6

RESISTANCE IN ZOONOTIC BACTERIA

6. Resistance in zoonotic bacteria

Highlights: In Denmark, antimicrobials are generally not recommended for treatment of diarrhoea in humans including salmonellosis and campylobacteriosis. This is due to the self-limiting nature of the diseases. If needed, it is recommended that patients are treated with macrolides (azithromycin and erythromycin).

Isolates from animals and meat for susceptibility testing are mainly collected by repeated, representative, national surveys conducted at Danish slaughter houses.

In 2018, the level of azithromycin resistance in *Salmonella* Typhimurium isolates from humans was less than 1%, and 5% in Danish pork. No erythromycin resistance was found in *Campylobacter jejuni* isolates from animals or human patients in 2018.

Resistance to quinolones remained the most common resistance type found in *C. jejuni* from all populations: broilers, cattle and humans. Around one third of all isolates of animal origin and domestically acquired human cases were resistant to ciprofloxacin, whereas 83% of the travel associated human isolates were ciprofloxacin resistant. The majority of ciprofloxacin resistant isolates from poultry and humans were also resistant to tetracycline.

Tetracycline resistance in *C. jejuni* from broilers increased significantly from 16% in 2017 to 32% in 2018 and this increase coincided with an increase among the human isolates of domestic origin from 22% in 2017 to 34% in 2018. Resistance to both tetracycline and ciprofloxacin also increased in isolates from broilers.

Salmonella Typhimurium and *S.* Derby were the two most prevalent serotypes isolated from Danish pigs and pork. About two thirds of the *S.* Typhimurium isolates were monophasic and a similar pattern was observed among the human *S.* Typhimurium isolates. The dominance of clonally related monophasic *S.* Typhimurium isolates influences the resistance patterns in all populations causing high levels of resistance to tetracycline, ampicillin and sulfonamide. About half of all *Salmonella* isolated from Danish pigs and pork were *S.* Derby, where lower levels of resistance were observed.

Of the antimicrobials of critical importance in Denmark, resistance to colistin, 3rd generation cephalosporins and carbapenems remained low or absent in isolates from all populations and fluoroquinolone resistance has not been identified in *S.* Typhimurium from Danish pigs and pork since 2010 and 2007, respectively.

Among human cases resistance to fluoroquinolones remained high among *S.* Typhimurium isolates from travel 25% versus 4% in isolates from cases acquired in Denmark. Resistance to 3rd generation cephalosporins and carbapenems remained very low, less than 1%, in *S.* Typhimurium from human cases and was not found in the *Salmonella* isolates from Danish pigs and pork.

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 the Annual Report on Zoonoses in Denmark 2018 [www.food.dtu.dk].

Campylobacter and *Salmonella* surveillance has been part of the DANMAP programme since 1995, where isolates from broilers, cattle and pigs as well as from human cases were susceptibility tested. Isolates from samples of fresh meat were included from 1997. Since 2014, sampling and testing of *Campylobacter and Salmonella* in animals and foods have been done in accordance with the EU harmonised monitoring of antimicrobial resistance [Decision 2013/652/EU] and supplemented by additional surveillance addressing national objectives.

Zoonotic bacteria resistant to antimicrobials used for treatment of human patients have consequences for the patient and society by prolonging and/or increasing the severity of the disease. Resistance to antimicrobials not used for treatment of the given disease has less direct importance, but may impact public health either by: i) horizontal transfer of resistance genes to other pathogens resulting in treatment failure of these or ii) by facilitating selection of strains harbouring resistance genes of public health importance, if resistance to both (e.g. co-resistance) are present. The relative importance of the indirect consequences are unknown.

In Denmark, antimicrobials are not recommended for treatment of diarrhoea in patients unless there is prolonged duration or the patient is severely ill. Macrolides (azithromycin and erythromycin) are recommended as drug of choice if treatment is required, regardless of the nature of the disease. [Promedicin: http://pro.medicin.dk].

6.2 *Campylobacter*

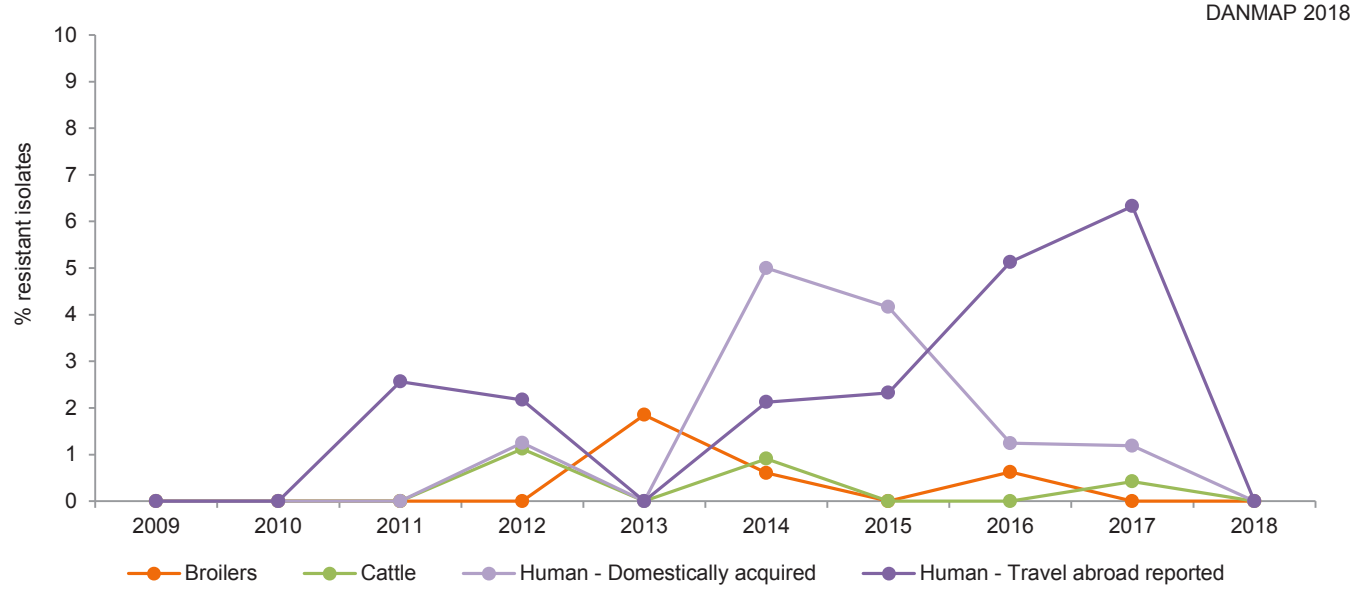
Thermotolerant *Campylobacter* spp. are the most commonly reported cause of gastrointestinal bacterial infections in humans in the EU [ECDC/EFSA 2018. EFSA journal 16(12):5500]. In Denmark, campylobacteriosis is also the most common cause of bacterial gastroenteritis with an estimated 40-60,000 food borne cases per year. Around 7% of these patients seek health care leading to approximately 4,000-5,000 laboratory confirmed cases per year [Pires 2014. DTU report, ISBN: 978-87-93109-31-5]. In 2018, a total of 4,546 human laboratory confirmed cases of campylobacteriosis were reported (78.5 per 100,000 inhabitants) [Annual Report on Zoonoses in Denmark 2018].

Around one third of human *Campylobacter* cases are travel associated. The most common source of domestically acquired cases is poultry meat. Cattle is also an important source and transmission from cattle happens through meat, unpasteurized milk, the environment, and direct contact. Dogs, other food sources and the environment are also sources of *Campylobacter* infections [Kuhn et al. 2018. Clinical Epidemiology 10:1695; Pires 2017. DTU report ISBN: 978-87-93565-10-4].

