
Ciprofloxacin	0	0	4	14	6
Colistin	0	0	2	5	2
Gentamicin	16	7	3	6	2
Meropenem	0	0	0	0	0
Nalidixic acid	0	0	3	3	2
Sulfonamide	80	83	73	55	66
Tetracycline	80	73	75	57	70
Tigecycline	0	0	4	0	2
Trimethoprim	18	12	12	12	9
Fully sensitive (%)	13	12	14	31	21
Number of isolates	45	59	97	65	271

Note: Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas S. 4,[5],12:i:-.Total number of human cases includes infections with unknown travel status. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel (Table 9.3). None of the colistin resistant strains harboured *mcr* genes

(66), ST34 (9) and ST36 (9). Eighty-nine monophasic isolates were associated with four outbreaks that encompassed 57 (ST34), 14 (ST34), 13 (ST5296), and 5 (ST34) cases. With a few exceptions, the outbreak strains were ASuT-resistant.

Since 2014, macrolide resistance in *Salmonella* has been monitored using azithromycin. In 2019, azithromycin resistance was observed in two *S.* Typhimurium isolates from Danish pork (3%) and in 2% of *S.* Typhimurium isolates from domestically acquired human cases (Table 6.2 and Figure 6.3).

From Danish pigs and pork, between zero and three azithromycin-resistant *S.* Typhimurium isolates per year were detected, and resistance never exceeded 6% annually during 2014-2019 (Figure 6.3). This indicates that the actual prevalence of azithromycin resistance in pigs and pork is close to the detection limit offered by the current sampling scheme.

During the last ten years, ciprofloxacin resistance in *S.* Typhimurium from Danish pigs and pork has rarely been observed, and, in 2019, none of 105 isolates were resistant to ciprofloxacin. The level of ciprofloxacin resistance in isolates from domestically acquired and travel associated human cases was 4% and 14%, respectively. These figures are in line with the observations in the previous years (Figure 6.3).

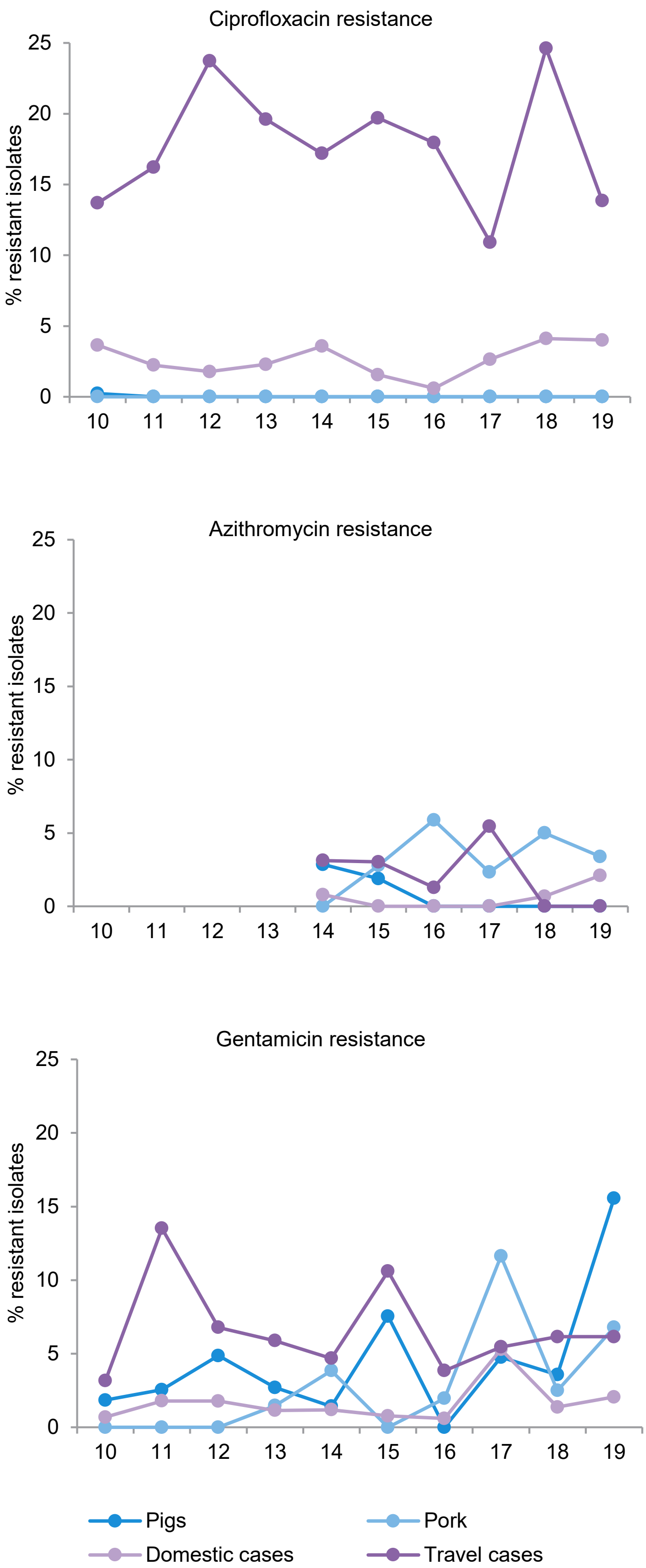
The levels of resistance to gentamycin in isolates from pigs were 16% with 7% resistance in domestically produced pork. This is an increase from previous years and coincides with an increase in use of neomycin in pigs that followed the discontinued use of colistin in 2017. The trend is very uncertain, due to the small number of isolates from pigs and pork, and the coming years will provide more information.

The levels of gentamycin resistance in domestically acquired human isolates have been stable over the last years, and, in 2019, two percent of the isolates were resistant.

