9

MATERIALS AND METHODS

9. Materials and methods

9.1 General information

For the DANMAP 2018 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on general practitioners from the Danish Medical Association [www.laeger.dk].

The epidemiological unit for pigs and cattle was defined at the individual farm level, meaning that only one isolate per bacterial species per farm was included in the report. The individual flock of broilers was defined as the epidemiological unit, and for food, the epidemiological unit was defined as the individual meat sample.

For humans, the epidemiological unit was defined as the individual patient and the first isolate per species per patient per year was included. An overview of all antimicrobial agents registered for humans and animals in Denmark is presented in Table 3.2.

9.2 Data on antimicrobial consumption in animals

9.2.1 Data

In Denmark, all antimicrobial agents used for treatment are available on prescription only. Until 2007, antimicrobial agents were exclusively sold by pharmacies or as medicated feed from the feed mills. However, since April 2007, the monopoly was suspended and private companies (four in 2018) were given license to sell prescribed veterinary medicinal products for animals, when following strict guidelines, identical to those applied to pharmacies. Furthermore, in 2007 price setting of antibiotic was liberalised, which allowed for discounts to veterinarians, when buying larger quantities.

A pharmacy or company either sells the medicine to veterinarians for use in their practice or for re-sale to farmers, or sells the medicine directly to the animal holder on presentation of a prescription. By law, veterinarians are allowed only very small profits on their sale of medicine (5%), to limit the economic incentive to overprescribe.

In 2018, 97% of antimicrobial agents were purchased through pharmacies and the drug trading companies, while 3% were purchased from the feed mills. These numbers did not include prescribed zinc oxide from the feeding mills for the pigs. For cattle, 83% of antimicrobial agents used in 2018 were purchased from pharmacies, whereas 10 years ago two thirds of the antimicrobial agents used in cattle was purchased through the veterinarian. In aquaculture, approximately two thirds is purchased through the feed mills.

Data on all sales of veterinary prescription medicine from the pharmacies, private companies, feed mills and veterinarians are sent electronically to a central database called VetStat, which is hosted by the Danish Veterinary and Food Administration. Prior to 2001, all data on antimicrobial sales were derived from pharmaceutical companies. Veterinarians are required by law to report all use of antibiotics and prescriptions for production animals to VetStat monthly. For most veterinarians, the registration of data is linked to the writing of invoices. The electronic registration of the sales at the pharmacies is linked to the billing process and stock accounts at the pharmacy, which ensures a very high data quality regarding amounts and type of drugs. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at SSI and to VetStat. However, VetStat does not have any validation on data entry and slight typing errors from vets may occur.

In addition, data on coccidiostatics as feed additives (non-prescription) and antimicrobial growth promoters (not in use since 2000) have also been collected by VetStat, providing an almost complete register of all antimicrobial agents used for animals in Denmark for the past twenty years. In very rare instances, medicines are prescribed on special license and will not be included in VetStat (i.e. medicines not approved for marketing in Denmark).

The VetStat database contains detailed information about source and consumption for each prescription item: date of sale, identity of prescribing veterinarian, source ID (identity of the pharmacy, feed mill, or veterinarian practice reporting), package identity code and amount, animal species, age group, disease category and code for farm-identity (CHR Danish Central Husbandry Register). The package code is a unique identifier, relating to all information on the medicinal product, such as active ingredient, content as number of unit doses (e.g. number of tablets), package size, and code of the antimicrobial agent in the Veterinary Anatomical Therapeutic Chemical (ATCvet) classification system.

Knowledge of the target animal species enables the presentation of consumption data in "defined animal daily doses" (DADD) a national veterinary equivalent to the international defined daily doses (DDDs) system applied in the human field [www. whocc.no]. The data presented in DANMAP 2018 were extracted from VetStat on 3th March 2019.

9.2.2 Methods

In DANMAP, we report use of antimicrobials dispersed in different animal populations. As a first step, the amount of antimicrobial agents used in animals is measured in kg active compound, to enable an overall crude comparison of consumption in different animal species and in the veterinary and human sectors.

A more detailed comparison of antimicrobial use is performed, taking into account their potency, formulation, route of administration and the age of the animals (where relevant), by generating defined animal daily doses (DADDs). For these calculations, we select data with relevant animal and age group codes, e.g. pigs and weaners and relevant codes for dispensation. For example, when calculating the antimicrobial use for systemic treatment in pigs, we exclude antimicrobials dispensed as tablets, products for topical use, intramammaries and gynaecologicals. This is described in the footnotes for figures and tables in chapter 4.