Macrolides are used to treat infections in animals. In 2018, 12,951 kg of macrolide was prescribed for animals. The major-

Figure 6.1 Erythromycin resistance (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark

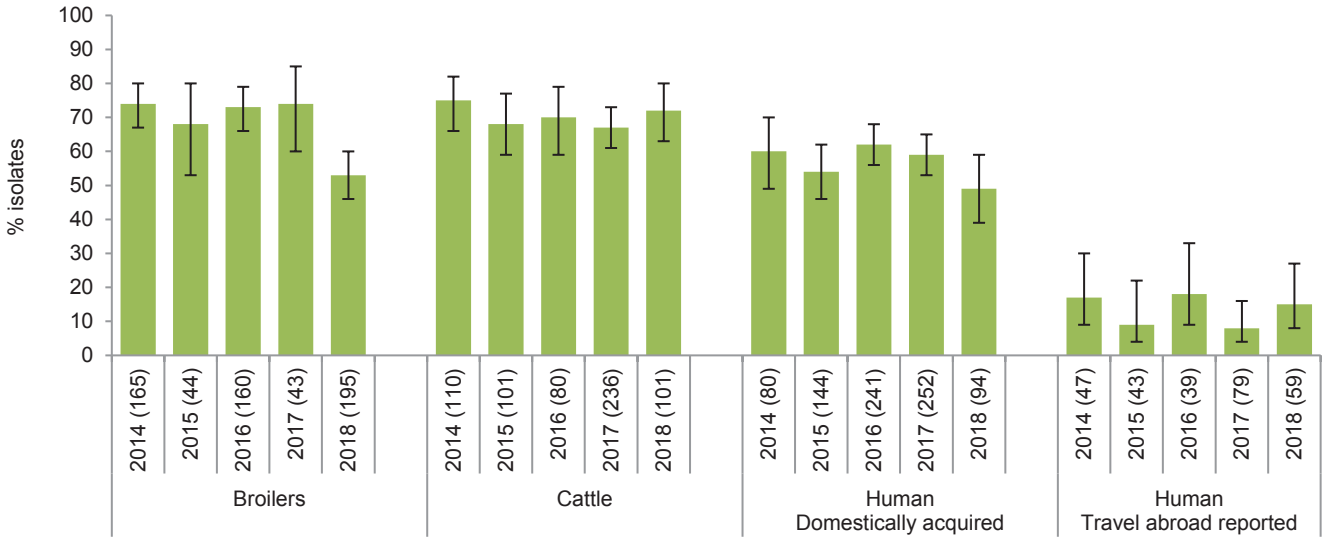
DANMAP 2018



Note: A human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease

Figure 6.2 Distribution (%) of fully sensitive *Campylobacter jejuni* from broilers, cattle and human cases, Denmark

DANMAP 2018



Note: The number of isolates included each year is shown in parentheses, confidence intervals are calculated as 95% binomial proportions presenting wilson intervals. An human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. An isolate is considered fully sensitive if susceptible to all antimicrobial agents included in the test panel

ity (93%) of this was used in pigs, whereas cattle and poultry used 245 kg and 162 kg, respectively. Fluoroquinolones are not used in food production animals and are only prescribed to pets and humans in Denmark.

In 2018, the animal *Campylobacter* isolates for DANMAP were obtained by sampling of randomly selected broiler caeca and cattle caeca at slaughter. In 2018, 836 broiler flocks and 154 cattle <1 year of age were sampled at slaughter. One isolate per farm was susceptibility tested. Sampling, isolation and susceptibility methods followed EFSAs recommendations for animal and food isolates.

In humans, campylobacteriosis is a notifiable disease. For the purpose of DANMAP a selection of isolates from reported human *C. jejuni* cases are susceptibility tested. In 2018, *Campylobacter* isolates were submitted to Statens Serum Institut (SSI) by three clinical microbiological laboratories. The isolates were geographically dispersed and representative of both urban and rural areas of Denmark. Travel history of the patient was collected by the general practitioner when possible, and reported to the diagnostic laboratory with submission of patient samples. The isolates were species typed at SSI and a total of 193 *C. jejuni* isolates were susceptibility tested in accordance with the ECDC recommendations. Only one isolate per patient was tested.

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*

Macrolide resistance in *Campylobacter* is monitored using erythromycin. No erythromycin resistance was observed in *C. jejuni* from animals or humans in 2018 (Table 6.1).

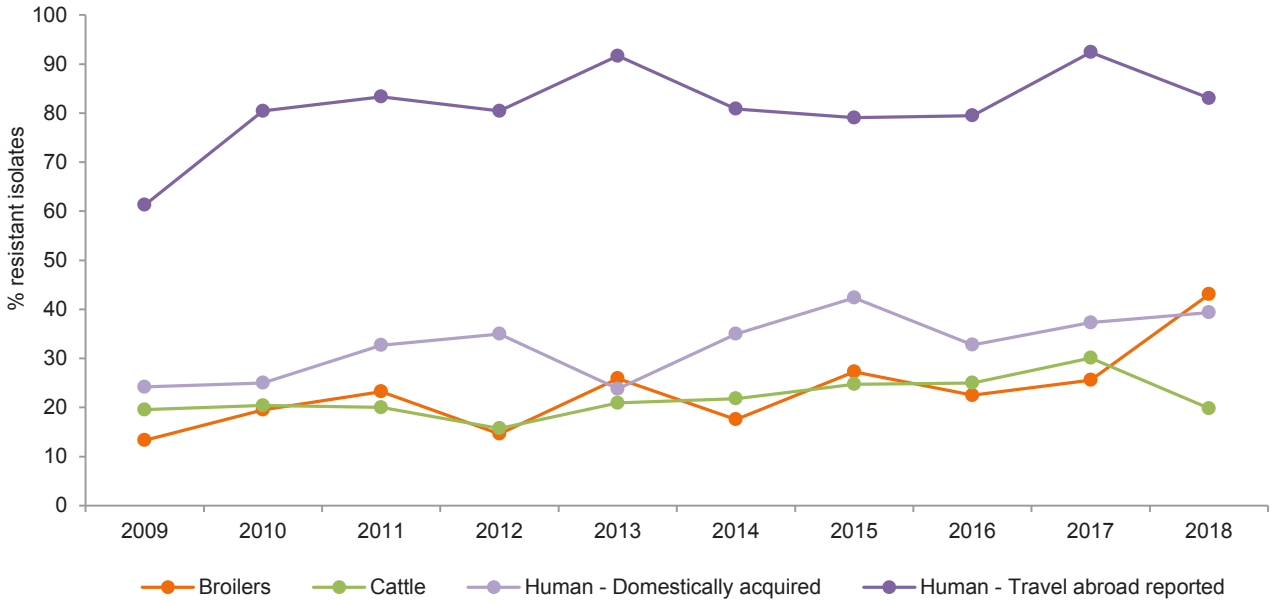
Over the last decade, macrolide resistance was slightly more common in isolates from humans than in animals, but only in low levels. Macrolide resistance never exceed 7% in any year and only a few erythromycin resistant *C. jejuni* isolates were identified in poultry and cattle in the last decade, varying between zero and two resistant isolates per year (Figure 6.1). This indicates that the actual prevalence of macrolide resistance is very close to the limit of detection by the curren sampling scheme. Based on the available number of isolates, we are 95% confident that macrolide resistance is not exhibited by more than 1.5% and 3% *C. jejuni* from broilers and cattle, respectively (see section 9.7).

The proportions of fully sensitive *C. jejuni* from broilers, cattle and humans are presented in Figure 6.2.

Among the domestically acquired human infections, 49% were fully susceptible to all antimicrobials tested (Table 6.1 and Figure 6.2). This is the lowest number of fully sensitive *C. jejuni* reported within the last five years, mainly due to a significant increase in tetracycline resistance, from 22% in 2017 to 34% in 2018 (Table 6.1). The number of fully susceptible strains from patients with a known history of travel was significantly lower than the corresponding number of domestically acquired cases. Similar to previous years, the occurrence of resistance to ciprofloxacin and tetracycline was significantly higher in

Figure 6.3 Ciprofloxacin resistance (%) among *Campylobacter jejuni* from broilers, cattle and human cases, Denmark

DANMAP 2018



Note: An human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease

travel associated *C. jejuni* isolates (83% and 63%, respectively) compared to isolates from domestically acquired infections (39% and 34%, respectively).

Among the human isolates, the most frequent resistance profile was resistance to quinolones only (30/193) or quinolones in combination with tetracycline (64/193), see the AMR profile distributions in web annex Table A6.3.

In broilers, the level of fully susceptible isolates also decreased in 2018 reflecting an increase in isolates with co-resistance to ciprofloxacin and tetracycline; from 16% in 2017 to 28% in 2018. Tetracycline resistance in *C. jejuni* from broilers increased significantly from 16% in 2017 to 32% in 2018. This increase coincided with an increase among the human isolates.

A significant increase was observed in ciprofloxacin resistance in *C. jejuni* from broilers this year and a slow, but statistically significant increasing trend has been observed over the last 10 years (Figure 6.3). An increase in ciprofloxacin resistance is similar to what is observed internationally [EFSA/ECDC 2019. EFSA journal 17(2):5598]. Ciprofloxacin resistance levels were similar in domestically acquired human cases and the main *Campylobacter* sources: broilers and cattle.

In cattle, the level of fully susceptible isolates was slightly higher in 2018 than in 2016 and 2017 (Figure 6.2). The level of resistance to ciprofloxacin dropped significantly from 30% to 20%. This will be monitored in the coming years to establish whether this positive development continues. Tetracycline resistance level remained the same as in 2017 (Table 6.1).