Among human cases, the level of cephalosporin resistance was overall <1% for both cefotaxime and ceftazidime, and resistance to 3rd generation cephalosporins was only observed in isolates from travel-related cases. As in the previous years, none of the isolates from pigs or pork were resistant to 3rd generation cephalosporins. Meropenem (carbapenem) resistance was not observed in animal, food or human isolates. The findings for pigs and pork provide 95% confidence that the true prevalence of 3rd generation cephalosporins and carbapenem is less than 2.8% in pigs and pork in Denmark (see section 9.7).

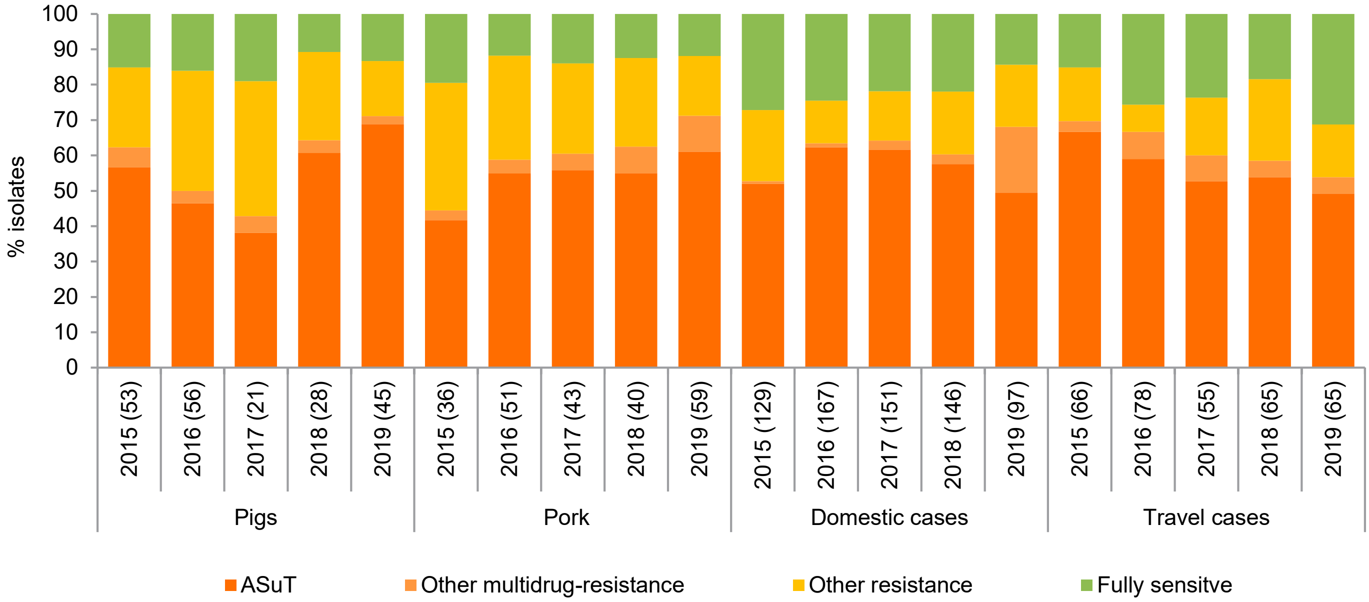
Resistance to tigecycline and colistin in *S.* Typhimurium is rare in Denmark and was not found in pigs or pork in 2019. Two percent of the human cases were resistant to tigecycline (MIC value>= 4 mg/L), and 2% was resistant to colistin. The colistin-resistant human isolates had MIC values that were close to the ECOFF, and none of the isolates harboured *mcr* genes.

Figure 6.3 Ciprofloxacin, azithromycin and gentamycin resistance (%) among *S.* Typhimurium from pigs, domestic pork and human cases, Denmark DANMAP 2019



Note: Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas *S.* 4,[5],12:i:-. An isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. Total number of human cases includes infections of unknown origin

Figure 6.4 Distribution (%) of multidrug-resistant, resistant and fully sensitive *S.* Typhimurium from pigs, domestic pork and human cases, Denmark DANMAP 2019



Note: Number of isolates included each year is presented in the parenthesis. Includes isolates verified as monophasic variants of *S.* Typhimurium with antigenic formulas *S.* 4,[5],12:i:-.An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel, and multidrug-resistant if resistant to 3 or more of the 12 antimicrobial classes included in the test panel (Table 9.3). ASuT are multidrug-resistant isolates resistant to ampicillin, sulfonamide and tetracycline

Most of the isolates were resistant to one or several antimicrobials. Less than 13% of the isolates from pigs and pork and 14% of the isolates from human domestic cases were fully sensitive to all tested antimicrobials, and the number of fully sensitive isolates from travel-associated cases was 31% (Table 6.2).

Over the last five years, the occurrence of multidrug-resistance among *S.* Typhimurium from Danish pork has increased significantly (Figure 6.4). As in previous years, the ASuT phenotype was the most frequent resistance profile among *S.* Typhimurium from both pigs and pork as well as from human cases (Figure 6.4). The observed levels were similar to most European countries and especially high among the monophasic isolates. See the AMR profile distribution in the web annex (Table A6.7).

The marked reduction in usage of tetracycline over the last ten years in pigs is still not reflected in the 2019 levels of resistance in *S.* Typhimurium from pigs and pork. In pigs, tetracycline resistance continued to increase from 47% in 2010 to 75% in 2018 and 80% in 2019, probably due to spread of *Salmonella* clones. The increased use since 2017 of macrolides for weaner and finisher pigs did not result in a measurable increase in resistance to azithromycin, but this will be monitored closely in the coming years.