Numerator - DADD

Defined animal daily dose (DADD) is the average maintenance dose per day for a drug used for its main indication in the appropriate animal species. The DADD is not defined at product level but for each antimicrobial agent, administration route and animal species and when appropriate, also age group. DADD has been specifically defined for use in DANMAP and does not always completely match the "prescribed daily dose" or the recommended dosage in the Summaries of Product Characteristics (SPC).

The following principles are applied when setting the DADDs:

1. Minor inconsistencies are corrected (e.g. due to rounding of numbers);
2. Approved dosage for the most widely used antimicrobial products is given priority above dosage for products that are rarely used;
3. Approved dosage for older products within the group is maintained as the common DADD even if a new product is approved with a higher dosage;
4. If the dosage for a group shows large variation in approved dosages of the products, the dosages provided by "The Veterinary Formulary" [British Veterinary Association 2005, 6th edition] are applied;
5. Dosages may vary within active compound and administration route, if different dosages have been approved for different age groups, indications or formulations.

When principle 3 and 4 are conflicting, principle 5 is applied.

Denominator - biomass

Trends in antimicrobial use in pigs are presented in DADD per 1,000 animals per day (DAPD). The number of animals in a population (in epidemiological terms: the population at risk) is represented by their live biomass. The biomass of a species is calculated, taking into account average live bodyweight and the average lifespan in each age group. The estimation of live biomass and thus the number of standard animals at risk per day depends on the available data sources for each species.

Pig production: The estimation was based on the number of pigs produced, including exports at different ages, productivity data for each year [Statistics Denmark; Danish Agriculture and Food Council] and census data for breeding animals [Statistics Denmark]. The average weight and life span for the growing animals (piglets, weaners and finishers) was estimated from the annual productivity numbers [Danish Agriculture and Food Council]. For DANMAP 2018, productivity data from 2017 were used to estimate the biomasses for pigs, since the 2018 productivity data were not available when estimates were calculated. The estimation methods were developed in cooperation with Danish Agriculture and Food Council. There are no statistics on average weight of breeding animals available, so an estimated average weight had to be assumed. However, the size of the breeding animals has probably increased over the last decade, but this could not be accounted for.

Cattle production: The live biomass of the cattle population is estimated from census data [Statistics Denmark 2019] and the average live weight of the different age groups. The Danish cattle population is mainly dairy, particularly Holstein Friesian, but also other breeds such as Jersey and a small population of beef cattle. Most of the cattle slaughtered are dairy cows and bull calves of dairy origin. The average live weight was estimated for 10 different age and gender categories.

Broiler (Gallus gallus): The live biomass was estimated based on number of broilers produced [Statistics Denmark; Danish Agriculture and Food Council], an average live weight at slaughter of 1.97 kg after an estimated average life span of 30 days. The mean live biomass per broiler is assumed to be half of the weight at slaughter.

Turkey production: The live biomass is estimated based on the number of turkeys produced [Statistics Denmark; Danish Agriculture and Food Council] and an average live weight at slaughter of 21 kg for male turkeys and 11 kg for hens after an estimated average life span of 20 weeks and 15.5 weeks, respectively [Danish Agro; S. Astrup, personal communication]. The estimated mean live biomass per turkey is assumed to be half of the weight at slaughter.

Fur animals: The live biomass of mink is estimated from production data [Kopenhagen Fur] and carried out as described by Jensen et al., 2016 [Prev Vet Med. 26:170].

Pet animals: Only dogs and cats are taken into account, as the other population sizes are negligible in Denmark, and relatively rare in veterinary practice. The population is based on census data [Statistics Denmark, 2000] estimating 650,000 cats and 550,000 dogs. The number of dogs in Denmark has been relatively stable during the last ten years [Danish Dog register, 2012]. The average live weight for cats and dogs were estimated to 4 kg and 20 kg, respectively (based on pedigree registration data).

Aquaculture: The estimation is based on data from the Danish AgriFish Agency (Ministry of Environment and Food) on produced amounts in each subtype of production, and information

on the typical lifespan, entrance and exit body weights. The estimation was calculated in cooperation with Danish Aquaculture [N.H. Henriksen, Danish Aquaculture].