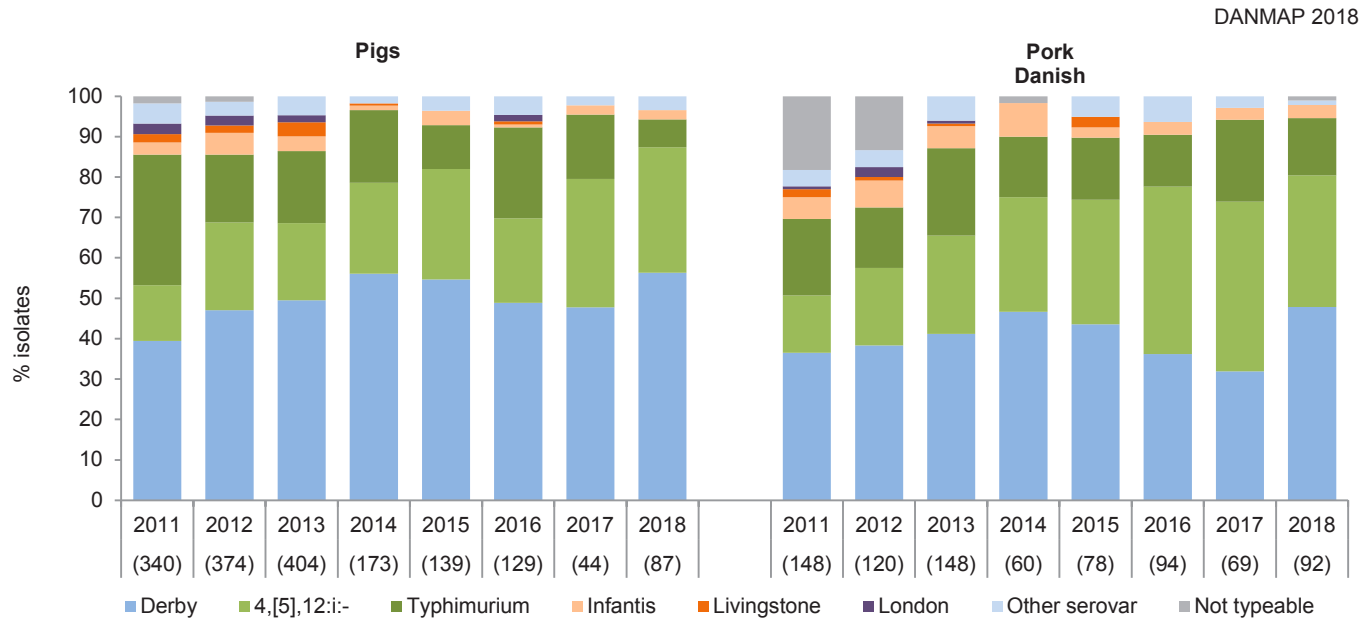
Fluoroquinolones are not used in food production animals in Denmark - indicating that the continued increase in ciprofloxacin resistance observed in Denmark is driven by something other than the direct usage of fluoroquinolones. In general, the Danish poultry sector uses very little antimicrobials, but tetracycline is the most common antimicrobial used in poultry (Table 4.1). The high level of *C. jejuni* isolates with both ciprofloxacin and tetracycline resistance suggests the pos-

Table 6.1 Resistance (%) in *Campylobacter jejuni* isolates from broilers, cattle and and human cases, Denmark DANMAP 2018

	Broilers	Cattle	Human		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%
Ciprofloxacin	43	20	39	83	55
Erythromycin	0	0	0	0	0
Gentamicin	0	0	0	0	0
Nalidixic acid	43	20	38	83	54
Streptomycin	5	4	4	12	8
Tetracycline	32	8	34	63	45
Fully sensitive (%)	53	72	49	15	38
Number of isolates	195	101	94	59	193

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

Figure 6.4 Relative distribution (%) of *Salmonella* serotypes from pigs and pork, Denmark



Note: Number of isolates included each year is presented in the parenthesis. Isolates from pigs originate from caecum samples collected at slaughter and isolates from pork originate from carcass swabs collected after slaughter for the national control programme

sibility of co-selection of ciprofloxacin resistance by the use of tetracycline in poultry. Whether that is what happens in reality, warrants further investigation.

As the previous years, no resistance to gentamicin was observed in 2018 (Table 6.1), providing 95% confidence that resistance to the antimicrobial is only present in 1.5% or less of the *C. jejuni* isolates from broilers. Gentamicin resistance was not observed in any of the human isolates.

6.3 *Salmonella*

Salmonella is the second most frequent zoonotic bacterial pathogen in humans in Denmark and can have a severe impact on both animal and human health [Annual Report on Zoonoses in Denmark 2018].

In Denmark, *S.* Typhimurium and *S.* Enteritidis are the serotypes that most frequently are associated with human illness. Human cases caused by *S.* Enteritidis are frequently associated with consumption of contaminated eggs, whereas *S.* Typhimurium cases often are associated with contaminated pork. Many *Salmonella* cases are travel associated and in 2018, 45% of cases reported travel history in association with their illness.

Salmonella isolates for DANMAP 2018 were obtained from national surveillance and control programmes. Pig isolates originated from slaughterhouses, where representative samples from healthy pigs (caecum) and pork (carcass swabs) are collected. Salmonellosis is a notifiable disease in humans and isolates from the reported *S.* Typhimurium cases are susceptibility tested and included in the DANMAP report. Only one isolate per farm, meat sample or human case was included in this report. For further details see chapter 9.

The occurrence of *Salmonella* in broilers, layers and cattle are monitored in Denmark each year. However, these isolates are not included in DANMAP 2018, as only few isolates were found and thus, fall below the inclusion threshold for DANMAP of 15 isolates per population. The occurrence of resistance in human *Salmonella* isolates other than Typhimurium is also monitored, but the results are not included in the DANMAP report. However, the data are reported to EFSA and ECDC, and are included in the European Union summary report on antimicrobial resistance, 2018.

The DANMAP report focuses on the resistance in *S.* Typhimurium and its monophasic variants. However, resistance in other *Salmonella* serotypes from pigs and pork is also monitored from 2011 and onwards, which is the year Denmark started to susceptibility test all serotypes according to EU legislation.

In DANMAP, *S.* Typhimurium includes the monophasic variants with antigenic formulas *S.* 4, [5],12:i:-, unless otherwise stated.

The antimicrobials recommended by EFSA were used for susceptibility testing. MIC distributions and occurrence of resistance among isolates from pigs, pork and humans are presented in the web annex (Tables A6.4 - A6.8).

6.3.1 *Salmonella* in Danish pigs and pork - all serotypes

From 553 representative pig caeca and 18,994 pig carcass's (pork) sampled at Danish slaughterhouses, 179 *Salmonella* isolates were obtained. A total of 161 of these were tested for antimicrobial resistance. As in the previous years, *S.* Typhimurium and *S.* Derby were the most common serotypes, representing 94% of all the isolates (Figure 6.4). In recent years, the relative occurrence of *S.* Derby has decreased, and in 2015 *S.* Typhimurium replaced *S.* Derby as the most prevalent serotype from domestically produced pork. In 2018, *S.* Derby was again the most dominant serovar, albeit with a small majority only.

A total of 32% of the *Salmonella* isolates from pigs were multidrug-resistant (defined as resistance to 3 or more of the 12 antimicrobial classes in the test panel, see Table 9.5). As 16% of the pig caeca was *Salmonella* positive, this indicates that approximately 900.000 of the 18 million pigs slaughtered in Denmark during 2018 carried multidrug-resistant *Salmonella*.

6.3.2 *S.* Typhimurium in Danish pigs and pork

S. Typhimurium remains the most important zoonotic serotype originating from pigs in Denmark. A total of 76 *S.* Typhimurium including 19 diphasic and 57 monophasic variants, were isolated from Danish pigs and pork in 2018. Most of the susceptibility-tested isolates were resistant to one or several antimicrobials and only 11% and 13% of the isolates from pigs and pork were fully susceptible to all antimicrobial agents in the test panel (Table 6.2).

As in the previous years, resistance to ampicillin, tetracycline and sulfonamide were common in the *S.* Typhimurium isolates from pigs and pork. Over the last five years, resistance to trimethoprim and chloramphenicol have increased in isolates from pork and in 2018, they were more frequent in isolates from pork than from pigs. Resistance to ampicillin and sulfonamide increased significantly in *S.* Typhimurium from pigs from 2017 to 2018 (48% vs. 75% and 52% vs. 82%, respectively). This follows the trend seen over the last 10 years where statistically significant increases in resistance to ampicillin, sulfonamide and tetracycline have occurred in isolates from both pigs and pork (Figure 6.5).

This year, 57% of the *S.* Typhimurium isolates from pigs and pork carried the ASuT resistance profile and 63% were multidrug-resistant (Figure 6.6), see the AMR profile distributions in web annex Table A6.9. One diphasic isolate was resistant to tigecycline (Table 6.2) with a MIC value of 4 µg/ml. Tigecycline resistance is rare in Denmark and was only previously detected in DANMAP samples from pigs in 2014, where two isolates were resistant (MIC = 2 µg/ml). The 2018 isolate was multidrug-resistant and further resistant to: ampicillin, chloramphenicol, sulfonamide, tetracycline and trimethoprim.

Two multidrug-resistant monophasic isolates from pork were resistant to azithromycin. They were both also resistant to ampicillin, sulfonamide, tetracycline and trimethoprim and one isolate further to chloramphenicol. Azithromycin is an important antimicrobial for treatment requiring diarrhoea in humans in Denmark.