6.3.2 Resistance in other *Salmonella* serotypes

***S.* Derby** is common in pigs, but the high prevalence is not reflected in the number of human cases. In pigs, a decrease in fully sensitive isolates was observed from 70% in 2018 to 55% in 2019 (Table 6.3). As expected, resistance to tetracycline, sulfonamides, trimethoprim and ampicillin was most common, either alone or in combination. Gentamycin resistance was found in 1-2% of the S. Derby isolates. Resistance to ciprofloxacin, azithromycin or other antimicrobials of critical importance was not detected (Table 6.3).

Twenty-one human S. Derby isolates were susceptibility tested in 2019. Eleven isolates were part of an outbreak caused by a fully sensitive strain. Two travel-associated isolates exhibited resistance to ampicillin, chloramphenicol, ciprofloxacin, sulfonamide, tetracycline and trimethoprim. One isolate was resistant to tetracycline, and one isolate was resistant to sulfonamides.

***S.* Infantis** isolates from 22 sporadic human cases were susceptibility tested, 17 of which were fully sensitive. Two isolates from domestic cases were resistant to cefotaxime and ceftazidime. Only eight *S.* Infantis were isolated from pigs and pork, seven of which were fully sensitive and one of which had an ASuT profile supplemented with trimethoprim resistance.

***S.* Dublin** is cattle associated. A total of 24 human isolates of *S.* Dublin were susceptibility tested. *S.* Dublin is intrinsically (naturally) resistant to colistin. Most of the isolates, 21, were fully sensitive, and resistance to 3rd generation cephalosporins was not observed. Two strains exhibited multi-resistance that included ciprofloxacin resistance (one isolate) and azithromycin resistance (one isolate). Most of the *S.* Dublin isolates came from cases with no information on travel.

***S.* Enteritidis** is a common cause of salmonellosis in Denmark, where it is often associated with travel. In 2019, 32 *S.* Enteritidis isolates were susceptibility tested. Like *S.* Dublin, *S.* Enteritidis is intrinsically resistant to colistin. Resistance to ciprofloxacin is common in *S.* Enteritidis, with 17 of the 32 isolates being resistant. Four of the susceptibility tested isolates of *S.* Enteritidis were from domestic cases. In 2019, *S.* Enteritidis was found in 11 batches of imported duck meat. Three of the isolates were resistant to ciprofloxacin and the remaining isolates were fully sensitive.

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Table 6.3 Resistance (%) in *Salmonella Derby* isolates from pigs and pork, Denmark DANMAP 2019

	Pigs	Pork
Antimicrobial agent	Danish %	Danish %
Ampicillin	13	16
Azithromycin	0	0
Cefotaxime	0	0
Ceftazidime	0	0
Chloramphenicol	4	2
Ciprofloxacin	0	0
Colistin	0	0
Gentamicin	1	2
Meropenem	0	0
Nalidixic acid	0	0
Sulfonamide	21	25
Tetracycline	31	21
Tigecycline	0	0
Trimethoprim	21	23
Fully sensitive (%)	55	64
Number of isolates	67	56

Note: An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel (Table 9.3)

Textbox 6.1

Antimicrobial resistance in clinical isolates from dogs and cats in Denmark

Background: Most countries, including Denmark, have no systematic surveillance of antimicrobial resistance (AMR) in companion animals and no systematic way of detecting trends and new or emerging resistance problems to guide antimicrobial use practices and policies. In small animal practice today, *Escherichia coli* and *Staphylococcus pseudintermedius* are the most common bacterial pathogens encountered, causing mainly urinary tract and skin infections, respectively. Among these, the increasing occurrence of methicillin-resistant *S. pseudintermedius* (MRSP) and extended-spectrum beta-lactamase (ESBL)-producing *E. coli* is particularly worrying, since they can be resistant to all antimicrobial drugs approved for veterinary use [1]. Furthermore, they can be transmitted to humans, posing a potential risk to household contacts and veterinary staff.

Materials and methods: Antimicrobial susceptibility data were obtained for 1,177 *E. coli* and 1,763 *S. pseudintermedius* isolates from canine and feline clinical specimens submitted to Sund Vet Diagnostik, the veterinary diagnostic microbiology laboratory at the University of Copenhagen. This laboratory processes veterinary samples from all over Denmark, including from referral practices. Isolates were obtained in 2011-2012 and 2016-2019 from predominantly urinary tract infections and skin/ear infections (Table 1). The two time periods were selected to enable comparison of resistance levels before and after the national guidelines for antimicrobial treatment of companion animals were launched in late 2012 [2]. Isolates were tested by broth microdilution using commercial plates (SensiTitre, Thermo Fisher Scientific) according to the Clinical Laboratory Standards Institute standards for susceptibility testing of veterinary pathogens [3].

Table 1 Origin of *E. coli* and *S. pseudintermedius* isolates obtained from clinical specimens in Sund Vet Diagnostik, 2011-2012 and 2016-2019 DANMAP 2019

	Staphylococcus pseudintermedius		*Escherichia coli*	
Origin	Dogs	Cats	Dogs	Cats
Skin, wounds and ears	1358	19	230	13
Urinary tract	111	7	587	149
Other	264	4	176	22
Total	1733	30	993	184

Results: Overall, resistance levels were stable with no major fluctuations over the 8-year time period (Table 2). In *E. coli*, imipenem resistance was not detected, indicating the absence of carbapenemase-producers. Between 4% and 7% of *E. coli* isolates were resistant to cefpodoxime, which is an indicator of ESBL- and AmpC-production. Based on sequence-based typing of a subset of isolates, CTX-M-1, CTX-M-15, and CMY-2 are the most common ESBL/AmpC enzymes in clinical *E. coli* from dogs in Denmark. Approximately 25% and 10% of *E. coli* isolates were resistant to ampicillin and potentiated sulfonamides, respectively (Table 2).