Treatment proportion - DAPD

The treatment proportion is calculated as the number of DADDs administered to an animal species during a year (in thousands) divided by the number of standard animals at risk per day (DADD per 1000 animals per day). The number of standard animals at risk per day takes into account species differences in average body-mass and lifespan. When relevant, the numbers of DADDs and standard animals at risk are estimated for specific age groups, or simply as number of doses (DADDs) used to treat one kg of animal divided with the total estimated biomass (in tonnes).

DAPD is a statistical measure, which provides a rough estimate of the proportion of animals treated daily with an average maintenance dose of a particular antimicrobial agent. For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day. The DAPD is also referred to as the treatment proportion or treatment intensity. In principle, the metric DAPD is parallel to the metric used in pharmaco-epidemiology for the human sector, defined daily dose per 1000 inhabitants per day (DID), see section 9.8.2.

In 2018, DAPD calculations were carried out for pigs, cattle and fur animals.

Due to a relative high number of pigs exported around 30 kg; an adjusted measure of the average antimicrobial use in all age groups was calculated ($DAPD_{adj}$). The adjustment is based on the assumption that pigs exported at 30 kg, on average, would have received the same amount of antimicrobial agents as other pigs from farrowing to slaughter.

Antimicrobial use per pig produced (adjusted) is calculated as: [DADDs + DADDw + (1+Q)*DADDf] / (biomass-days-total + Nw* AN_{BDW} (kg*days)), where
DADDs= the amount of antimicrobial agents used in sows
DADDw = the amount of antimicrobial agents used in weaners
DADDf = the amount of antimicrobial agents used in finishers
Q is the proportion of weaning pigs exported around 30 kg
Nw= the number of pigs exported at 30 kg bodyweight
AN_{BDW}=Average number of biomass days contributed by a weaner pig

9.3 Collection of bacterial isolates - animals and meat

9.3.1 Animals

Since 2014, most isolates available for DANMAP have been collected in accordance with the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria [Decision 2013/652/EU]. The legislation requires, in addition to sampling for the national *Salmonella* control programmes in poultry farms, sampling of broilers and fattening turkeys at slaughter in even years (2014-2020) and sampling of fattening pigs and cattle <1 year at slaughter in odd years (2015-2019).

In 2018, most of the sampling for DANMAP was allocated to the mandatory sampling of broilers (examined for *Campylobacter jejuni*, indicator *E. coli* and ESBL/AmpC/Carbapenemase-producing *E. coli*). Additionally, sampling of slaughter pigs (examined for *Salmonella* and indicator *E. coli*) and cattle <1 year (examined for *Campylobacter jejuni* and indicator *E. coli*) was carried out (Table 9.1).

Meat inspection staff or abattoir personnel at the slaughterhouses collected caecal samples from healthy pigs, cattle (<1 year) and broilers. For broilers, the samples were collected throughout 2018, but the majority of samples were taken during June to November; in order to collect isolates during the expected high-prevalence period of *Campylobacter*. For broilers, the sampling took place at the two major Danish slaughterhouses and at one minor slaughterhouse. For pigs and cattle, the samples were collected throughout 2018. The sampling was stratified per slaughterhouse by allocating the number of samples from domestically produced animals collected per slaughterhouse proportionally to the annual throughput of the slaughterhouse.

Four intact caeca from each broiler flock were pooled into one sample. For pigs and cattle, samples contained 30-100 g caecal material from a single animal. All samples were processed at the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted, including antimicrobial susceptibility testing and *Salmonella* serotyping.

Salmonella from layers, broilers, turkeys and cattle are not included in DANMAP 2018 due to low numbers of isolates available from the national surveillance [Annual Report on Zoonoses in Denmark, 2018].

9.3.2 Meat

The EU harmonised monitoring requires, in addition to sampling for the national *Salmonella* control programmes at slaughter, sampling at retail of broiler meat in even years (2014-2020) and sampling of pork and beef in odd years (2015-2019) for detection of ESBL/AmpC-producing *E. coli* [Decision 2013/652/EU].

In 2018, ESBL/AmpC/Carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled broiler meat collected in Danish wholesale and retail outlets throughout the year by the regional DVFA officers (Table 9.1). Products with added saltwater or other types of marinade were excluded. Most the packages of broiler meat (n = 293) were selected without preselecting based on the country of origin as requested for the harmonised EU monitoring. In 2018, an additional 131 samples of imported meat was collected. The number of establishments

and samples selected by each regional DVFA control unit was proportional to the number of establishments in the region in relation to the total number of establishments in the country. One unit of product (minimum of 200 g) was collected and all samples were processed at the DVFA laboratory.