None of the *S.* Typhimurium isolates from pigs or domestic pork were resistant to quinolones, cephalosporins, colistin or carbapenems. Based on the available number of isolates, we are 95% confident that these resistances are not present in more than 10% of *S.* Typhimurium isolates from pigs and 7% *S.* Typhimurium from pork (see section 9.7).

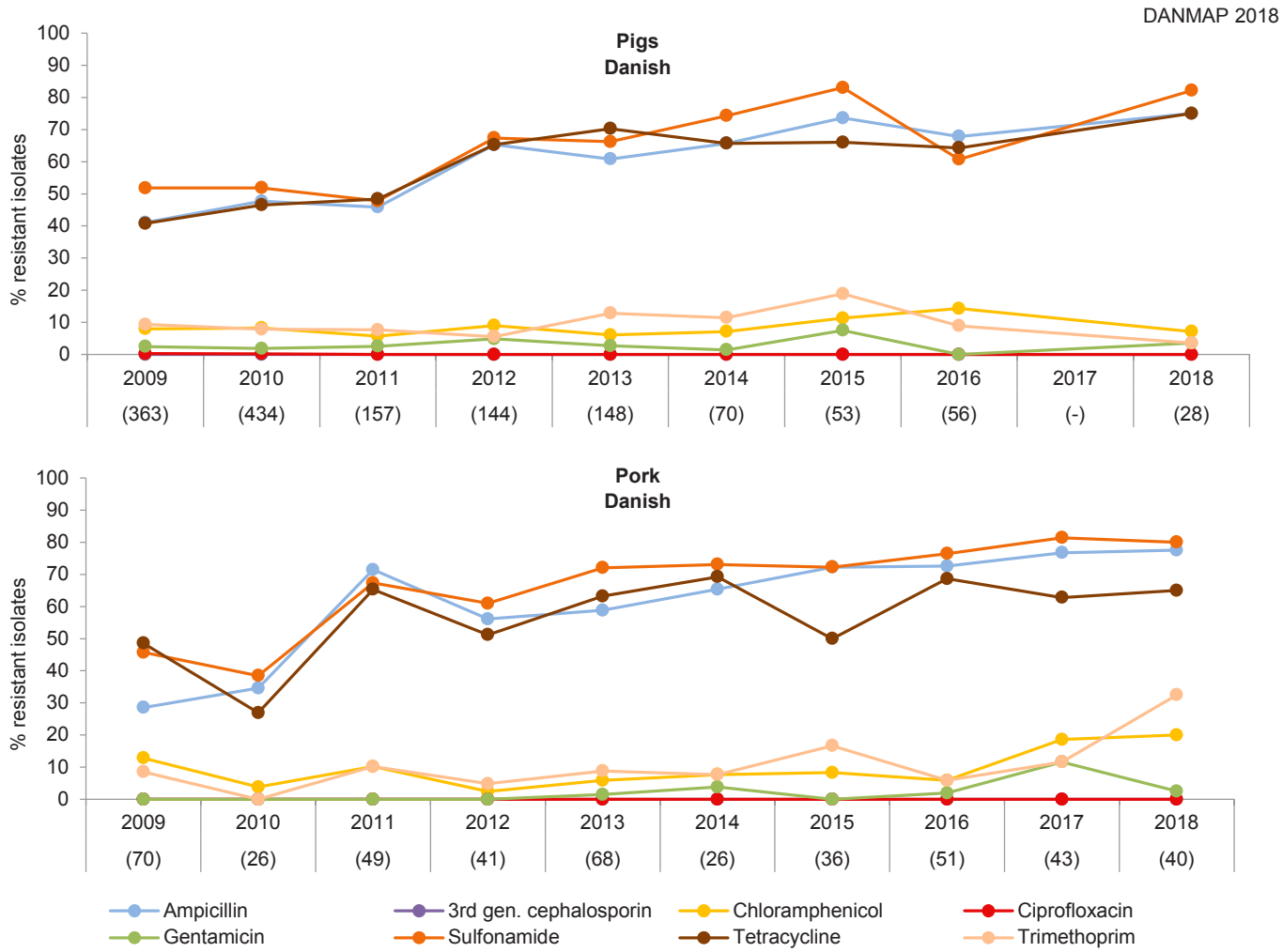
Tetracyclines, macrolides, pleuromutilins and beta-lactamase sensitive penicillins are the main antimicrobial agents used in pigs in Denmark (Figure 4.4). The distinct reduction in usage of tetracycline over the last 4-5 years in pigs were still not reflected in the 2018 levels of resistance in *S.* Typhimurium from Danish pigs and pork.

Table 6.2 Resistance (%) in *Salmonella* Typhimurium isolates from pigs, pork and human cases, Denmark DANMAP 2018

	Pigs	Pork	Human		
	Danish	Danish	Domestically acquired	Travel abroad reported	Total
Antimicrobial agent	%	%	%	%	%
Ampicillin	75	78	67	69	64
Azithromycin	0	5	<1	0	<1
Cefotaxime	0	0	<1	3	<1
Ceftazidime	0	0	<1	2	<1
Chloramphenicol	7	20	8	23	10
Ciprofloxacin	0	0	4	25	8
Colistin	0	0	1	2	2
Gentamicin	4	3	1	6	2
Meropenem	0	0	0	0	0
Nalidixic acid	0	0	2	11	4
Sulfonamide	82	80	66	63	62
Tetracycline	75	65	70	72	71
Tigecycline[a]	0	3	1	3	2
Trimethoprim	4	33	10	22	10
Fully sensitive (%)	11	13	22	8	22
Number of isolates	28	40	146	65	305

Note. Includes isolates verified as monophasic variants of *S.* Typhimurium with antigeneic 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
a) All human isolates classified as tigecycline resistant had a MIC value that was one dilution step higher than the ECOFF

Figure 6.5 Resistance (%) among *Salmonella* Typhimurium from pigs and pork, Denmark



Note: Number of isolates included each year is presented in the parenthesis and '-' indicate data that less than 25 isolates (n = 21) from pigs were included for 2017. Includes isolates verified as monophasic variants of S. Typhimurium with antigeneic formulas S. 4,[5],12:i:-. Isolates from pigs originate from caecum samples collected at slaughter (2011-2018) and boot swabs collected at farms (2009-2013). Isolates from pork originate from carcass swabs collected after slaughter for the national control programme

The 2018 increase in the use of macrolides for weaner and finisher pigs did not result in increased resistance to azithromycin (Figure 4.4). However, this will be monitored in the coming years.

6.3.3 Resistance in other relevant *Salmonella* serotypes in Danish pigs and pork

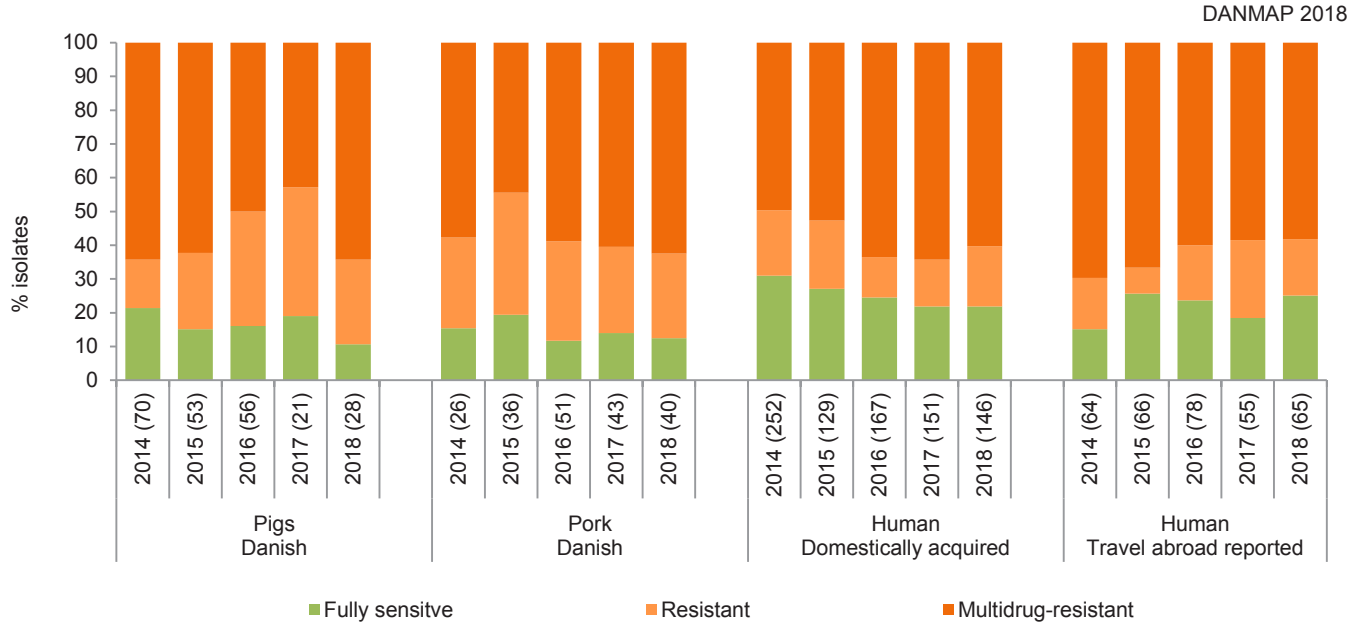
S. Derby was isolated from 49 slaughter pigs and from 44 pork samples. *S.* Derby is common among pigs, but only 12 human cases were reported in Denmark in 2018. In the last couple of years, a declining trend of fully susceptible *S.* Derby isolates has been observed. In 2015, 72% of *S.* Derby isolates were susceptible to all antimicrobials tested, in 2016 the proportion was 63%, and in 2017 58% were fully susceptible. This year, 70% of *S.* Derby isolates were again fully susceptible to all antimicrobials tested, bouncing back to 2015 levels. Resistance to tetracycline, sulfonamides, trimethoprim and ampicillin were most common, either alone or in combination. Only 13% of isolates were multidrug-resistant, one of these was further resistant to gentamicin and four to chloramphenicol.