In the national treatment guidelines, these agents are recommended as first choice for lower urinary tract infection (UTI) in dogs and cats. Consumption of potentiated sulfonamides for pets has decreased by 25% between 2011 and 2018, likely due to withdrawal from the Danish market in 2014 of the only sulfonamide/trimethoprim product licensed for these animals. This may explain the significant reduction in levels of resistance to sulfamethoxazole/trimethoprim (15 vs. 9%, $p<0.001$). Today, amoxicillin is the only registered first choice antibiotic for treatment of UTI. The high level of resistance to ampicillin, which is a surrogate antibiotic for testing amoxicillin susceptibility, emphasizes the need for culture and susceptibility testing to guide therapy for this common condition.

Between 6% and 8% of the *S. pseudintermedius* isolates were likely to be MRSP, as they exhibited resistance to oxacillin. Since 2005, the almost pan-resistant MRSP clonal complex CC71 has spread globally among dogs and to a lesser extent among cats [4]. Although this clone also occurs in Denmark, the most prevalent clone in Danish pets is CC258, which is typically susceptible to more drugs than CC71 [5]. Clindamycin is the systemic treatment of choice for superficial canine skin infections, and

continued ... Textbox 6.1

25-28% of *S. pseudintermedius* isolates were resistant to this drug. Even higher percentages of resistance were observed for antibiotics that are not used for treatment of *S. pseudintermedius* infections, such as doxycycline (29-33%) and erythromycin (26-28%), and the already high level of resistance to ampicillin increased even further (58% vs 70%, p<0.001). Notably, most skin and ear infections can be managed by antiseptic topical treatment (e.g. shampoo, gels) without the need for systemic antimicrobial therapy. However, more severe infections including deep pyoderma require systemic therapy, and susceptibility testing is highly recommended in these cases, also in consideration of the treatment period, which is often longer than three weeks.

In order to assess the representativeness of our data, we recently studied resistance in clinical *S. pseudintermedius* from dogs with first-time pyoderma that were not previously treated with antibiotics. Only 14% of isolates in that study were resistant to clindamycin [6], suggesting that the data reported here are likely to overestimate resistance levels encountered in primary practices. We also suspect that the overall lower resistance levels observed in *E. coli* in 2016-2017, as compared to the periods before and after (Table 2), could be due to a higher proportion of isolates from primary practices in that period.

Table 2 Percentage of antimicrobial resistant clinical *E. coli* and *S. pseudintermedius* isolates from dogs and cats in Denmark
DANMAP 2019

	Escherichia coli			*Staphylococcus pseudintermedius*		
	2011-2012	2016-2017	2018-2019	2011-2012	2016-2017	2018-2019
	(N=342)	(N=394)	(N=441)	(N=675)	(N=486)	(N=602)
Antimicrobial agent	%	%	%	%	%	%
Amikacin	1	2	2	1	1	1
Ampicillin(a)	28	14	25	58	59	70
Amoxicillin/clavulanic acid(b)	9	4	5	-	8	7
Cefazolin	-	-	-	6	8	7
Cefpodoxime	7	4	5	-	-	-
Chloramphenicol	4	4	4	14	16	21
Clindamycin	-	-	-	28	25	27
Doxycycline(b)	9	7	8	-	33	29
Enrofloxacin	8	3	4	3	3	2
Erythromycin	-	-	-	28	26	28
Gentamicin	4	4	4	3	3	2
Imipenem	0	0	0	-	-	-
Marbofloxacine	8	3	3	3	3	3
Oxacillin	-	-	-	6	8	6
Sulfamethoxazole/trimethoprim	15	7	9	6	5	6

a) Susceptibility data for ampicillin and amoxicillin/clavulanic acid in *E. coli* have been determined only for isolates from urinary tract infections, as isolates from other infections are unequivocally classified as resistant to these drugs according to CLSI breakpoints
b) Amoxicillin/clavulanic acid and doxycycline susceptibility data from 2011-12 for *S. pseudintermedius* were not included, as concentrations in the MIC panels used cannot be used for interpretation according to the breakpoints available today

Discussion and conclusion: It is difficult to relate AMR in companion animals to national trends of antimicrobial consumption, because VetStat data on antimicrobial usage in companion animals are less complete than for production animals. A detailed investigation of consumption patterns in DANMAP 2016 indicated a small overall decrease in total antimicrobial consumption for companion animals, including a substantial decrease in the use of 3rd generation cephalosporins following the launch of the national treatment guidelines in 2012. This positive trend has continued since 2016, but companion animals still account for 12% and 89% of total kg active ingredient of all 3rd and 4th generation cephalosporins and fluoroquinolones used for animals, respectively. Several studies have hypothesized that exposure to these two antimicrobial classes is a risk factor for colonization or infection with MRSP- and ESBL-producing *E. coli*. Accordingly, a further reduction in their use should be pursued to control the occurrence of these multidrug-resistant bacteria in the future.

In conclusion, AMR levels in clinical *E. coli* and *S. pseudintermedius* isolates from dogs and cats have remained stable for most antimicrobials since 2011. MRSP- and ESBL-producing *E. coli* still occur at fairly low frequency, and it is important to maintain this trend via continued focus on responsible antimicrobial use in small animal practice. A largely untapped resource for implementing responsible antimicrobial use is the implementation of antimicrobial stewardship programmes at the clinic level [7]. Guidelines for developing such programmes tailored to type and size of clinic have now become available in small animal veterinary medicine [8].