The *Salmonella* isolates from pork originate from the national control programme at the slaughterhouses (Table 9.1), where the carcasses are swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10cm). The numbers of swabs collected depend on the slaughterhouse capacity. All samples were processed at Industry laboratories. Isolates from all *Salmonella* positive samples were send to the DVFA laboratory, where one isolate per sample was serotyped and susceptibility tested.

Salmonella from broiler meat and beef are not included in DANMAP 2018 due to low numbers of isolates available from the national surveillance programmes [Annual Report on Zoonoses in Denmark, 2017]. *Campylobacter* from broiler meat for DANMAP originate from the national control program: Intensified Control of *Salmonella* and *Campylobacter* in fresh meat. However, very few *Campylobacter* isolates from domestically produced broiler meat was susceptibility tested in 2018 and therefore not included in DANMAP 2018.

9.4 Microbiological methods – isolates from animals and meat

9.4.1 *Salmonella*

Salmonella was isolated in accordance with the methods issued by the NMKL [NMKL No. 187, 2007] or ISO 6579-1 [ISO6579-1:2017]. Serotyping of isolates was performed by whole genome sequencing using the Illumina MiSeq platform, paired-end sequencing 2x250 cycles. For bioinformatics, a CGE service (Centre for Genomic Epidemiology, DTU) for *Salmonella* serotyping was applied based on the genetic background for antigenic formulas given by the White-Kauffmann-Le Minor scheme. Only one isolate per serotype was selected from each herd, flock or slaughter batch.

9.4.2 *Campylobacter*

Campylobacter from broilers and cattle was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] followed by species-determination by BAX® rtPCR assay. Pre-enrichment in Bolton broth was used for cattle samples, whereas direct spread of caecal sample on to selective agar was used for broiler samples. Only one *Campylobacter jejuni* isolate per broiler flock, cattle herd or per batch of fresh meat was selected.

9.4.3 Indicator *Escherichia coli*

Indicator *E. coli* from broilers, pigs and cattle was isolated by direct spread of caecal sample material onto violet red bile

Table 9.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contribute isolates to DANMAP 2018

DANMAP 2018

Bacteria	Origin of isolates	Legislative reporting frequency (2013/652/EU)	Number of tested and positive samples in 2018
Salmonella spp.	On-farm samples from laying hens (production flocks)	Even years	4,245 flocks (35 positive)
	On-farm samples from broilers (production flocks)	Even years	454 flocks (12 positive)
	Caecal samples from fattening pigs		553 animals (87 positive)
	Neck skin samples from broilers	Even years	249 flocks (1 positive)
	Carcass swabs from fattening pigs (a)	Odd years	18,994 animals (0.8% positive)
	Carcass swabs from cattle <1 year (a)	Odd years	6,356 animals (0.2% positive)
Campylobacter jejuni	Caecal samples from broilers	Even years	836 flocks (256 positive)
	Caecal samples from cattle <1 yr		154 animals (111 positive)
Indicator *E. coli*	Caecal samples from broilers	Even years	186 flocks (174 positive)
	Caecal samples from fattening pigs	Odd years	154 animals (150 positive)
	Caecal samples from cattle <1 yr	Odd years	198 animals (187 positive)
Specific monitoring of ESBL/AmpC and Carba – producing *E. coli*	Caecal samples from broilers	Even years	837 flocks (124 positive)
	Fresh broiler meat at retail (domestic origin)	Even years	244 units (36 positive)
	Fresh broiler meat at retail (Imports)	Even years	180 units (82 positive)
	WGS data for collected ESBL/AmpC isolates		189 isolates

Note: Carcasses swabs collected at slaughterhouse slaughtering more than 30,000 pigs or 7,500 cattle, swab samples are analysed in pools of 5 samples. When estimating the prevalence of *Salmonella*, both the loss of sensitivity and the probability of more than one sample being positive in each pool are taken into consideration

Testing for carbapenemase producing *E. coli* is voluntary according to regulation 2013/652/EU. Carbapenemase producing E. coli was not detected in any of the analysed sample. Most the packages of broiler meat (n = 293) were selected without pre-selecting based on the country of origin as requested for the harmonised EU monitoring. In 2018, an additional 131 samples of imported meat was collected and analysed for ESBL/AmpC and Carbapenemase-producing *E. coli*

agar incubated for 24h at 44°C. Presumptive *E. coli* was identified on TBX agar incubated at 44°C o/n. Only one indicator *E. coli* isolate per flock or herd was selected. For specific isolation of ESBL/AmpC and carbapenemase-producing *E. coli* from meat and caecal samples, the present EURL-AR laboratory protocols describing the selective enrichment procedures was applied in accordance with the EU harmonised monitoring. Only one ESBL/AmpC-producing *E. coli* isolate per broiler flock and meat sample was selected (no isolates of carbapenemase-producing *E. coli* was detected).