Resistance to ciprofloxacin without simultaneous resistance to nalidixic acid was found in one isolate, suggesting plasmid-mediated quinolone resistance (PMQR). WGS of the isolate was examined through ResFinder 4.0, confirming the presence of the PMQR-gene *qnrS1*. This is the first registered case of PMQR in *Salmonella* from Danish pigs. The isolate was further resistant to chloramphenicol and ampicillin, introducing a possible risk of spread through co-selection, as ampicillin especially is commonly used in the pig production.

6.3.4 *Salmonella* in humans

A total of 1,168 human laboratory-confirmed cases of salmonellosis were reported (20.2 cases per 100,000 inhabitants). The most common serotypes were *S.* Typhimurium (including the monophasic variants) and *S.* Enteritidis with 5.3 and 4.6 cases per 100,000 inhabitants, respectively [Annual report on Zoonoses in Denmark 2018].

Figure 6.6 Relative distributions (%) of fully sensitive, resistant and multidrug-resistant *Salmonella* Typhimurium from pigs, pork and human cases, Denmark



Note: Number of isolates included each year is presented in the parenthesis. Includes isolates verified as monophasic variants of *S*. Typhimurium with antigeneic formulas *S*. 4,[5],12:i:-. An human isolate is categorised as domestically acquired if the patient did not travel outside Denmark one week prior to the onset of disease. 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 (Table 9.3)

6.3.5 *S.* Typhimurium in humans

The serotypes of *Salmonella* were derived from whole genome DNA sequence data. *S.* Typhimurium, including the monophasic variants, were most commonly identified among the human cases (306 cases) and MIC data from 305 of these isolates were included in this report. The monophasic variants represented 64% of the *S.* Typhimurium cases (196 monophasic and 109 diphasic). Information on patient travel history was available for 69% of the 305 cases, 21% of the cases were categorised as travel associated, 48% were acquired in Denmark, and the remaining cases had unknown travel status (Table 6.2). A total of 89 human cases were considered 'outbreak-related' of which 65 cases were associated with the monophasic variant. Two large outbreaks caused by the monophasic variant were recorded compromising 43 (ASuT) and 17 (fully sensitive) patients respectively. The other outbreak related cases compromised less than eight cases. All outbreaks, except one affecting six patients, were domestic.

As in the previous years, high levels (62-71%) of resistance to sulfonamide, ampicillin and tetracycline were observed (Table 6.2). Isolates that exclusively exhibited resistance towards these three antimicrobials were observed in 126 isolates of which 120 were monophasic. The large number of monophasic isolates exhibiting the ASuT phenotype is associated with a successful clone that has emerged worldwide over the last few years. See the AMR profile distribution in the web annex Table A6.9.

The levels of resistance in isolates from domestically acquired cases are overall at the same levels as in the previous years (Figure 6.7). The level of trimethoprim resistance increased from 1% in 2017 to 10% in 2018, a level that is in accordance with the levels observed in 2015 and 2016.

The level of resistance in isolates from human cases associated with travel were also in line with the observed levels in the previous years and were significantly higher than the level observed among isolates from domestic cases (Figure 6.7). Fluoroquinolone resistance increased from 11% to 25% from 2017 to 2018 in isolates from travel associated cases.

Resistance to colistin was observed in isolates from both domestically acquired (1%), and travel associated human cases (2%) and gentamicin resistance was also found in both domestic (1%) and travel associated isolates (6%).

The level of cephalosporin resistance was overall <1% for both cefotaxime and ceftazidime. Carbapenem resistance was not observed in any of the tested strains.

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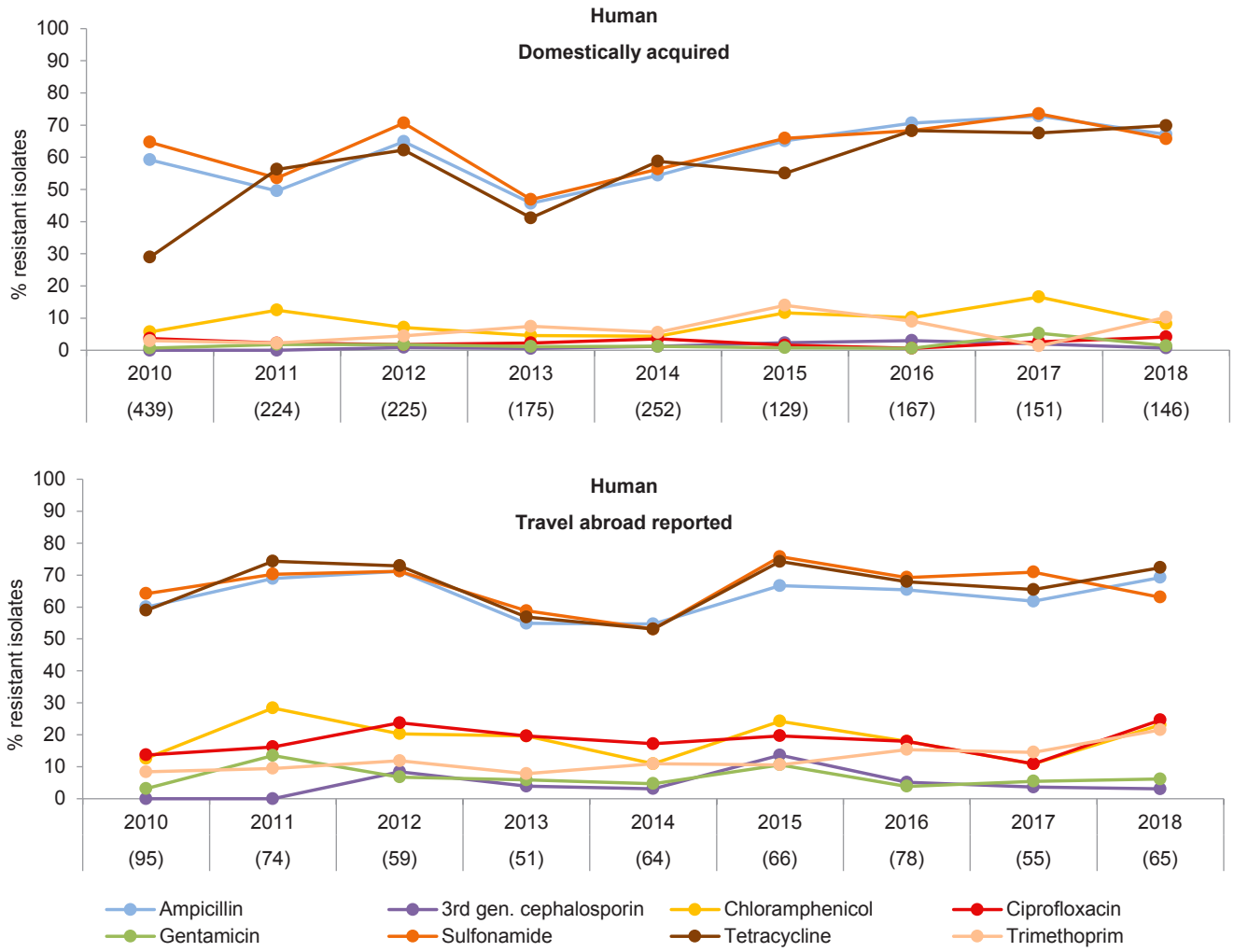
Figure 6.7 Resistance (%) among *Salmonella* Typhimurium from human cases, Denmark

DANMAP 2018



Note: Number of isolates included each year is presented in the parenthesis. Includes isolates verified as monophasic variants of *S*. Typhimurium with antigeneic formulas *S*. 4,[5],12:i:-. An isolate is categorised as 'domestic' if the patient did not travel outside Denmark one week prior to the onset of the disease

Textbox 6.1
Antimicrobial resistance in *Salmonella* from the national control programme in sow, multiplier and breeder pig herds

Background: Serological surveillance of *Salmonella* in Danish pig herds has been performed since 1995 (Order no. 1426 of 30/11/2018). Pigs from breeding and multiplying herds are blood sampled monthly and slaughter pig herds are monitored continuously by serologic testing of "meat juice" collected at slaughter. The number of samples and frequency of sampling are determined by the size of the herd and previous levels of sero-positive samples (see Annual Report on Zoonoses in Denmark for details). If the level of sero-positive samples exceeds the specified cut-off level, the herd and its supplier herds will be considered for on-farm bacteriologic confirmatory testing according to the criteria specified in the legislation. All *Salmonella* isolates recovered from these visits are susceptibility tested as part of the surveillance of critical resistance (Order no. 1426 of 30/11/2018). The farm derived isolates were included in DANMAP until 2014, when confirmatory testing of slaughter pig herds was discontinued. This and other changes to the legislation resulted in a non-representative sample of the remaining herds and the isolates were excluded from DANMAP.