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Textbox 6.2

MRSA surveys in animals

In 2019, the Danish Veterinary and Food Administration (DVFA) conducted MRSA surveys in conventional breeding pig herds, dairy cattle herds, veal calf herds, broiler flocks, horses and mink feed. For pigs, dairy cattle, veal calves and horses, 25 individual animals were tested per herd/farm and the samples analysed as pools of five samples, whereas sampling of poultry flocks was performed by collecting five sock samples per flock. Sampling of mink feed was done by swabs from ready-mixed feed. From mink feed, five pooled samples of five swabs were obtained from each batch of eight tonnes of feed. The results of the MRSA surveys are summarised in Table 1 and are presented at herd/flock/feed batch level (between-unit prevalence) rather than at the animal level (within-unit prevalence).

Table 1 Prevalence of MRSA in various animal populations and mink feed in Denmark, 2019 DANMAP 2019

Herd type	Number of sampled units*	Number of positive units	% positive units
Conventional breeding pigs	73	69	95
Dairy cattle	131	2	2
Veal calves	115	11	10
Horses	120	13	11
Broiler chicken	83	0	0
Mink feed	10	1	10

*Unit = flock/ herd/ feed batch

The prevalence of MRSA in conventional breeding pig herds increased from 83% in 2018 to 95% in 2019. All isolates belonged to CC398. The prevalence among dairy herds remained low (6% in 2018). One isolate was *spa* type t127/CC1 while the other was *mecC* positive and *spa* type t843/CC130. The prevalence among horse herds was also stable (8% in 2018). Two positive horse samples were *mecC* (*spa* types t843/CC130 and t3256/CC130), while the remaining were CC398. Sampling from mink feed demonstrated one positive feed batch (*spa* type t011/CC398) and suggests that feed may be a route of introduction of MRSA in the mink flocks. In 2018, 25% of mink farms were positive for MRSA, and all but one farm/isolate were positive with *spa* types associated with CC398.

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Textbox 6.3

Antimicrobial resistance in mastitis isolates from dairy cows

Background and data source: Mastitis is the leading cause of antimicrobial usage in the dairy production worldwide. It is estimated that in Denmark, each year, every third dairy cow suffers from mastitis. Some of the major pathogens associated with clinical mastitis are *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Escherichia coli*. However, in Denmark, veterinarians are only allowed to prescribe penicillin for treatment of mastitis as long as no antibiotic resistance testing is carried out. The aim of this study was to provide new data of antimicrobial resistance occurrence in udder pathogens from Danish dairy cows with clinical mastitis. The isolates included were submitted from veterinary clinics throughout 2018 and 2019 to Center for Diagnostics, Technical University of Denmark, in relation to various research projects. The antimicrobial susceptibility testing was carried out using the broth microdilution method with SensiTitre. The panels and breakpoints applied for interpreting the minimum inhibitory concentrations (MICs) were those routinely applied at Center for Diagnostics, Technical University of Denmark [1].

Results and discussion: The Gram-positive isolates generally exhibited low resistance levels to the tested antimicrobials (Table 1). The highest resistance level for the *S. aureus* isolates was to sulfamethoxazole (25%, 2018), otherwise low resistance levels were found (<20%). A single isolate was cefoxitin-resistant (8%, 2019), and this will be studied further for potential MRSA identification. All Streptococci isolates were susceptible to penicillin. However, several *S. uberis* isolates showed decreased susceptibility against penicillin and were classified as intermediate (data not shown). This trend is novel and will be investigated further in future projects. Additionally, the *S. uberis* isolates showed high resistance levels to streptomycin (84%, 2018 and 100%, 2019), cefoxitin (63%, 2018 and 50%, 2019) and tetracycline (26%, 2018 and 20%, 2019). For the *S. dysgalactiae* isolates, the highest resistance level was found in tetracycline (24%, 2018 and 19%, 2019) and otherwise, low resistance levels were detected.

Table 1 Resistance occurrence (%) among *S. aureus*, *S. uberis*, *S. dysgalactiae*, and *E. coli* isolates from dairy cows with clinical mastitis

DANMAP 2019

	AMP	AUG	APR	CHL	CIP	COL	ERY	FFN	FOT	FOX	GEN	NAL	NEO	PEN	SMX	SPE	STR	SXT	TET	TIA	TMP	XNL
	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
S.aureus																						
2018 (n = 12)	-	-	-	0	0	-	0	-	-	0	0	-	-	8	25	17	0	0	0	0	0	-
2019 (n = 12)	-	-	-	0	0	-	0	-	-	8	0	-	-	0	8	8	0	0	8	0	0	-
S. uberis																						
2018 (n = 19)	-	-	-	0	0	-	5	-	-	63	NA	-	-	0	-	5	84	0	26	5	0	-
2019 (n = 20)	-	-	-	0	0	-	15	-	-	50	NA	-	-	0	-	10	100	0	20	0	0	-
S. dysgalactiae																						
2018 (n = 17)	-	-	-	0	0	-	0	-	-	0	NA	-	-	0	-	0	6	0	24	0	0	-
2019 (n = 16)	-	-	-	0	0	-	6	-	-	0	NA	-	-	0	-	0	6	0	19	0	0	-
E. coli																						
2018 (n = 23)	4	0	0	4	0	4	-	0	0	-	0	4	0	-	17	4	4	-	4	-	4	0
2019 (n = 17)	6	6	0	0	0	0	-	0	0	-	0	0	0	-	6	6	12	-	0	-	0	0

AMP (ampicillin), AUG (amoxicillin + clavulanic acid), APR (apramycin), CHL (chloramphenicol), CIP (ciprofloxacin), COL (colistin), ERY (erythromycin), FFN (florfenicol), FOT (cefotaxime), FOX (cefoxitin), GEN (gentamicin), NAL (nalidixic acid), NEO (neomycin), PEN (penicillin), SMX (sulphamethoxazole), SPE (spectomycin), STR (streptomycin), SXT (sulphamethoxazole-trimethoprim), TET (tetracycline), TIA (tiamulin), TMP (trimethoprim), and XNL (ceftiourfur)

continued ... Textbox 6.3

The *E. coli* isolates exhibited low resistance levels to all tested agents, and no indication of ESBL producing *E. coli* was found in this study. A single isolate was colistin resistant, and this is currently under further investigation. This study showed no major changes in resistance levels from 2018 to 2019, however, we still emphasize the need for incessant surveillance of resistance occurrence and development among mastitis pathogens in the future.