9.5 Susceptibility testing – isolates from animals and meat

Antimicrobial susceptibility testing of *Salmonella*, *Campylobacter* and *E. coli* was performed as Minimum Inhibitory Concentration (MIC) determination using broth microdilution by Sensititre (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/ EU about the EU harmonised monitoring of antimicrobial resistance.

The relevant quality control strains were used at the laboratories: *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Campylobacter jejuni* ATCC 33560 and *Pseudomonas aeruginosa* ATCC 27853. Isolates from animals and meat were tested for antimicrobial susceptibility at the DVFA laboratory in Ringsted that is accredited by DANAK (the national body for accreditation).

Table 9.3 Definitions of antimicrobial classes for calculation of multidrug-resistance in *Salmonella* and indicator *E. coli* DANMAP 2018

Antimicrobial classes	*Salmonella* and *E. coli*
Beta-lactam penicillins	Ampicillin
Macrolides	Azithromycin
Cephalosporins	Cefotaxime and/or ceftazidime
Phenicols	Chloramphenicol
Quinolones	Ciprofloxacin and/or nalidixic acid
Polymycins	Colistin
Aminoglycosides	Gentamicin
Carbapenems	Meropenem
Sulfonamides	Sulfonamides
Tetracyclins	Tetracycline
Glycylcyclines	Tigecycline
Trimethoprim	Trimethoprim

Note: An isolates is considered multidrug-resistant if resistant to three or more of the defined antimicrobial classes and fully sensitive if susceptible to all antimicrobial agents included in the test panel

9.6 Whole genome sequencing – isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing (section 9.4.1), whole genome sequencing (WGS) and in silico bioinformatic tools were also used to detect the genetic background of the ESBL/AmpC and carbapenemase-producing *E. coli*. At the DVFA laboratory in Ringsted, strains were sequenced using the Illumina MiSeq platform followed by bioinformatics analysis at DTU National Food Institute from Centre for Genomic Epidemiology (www.genomicepidemiology.org; https://cge.cbs.dtu.dk//services/all.php) including:

1. De novo assembly using SPAdes [Bankevich et al. 2012. J Comput Biol. 19(5):455];
2. MLST using MLST Finder 2.0 [Larsen et al. 2012. J Clin Microbiol. 50(4):1355];
3. Detection of antimicrobial resistance genes using ResFinder 3.1 which includes chromosomal mutations leading to resistance to beta-lactams, quinolones and colistin as well as acquired resistance genes [Zankari et al. 2012. J Antimicrob Chemother. 67(11):2640; Zankari et al. 2017. J Antimicrob Chemother. 72(10):2764]; and
4. Detection of plasmid replicons using PlasmidFinder 2.0 [Carattoli et al. 2014. Antimicrob Agents Chemother. 58(7):3895].

9.7 Data handling – isolates from animals and meat

For the samples processed at the DFVA laboratory, sampling details and laboratory results were stored in the DVFA Laboratory system. Following validation by DVFA, data were send to National Food Institute at DTU (Excel sheets). Here data were harmonised and one isolate per epidemiological unit was selected for reporting. All data are stored in an Oracle database at isolate level (9i Enterprise Edition®). The database contains all antimicrobial data reported in DANMAP or to EFSA since 2007 (partial dataset from 2001-2006). Variables include: Bacterial species (subtype where applicable), date of sampling, animal species or food type, herd identifier and country of origin whenever possible.

9.7.1 Interpretation of MIC values

MIC values were retained as continuous variables in the database, from which binary variables were created using the relevant cut-off from 2018 for all years. Since 2007, data have been interpreted using EUCAST epidemiological cut-off values with a few exceptions described in Table 9.2. All MIC-distributions are presented in the web annex at at www.danmap.org/downloads/reports. Each of the tables provides information on the number of isolates, the applied interpretation of MIC-values and the estimated level of resistance. Confidence intervals are calculated as 95% binomial proportions presenting Wilson intervals.