The level of *Salmonella* is decreasing or remains at low levels in all animals in Denmark. This is a positive development, but it reduces the sensitivity of the *Salmonella* AMR surveillance. The number of isolates available for MIC testing from the annual surveys of slaughter pigs and meat is decreasing, hampering the ability to detect especially low level, new and emerging resistance.

The aim of this study was to investigate, whether the *Salmonella* isolates from sow, multiplier and breeder herds would enhance the current national surveillance of antimicrobial resistance in *Salmonella* from finisher pigs, if included as an additional surveillance component. To assess the surveillance value, we investigated the representativeness of the herds and AMR patterns for new information not already captured by the DANMAP slaughterhouse survey.

Data and data sources: The pen-faecal samples collected for the bacteriologic confirmatory testing were analysed at private industry laboratories and isolates were sent for serotyping and susceptibility testing at DTU National Food Institute (2014-2016) and the DVFA laboratory (2017-2018). For further details, see the Materials and methods in DANMAP 2014 and 2018.

The official Zoonosis Register (ZOOR) stores information on sampling and test results from the National *Salmonella* surveillance in the Danish pig production, and provided information on the herds where on-farm tests were performed during 2014-2018 (Accessed, 9. April 2019). From the DTU and DVFA Laboratory databases, the first isolate per serovar per year per herd from confirmatory testing in sow, multiplier and breeder herds during 2014-2018 was selected for inclusion. The test panel used in 2014 was slightly different than the panel used during 2015-2018 [DANMAP 2014, Table 9.1].

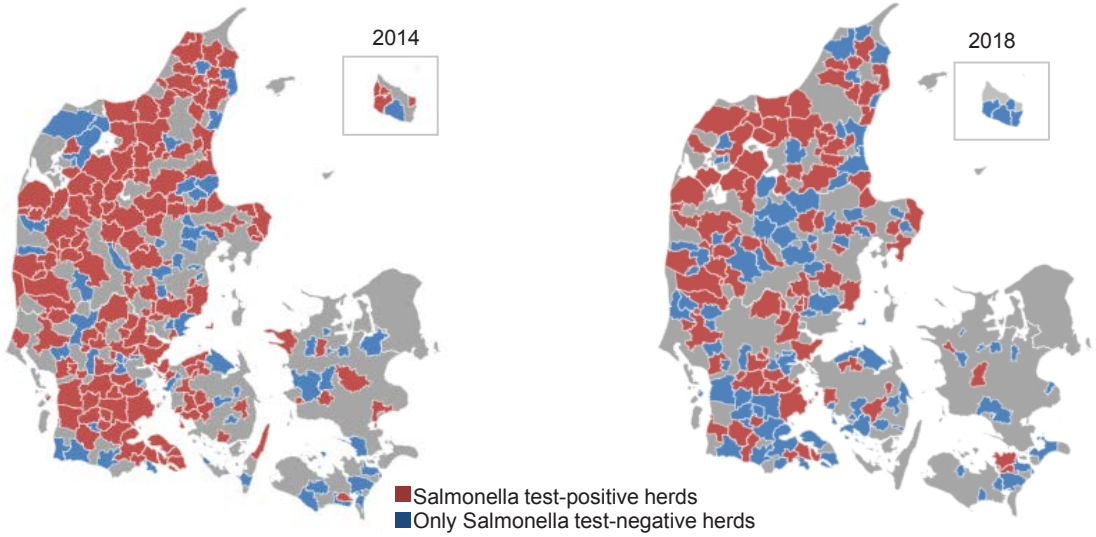
Results and discussion: Data from the official Zoonosis Register show that the annual number of performed confirmation tests in herds with sows, weaners and breeder pigs decreased during the five year period; from 625 tests (in 431 herds) during 2014 to 392 tests (in 298 herds) during 2018. The main reason for this decrease is that if herds are positive for *S.* Typhimurium (incl. monophasic variants), *S.* Derby, *S.* Infantis or *S.* Choleraesuis, they are considered positive for 60 months. As re-testing within this period is not performed, the size of the susceptible population is gradually reduced. Furthermore, testing is not performed in herds with recent sero-negative test results from pigs at slaughter or in herds found *Salmonella* positive with other serovars during the previous six months. These comprehensive exemption rules are implemented to optimise the use of resources and minimise cost for the private industry. However, it significantly reduces the number of available isolates for AMR testing and the continuously changing susceptible population makes it almost impossible to assess the representativeness of the isolates for AMR surveillance.

The geographical distribution of the tested herds visually reflected the general distribution of pig producers in Denmark. Most of the tested herds were located in Jutland, but herds from all regions were tested each year during the five year period. Figure 1 presents the regional distribution of the *Salmonella* tested sow, multiplier and breeder herds in 2014 and 2018. It shows zip-code areas without performed tests (grey), with only test-negative herds (blue) and with at least one *Salmonella* test-positive herd (red).

continued ... Textbox 6.1

Figure 1 Regional distribution (zip-code areas) of performed confirmatory tests for detection of *Salmonella* in sow, multiplier and breeder herds in 2014 and 2018, Denmark

DANMAP 2018



Note. Confirmatory testing were conducted in 431 herds in 2014 and 298 herds in 2018, where some herds were tested more than once. Zip-code areas without performed tests are coloured grey

When including only one serovar per herd per year, MIC data from a total of 955 isolates were available from the period 2014-2018. Only few herds are represented in more than one year for each serovar (4% for *S.* Typhimurium and 1% for *S.* Derby). Very few of these isolates were resistant to antimicrobial agents considered of critical importance for treatment of human infections. During the five years, azithromycin resistance was observed in 15 of 736 tested isolates (2%), and very few isolates were resistant to 3rd generation cephalosporins (1 of 945 isolates), fluoroquinolones (3 of 945 isolates) and colistin (4 of 955 isolates). Meropenem resistance was not observed in the 770 tested isolates.

The levels of resistance in *S.* Typhimurium incl. monophasic variants (473 isolates) and *S.* Derby (347 isolates) in the farm samples (Figure 2) are very similar to those of the slaughter pig caeca (Figure 6.5) during the five year period. In *S.* Typhimurium, stable and high levels of resistance to ampicillin, sulfonamide and tetracycline, low to moderate levels of resistance to trimethoprim and chloramphenicol and very low or no resistance towards the other compounds in the test panel were observed. Among the *S.* Derby isolates, the proportion of tetracycline resistance decreased significantly over the five years period, whereas resistance to the other compounds remained at the same low to moderate levels. No significant change in proportions of fully susceptible isolates were observed during 2015-2018 (Figure 2), but in all years the proportion of fully susceptible isolates was significantly higher among *S.* Derby compared to *S.* Typhimurium (63% vs. 15% in 2018, respectively).

Conclusions: The temporal and spatial patterns of sow, multiplier and breeder herds with *Salmonella* confirmatory tests in 2014-2018 are widely distributed at the national level. This allows consideration of the AMR results inclusion in DANMAP. However, as the rules for exemption from testing are very comprehensive and both affect herds that are presumed *Salmonella* negative as well as *Salmonella* positive, these data are not robust as evidence for changes in observed numbers of test-positive herds, changes in serovar distribution or changes in occurrence of antimicrobial resistance in *Salmonella* spp. over time. Nonetheless, the observed levels of resistance in *S.* Typhimurium and *S.* Derby from sow, multiplier and breeder herds from 2014-2018 supported the reported AMR levels in finisher pigs. Due to the findings and the uncertainty around denominator data, the component will not have sufficient surveillance value to be reported annually, but will be summarised every 3 to 5 years.