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Textbox 6.4

Resistance in bacteria from diagnostic submissions from pigs

Background and data source: Data on antimicrobial susceptibility of three important veterinary pathogens haemolytic *Escherichia coli*, *Streptococcus suis*, and *Actinobacillus pleuropneumoniae* were obtained from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup during 2019. The number of isolates belonging to other bacterial species was insufficient to deduct annual trends.

The antimicrobial susceptibility testing was carried out using the broth microdilution method with SensiTitre. Internationally approved clinical breakpoints were not available for most of the drug-bacterium combinations, so the occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases, SEGES. These breakpoints are mainly CLSI breakpoints, preferably porcine, but for *E. coli* the breakpoints are mostly human. When the applied breakpoints are adjusted according to new established breakpoints, these are used retrospectively in this text box.

MIC distributions and occurrence of resistance are presented in the web annex (Tables A6.8-A6.10).

Table 1 Resistance (%) among bacteria from diagnostic submissions from pigs, Denmark DANMAP 2019

Antimicrobial agent	*Actinobacillus pleuropneumoniae*	*Haemolytic Escherichia coli*	*Streptococcus suis*
	%	%	%
Amoxicillin/clavulanic acid	-	4	-
Ampicillin	1	62	-
Apramycin	-	12	-
Cefotaxime	-	0	-
Cefoxitin	-	-	14
Ceftiofur	0	0	-
Chloramphenicol	-	18	1
Ciprofloxacin	0	1	0
Colistin	-	0	-
Erythromycin	100	-	61
Florfenicol	0	10	1
Gentamicin	-	9	0
Nalidixic acid	-	6	-
Neomycin	-	20	-
Penicillin	1	-	0
Spectinomycin	0	51	27
Streptomycin	-	77	37
Sulfamethoxazole	-	-	86
Sulfonamide	-	75	-
Sulfonamid/trimethoprim	0	-	3
Tetracycline	0	69	69
Tiamulin	0	-	16
Tilmicosin	0	-	-
Trimethoprim	-	56	8
Tulathromycin	0	-	-
Number of isolates	116	262	147

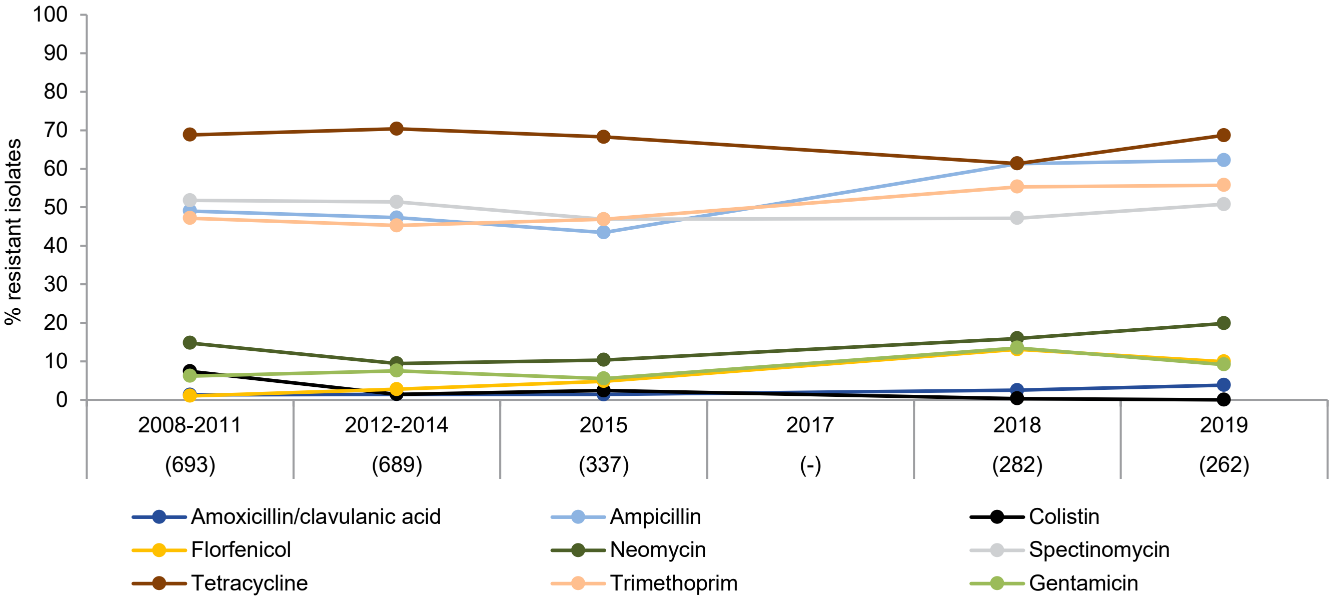
Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.8 - A6.10)

continued ... Textbox 6.4

E. coli - haemolytic pathogenic strains

Enterotoxigenic *E. coli* (ETEC) in combination with *Brachyspira pilosicoli* and *Lawsonia intracellularis* are the most prevalent causes of bacterial diarrhoeal in Danish pigs. Since 2014, PCR identification has been the most frequent method for identification of the diarrhoeal pathogens in Denmark, including identification of *E. coli* F4 and *E. coli* F18. Before 2018, the *E. coli* isolates were identified by serotyping at the SEGES laboratory, with the most virulent ETEC strains belonging to serovars O138, O139, O141, and O149, which are haemolytic and positive for enterotoxin. These strains are also mostly positive for F4 or F18 fimbrial adhesins, which are used for attachment to the intestinal mucosa. The haemolytic *E. coli* included in these analyses originated almost exclusively from porcine enteritis or oedema disease. The data from 2018 and 2019 presented F4 or F18 positive *E. coli*, while data for 2008-2017 presented the *E. coli* serovars O138, O139, O141, and O149. In general, the F18 positive strains belong to the serovars O138, O139 and O141, while serovar O149 carry the F4 fimbriae. However, this is not a clear cut correlation.

Figure 1 Resistance (%) among haemolytic _Escherichia coli_ from pigs, Denmark DANMAP 2019



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.8)

As in previous years, high resistance levels were recorded in 2019 for ampicillin, streptomycin, sulfonamide, tetracycline, trimethoprim, and spectinomycin (Figure 1). The level of resistance to these four antimicrobials remained at the same level throughout the past decade (Figure 1). The use of tetracycline has decreased significantly since 2016, but this is not reflected in the occurrence of tetracycline resistance in *E. coli*. There may be several explanations for this, including co-selection (ASSuT complex). Also, the use of tetracycline for diarrhoea might not have decreased as much as for other infections. Many of the other porcine pathogens are susceptible to most antimicrobials, while resistance is much more widespread in the haemolytic *E. coli* F4/F18. Thus, the use of tetracycline has decreased more in finishers than in weaners, but also within weaners, the use of tetracycline might have decreased more for respiratory diseases than for diarrhoea. Most cases of porcine diarrhoea that require treatment occur during the weaning period and tetracycline, neomycin, or aminopenicillin are the compounds of choice for *E. coli* infection. In pigs, the aminoglycosides are only used for gastrointestinal infections, and particularly the use of neomycin has been increasing in recent years, after a reintroduction in 2017. In 2018, a significant increase was observed in the level of resistance to neomycin, gentamicin and apramycin (Figure 1). For neomycin, the increase continued reaching 20% in 2019. For apramycin and gentamicin, the occurrence of resistance in *E. coli* was slightly, but not significantly lower in 2019 compared to 2018, but remained at a high level compared to previous years. The parallel increase of the gentamicin and apramycin is most likely a result of cross-resistance, as the vast majority of apramycin resistant isolates were also resistant to gentamicin (Table 1). A similar slight (non-significant) decrease was also noted for florfenicol (10% in 2019), after a continuous increase during the last decade (Figure 1).

Amoxacillin-clavulanic acid (amoxiclav) for oral treatment of pigs is a relatively new drug, and the use has been at a low level. However, an increasing trend in resistance to amoxiclav has been observed during the past decade, reaching 4% in 2020.

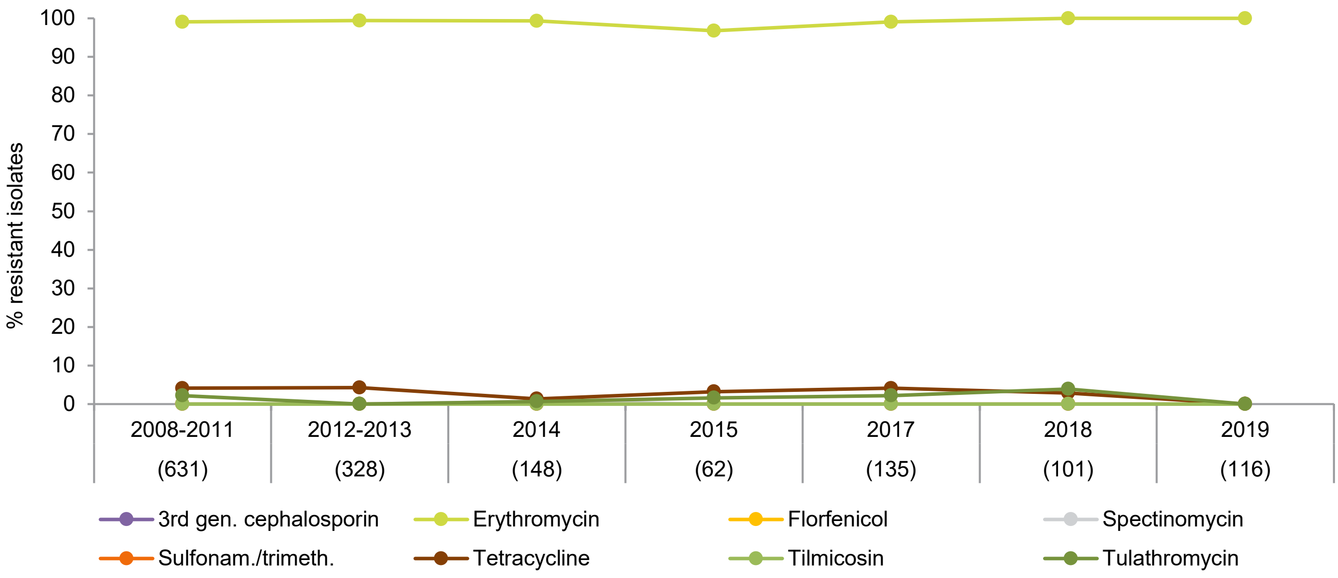
In 2017, the use of colistin was regulated by the authorities (given a high weight in the Yellow Card legislation), and the use of colistin has been close to zero since 2017. Resistance to colistin has since been decreasing (from a low level), and in 2019 no colistin resistant isolates were found.