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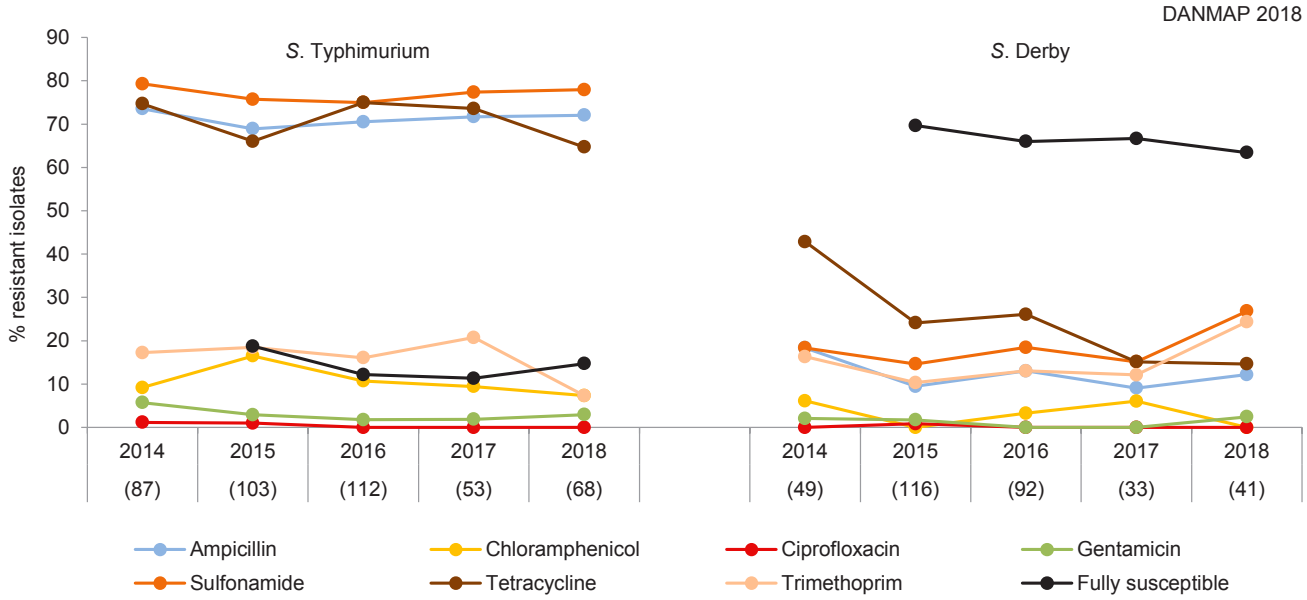
Figure 2 Resistance (%) among *Salmonella* Typhimurium and *Salmonella* Derby from sow, multiplier and breeder herds, Denmark



Note: Number of isolates included each year is presented in the parenthesis. Isolates originate from boot swabs collected at the farms.
S. Typhimurium includes isolates verified as monophasic variants with antigeneic formulas *S*. 4,[5],12:i:-

Textbox 6.2

Resistance in bacteria from diagnostic submissions from pigs

Background and data source: Data on antimicrobial susceptibility of three important veterinary pathogens *Escherichia coli* 0149, *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 2018. The number of isolates belonging to other bacterial species was too low to follow annual trends.

The antimicrobial susceptibility testing was carried out using the broth microdilution method with SensiTitre. Internationally approved clinical breakpoints are 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. Note that in 2019, the clinical breakpoint for colistin resistance was adjusted according to EUCAST clinical breakpoints for enterobactericae (>2 µg/ml; however >8 µg/ml was maintained for *Salmonella*), and these breakpoints are used retrospectively here.

MIC distributions and occurrence of resistance are presented in the web annex (Tables A6.10 - A6.12).

Table 1 Resistance (%) among bacteria from diagnostic submissions from pigs, Denmark DANMAP 2018

Antimicrobial agent	*Actinobacillus pleuropneumoniae* %	Haemolytic *Escherichia coli* %	*Streptococcus suis* %
Amoxicillin/clavulanic acid	-	2	-
Ampicillin	0	61	-
Apramycin	-	15	-
Cefotaxime	-	2	16
Ceftiofur	0	0	-
Chloramphenicol	-	21	0
Ciprofloxacin	0	0	2
Colistin	-	0	-
Erythromycin	100	-	49
Florfenicol	0	13	0
Gentamicin	-	13	0
Nalidixic acid	-	7	-
Neomycin	-	16	-
Penicillin	0	-	0
Spectinomycin	0	47	16
Streptomycin	-	74	36
Sulfametoxazol			70
Sulfonamide	-	67	-
Sulfonamide/trimethoprim	0	-	4
Tetracycline	3	61	48
Tiamulin	1	-	12
Tilmicosin	0	-	-
Trimethoprim	-	55	5
Tulathromycin	4	-	-
Number of isolates	102	282	111

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 - A6.12)

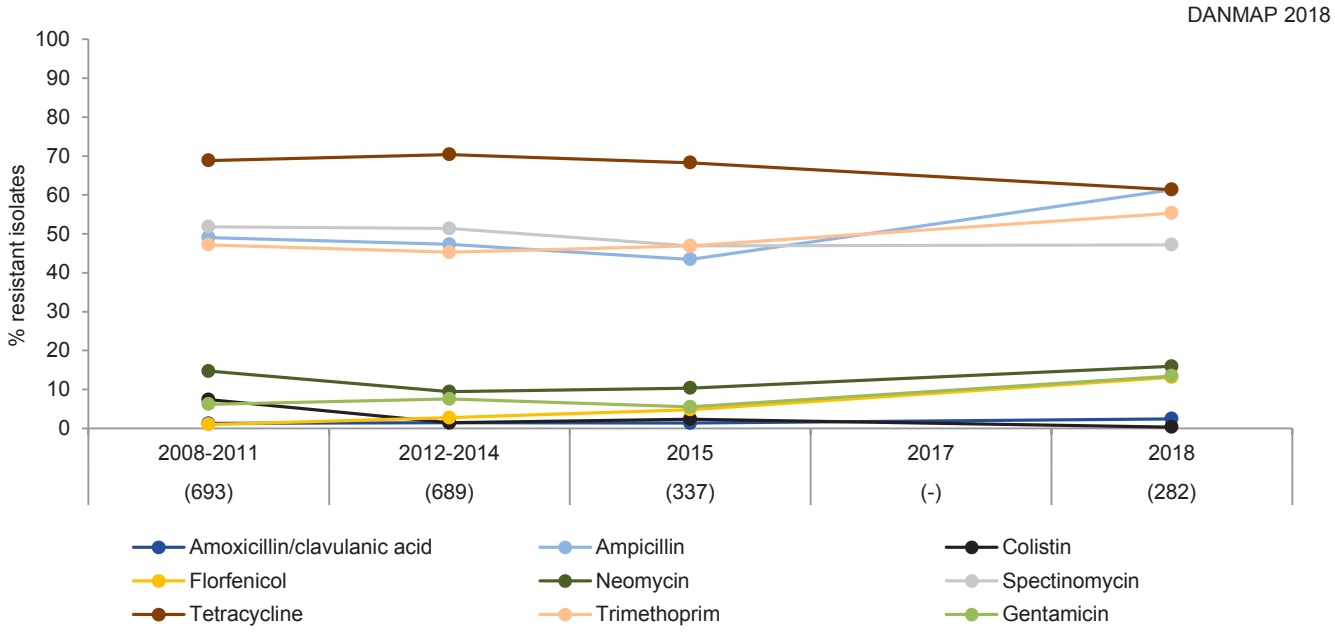
E. coli - haemolytic pathogenic strains

Enterotoxigenic *E. coli* (ETEC), *Brachyspira pilosicoli* and *Lawsonia intracellularis* are the most prevalent causes of bacterial diarrhoea in Danish pigs. Also, these bacteria are often found in combination. In previous years, the *E coli* isolates have been identified by serotyping, with the most virulent ETEC strains belonging to serovars O138, O139, O141, and O149, which are haemolytic and mostly positive for enterotoxin, and/or verotoxin 2e. Only a minor part of the haemolytic strains, 13% in 2018, were from edema disease cases (O139, $F18^+$ $VT2e^+$, enterotoxin negative). 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* reported here originates almost exclusively from porcine enteritis or edema disease.

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. Since 2018, almost all haemolytic *E. coli* strains sent for susceptibility testing have been typed for fimbrial adhesins and the strains are now rarely serotyped. 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. Furthermore, the fimbriae types are not available in the MIC dataset. Consequently, the pattern of antimicrobial resistance in *E.coli* in 2018, cannot be compared on serotype level to the occurrence of resistance in previous years. In Figure 1, we have chosen to compare with data for all haemolytic *E. coli* isolated from pigs in 2015 and previous years.