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumoniae causes severe pleuropneumonia in pigs, although severity varies between serotypes. Outbreaks usually require rapid onset of treatment to minimise losses. Fortunately, *A. pleuropneumoniae* has very low occurrence of resistance to most of the available antimicrobials. Almost all isolates are resistant to erythromycin but this compound is not available for veterinary use. Macrolides are frequently used for treatment of pneumonia, and the resistance to other macrolides is low (Figure 2). Tulathromycin is frequently used, but in recent years the occurrence of resistance increased nonsignificantly reaching 4% in 2018 (Table 1). However, in 2019, no tulathromycin resistant isolates were identified. Still, as resistance to tulathromycin does occur in Danish *A. pleuropneumoniae*, susceptibility testing is important to monitor the occurrence of resistance and reduce the risk of treatment failure.

No resistance to florfenicol, sulfonamide-trimethoprim and tilmicosin has been observed for the last decade, and the occurrence of resistance remained absent or very low to penicillin, spectinomycin and tiamulin (Table 1). It is also worth noting that no resistance to ciprofloxacin has been observed in Danish isolates for more than 10 years.

Figure 2 Resistance (%) among *Actinobacillus pleuropneumoniae* from pigs, Denmark DANMAP 2019



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.9)

continued ... Textbox 6.4

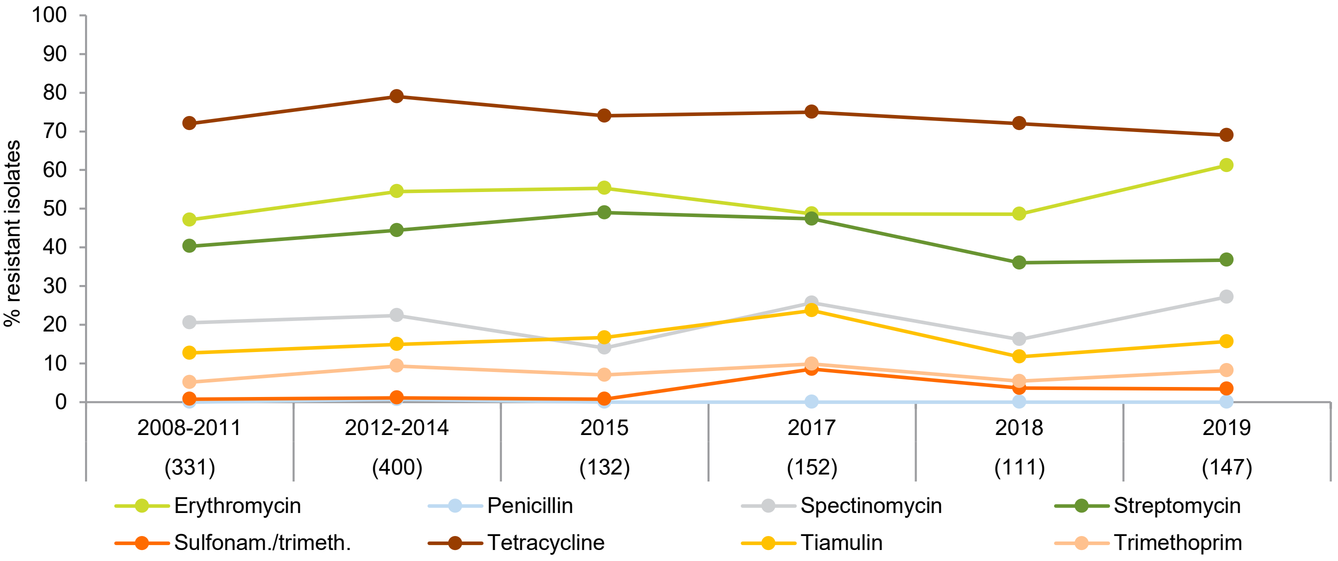
Streptococcus suis

Streptococcus suis may cause several different infectious conditions in pigs, such as meningitis, otitis media, arthritis, pneumonia, and septicaemia, and causes losses to the farmers due to increased mortality and veterinary costs.

This year the breakpoints for tetracycline and penicillin resistance were updated applying the CLSI breakpoints for porcine isolates. As in previous years, the highest levels of resistance was seen for tetracycline, streptomycin and erythromycin, but for erythromycin a significant increase was observed, reaching 61% in 2019 (Figure 3). The occurrence of resistance to tetracycline has remained stable around 70%, despite the decreases in use of tetracycline in pigs.

Resistance to sulfonamide is also very high (86% in 2019), but for pigs, sulfonamides are only available in combination with trimethoprim. Resistance to sulfonamide-trimethoprim remained at a low level, with 3.4% resistant isolates in 2019. For S. suis, there are several treatment options using compounds with very low levels of resistance (Table 1). Almost all isolates were susceptible to sulfonamide-trimethoprim, penicillin and florfenicol in 2019 and the past decade. Of these, penicillin and florfenicol are recommended 1st choice antimicrobials in the official guidelines. The occurrence of resistance to pleuromutilins (tiamulin) and spectinomycin remained at a moderate levels (Figure 3). For these compounds, considerable fluctuations were seen over the past decade, but mostly these fluctuations were non-significant. However, for spectinomycin the increase to 27% resistant isolates was significantly higher than the 2018 level, but similar to the level in 2017.

Figure 3 Resistance (%) among *Streptococcus suis* from pigs, Denmark DANMAP 2019



Note: Isolates from the routine diagnostic laboratory investigation of isolates from dead and diseased pigs submitted to SEGES Pig Research Centre's Laboratory for Pig Diseases in Kjellerup. Occurrences of resistant isolates are presented according to the clinical breakpoints that are currently in use at both DTU National Veterinary Institute and Laboratory for Pig Diseases. Clinical breakpoint and MIC distributions are presented in the web annex (Table A6.10)

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