As in previous years, high resistance levels were recorded in 2018 for ampicillin, streptomycin, sulphonamides, tetracyclines, trimethoprim, and spectinomycin (Figure 1). The level of resistance to tetracycline appeared (not statistically significant) lower in 2018 compared to 2015 (61% vs. 68%), suggesting a decreasing trend in parallel with the trend in tetracycline resistance in *E. coli* O149 reported in DANMAP 2017. Most cases of porcine diarrhoea that require treatment occur during the weaning period and tetracyclines, neomycin, or aminopenicillins are the compounds of choice in case of *E. coli* infection. However, the use of

Figure 1 Resistance (%) among haemolytic *Escherichia coli* from pigs, Denmark



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)

continued ... Textbox 6.2

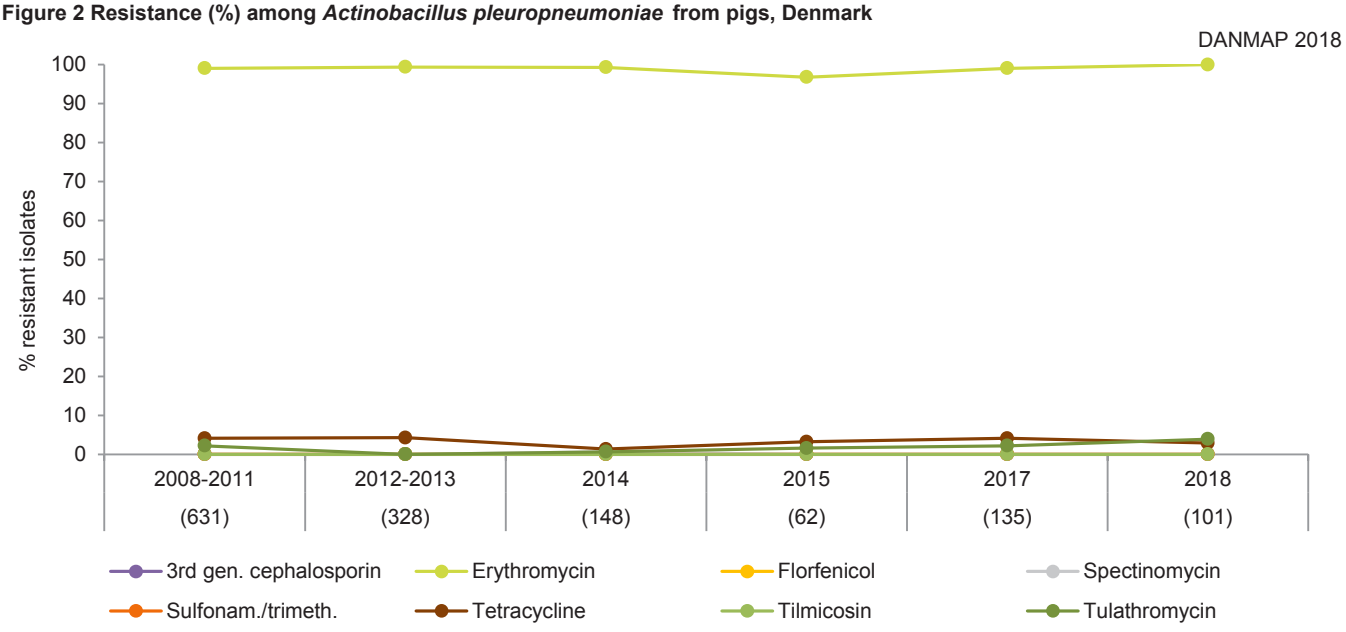
tetracyclines has decreased significantly from 2016 and onwards, most likely due to changes in the Yellow Card legislation (putting higher weights to tetracyclines, see Textbox 4.1). In 2018, a significant increase was observed in the level of resistance to ampicillin, neomycin, gentamicin and apramycin (Figure 1). The parallel increase in resistance among the two latter were most likely a result of cross resistance, as 78% of the apramycin resistant isolates were also resistant to gentamicin (Table 1). 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. It is uncertain whether the increasing level of resistance in these compounds and the apparent decrease in tetracycline resistance is due to natural variation or whether it is a trend following changes in the pattern of antimicrobial usage. A significant increase was also noted for florfenicol, as part of a gradual increase during the last decade, with 13% in 2018 (Figure 1). The reasons for this apparent steady increase need further investigation. Isolates that were resistant to florfenicol were also resistant to chloramphenicol, but resistance levels to chloramphenicol did not increase significantly.

For *E. coli*, the breakpoint for colistin was adjusted from >8 µg/ml to >2 µg/ml. This revealed that colistin resistance has decreased significantly in recent years from a very low level to absent in 2018. Colistin was never among the most frequently used compounds, but the usage of colistin declined close to zero during 2017, due to changes in official guidelines and the Yellow Card. The relatively high resistance levels to many compounds increase the benefits of susceptibility testing before treatment.

Actinobacillus pleuropneumoniae

Actinobacillus pleuropneumonia causes pleuropneumonia in pigs. It is a severe infection although severity varies with serotype. Therefore, outbreaks require rapid onset of treatment to minimise losses. Fortunately, *A. pleuropneumoniae* has a predictable resistance pattern, with very low occurrence of resistance to most compounds. Almost all isolates are resistant to erythromycin but have very low occurrence of resistance to other macrolides like tilmicosin, which are often used for treatment (Figure 2).

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



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 A6.11)

Tulathromycin is also frequently used, but the occurrence of resistance has increased numerically (not statistically significantly) in recent years from around 1-2% during 2008-2017 to 4 % in 2018 (Table 1). Although the resistance is still at a low level, alternatives should be considered for treatment, due to the risk of treatment failure, and because of the risk of continued increases in tulathromycin resistance. Multiple alternatives are available: No resistance to florfenicol, sulphonamide-trimethoprim and tilmicosin has been observed for the last decade, and the occurrence of resistance remains 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.

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. As in previous years, resistance was highest to macrolides (erythromycin), streptomycin, and tetracyclines (Figure 3). The observed fluctuations in tetracycline resistance were non-significant. However, there are several treatment options using compounds with very low levels of resistance (Table 1). As in 2017, all isolates were susceptible to both penicillin and florfenicol in 2018 and both of these are recommended first choice antimicrobials in the official guidelines. There was a high occurrence of resistance to sulphonamides, but a low level of resistance to trimethoprim, and even slightly lower resistance level for sulfonamide and trimethoprim in combination. The relative number of isolates resistant to sulfonamide-trimethoprim decreased (near significant) in 2018, with 3.6% resistant isolates, as compared to 8.6% in 2017. The occurrence of resistance to pleuromutilins (tiamulin) and spectinomycin is at a moderate level, but was significantly lower in the isolates tested in 2018, as compared to previous years (Figure 3).

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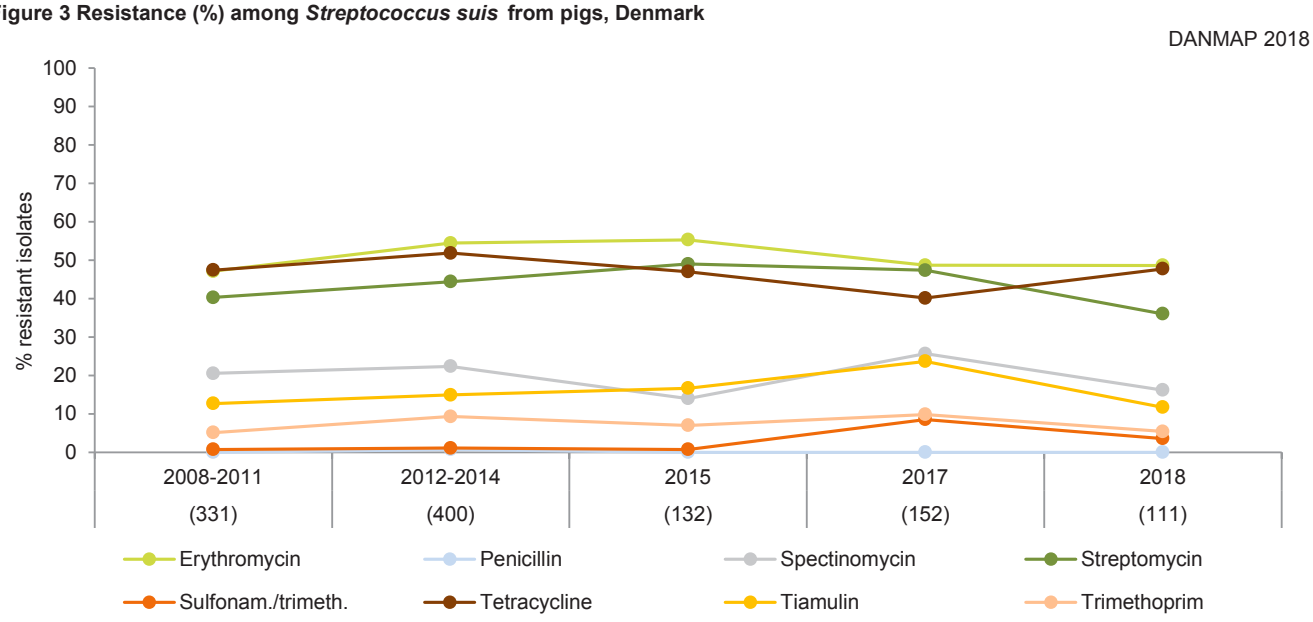
Figure 3 Resistance (%) among *Streptococcus suis* from pigs, Denmark



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 A6.12)