Table 9.2 Interpretation criteriae for MIC-testing by EUCAST epidemiological cut-off values and the corresponding EUCAST clinical breakpoints **DANMAP 2018**

	Salmonella		*E. coli*		*C. jejuni*		*Staphylococcus aureus*
	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	ECOFF µg/ml	Clinical breakpoint µg/ml	Clinical breakpoint µg/ml
Ampicillin	>8	>8	>8	>8			
Azithromycin	>16 (a)		>16 (a)				
Cefepime	>0.125 (a)	>4	>0.125	>4			
Cefotaxime	>0.5	>2	>0.25	>2			
Cefotaxime/clavulansyre	>0.5 (a)		>0.25 (a)				
Cefoxitin	>8		>8				>4
Ceftaroline							>1
Ceftazidime	>2	>4	>0.5	>4			
Ceftazidime/clavulansyre	>2 (a)		>0.5 (a)				
Ceftobiprole							>2
Chloramphenicol	>16	>8	>16	>8			
Ciprofloxacin	>0.064	>0.064	>0.064	>0.5	>0.5	>0.5	
Clindamycin							>0.5 (c)
Colistin	>2 (b)	>2	>2	>2			
Daptomycin							>1
Ertapenem	>0.064	>1	>0.064	>1			
Erythromycin					>4	>4	>2
Fusidic acid							>1
Gentamicin	>2	>4	>2	>4	>2		>1
Imipenem	>1	>8	>0.5	>8			
Kanamycin							>16
Linezolid							>4
Meropenem	>0.125	>8	>0.125	>8			
Mupirocin							>2
Nalidixic acid	>16		>16		>16		
Norfloxacin							>4
Penicillin							>0.125
Rifampicin							>0.5
Streptomycin					>4		
Sulfamethoxazole/Trimethoprim							>4
Sulfonamide	>256 (a)		>64				
Temocillin	>32 (a)		>32 (a)				
Tetracycline	>8		>8		>1	>2	>2
Tigecycline	>1	>2	>1	>2			
Trimethoprim	>2	>4	>2	>4			

Note: EUCAST epidemiological cut-off values (ECOFFs) and EUCAST clinical breakpoints listed unless noted
a) No current EUCAST ECOFF is available, apply complementary interpretative thresholds as suggested by EFSA [EFSA Supporting publication 2019:EN-1559]
b) The EUCAST ECOFF (>2) for colistin was applied for *S.* Typhimurium and other serotypes, except for *S.* Enteritidis and *S.* Dublin where ECOFF >8 was applied according to investigations presented in DANMAP 2011
c) Inducible clindamycin resistance is included

An isolates is considered multidrug-resistant if resistant to three or more of the antimicrobial classes defined in Table 9.3 and fully sensitive if susceptible to all antimicrobial agents included in the test panel.

9.7.2 ESBL/AmpC phenotypes

Classification of CPE, ESBL and AmpC phenotypes was done according to the scheme provided by EFSA [EFSA 2018. EFSA Journal 16(2):5182]:

1. CPE phenotype if meropenem MIC >0.12 µg/ml;
2. ESBL phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC <=8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime);
3. ESBL-AmpC phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ml and synergy (clavulanic acid and cefotaxime/ceftazidime);
4. AmpC phenotype if cefotaxime/ceftazidime MIC >1 µg/ml and meropenem MIC <=0.12 µg/ml and cefoxitin MIC >8 µg/ml and no synergy (clavulanic acid and cefotaxime/ceftazidime);
5. Other phenotype if not in 1-4.

Synergy is defined as ≥ 3 twofold concentration decrease in MIC for clavulanic acid combined with cefotaxime/ceftazidime vs the MIC of cefotaxime/ceftazidime alone.

9.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using SAS®Software, SAS Enterprise Guide 6.1 using univariable 2x2 Chi-square, or Fisher's Exact Tests as appropriate. All changes and differences yielding $p<0.05$ were commented on in the text, whereas the remaining data was visualised in figures or tables only.

Significance tests for trends in rates of resistance were performed by applying the Cochran-Armitage test. The significance levels were calculated using SAS Enterprise Guide 6.1. A p-value of <0.05 is generally considered significant. Presented in this report are results from trend analysis for five years- and ten years trend, respectively. The test was applied to several bacteria's resistance to a broad range of antimicrobials. One-sided tests were chosen because of preliminary expected trend directions.

Some types of resistances were looked for, but not found by the DANMAP monitoring system, yielding a prevalence of zero. It is not possible for surveys to prove freedom from diseases or resistances in populations, but with a defined confidence, surveys can identify the maximum possible prevalence given that the survey failed to find any positives [Textbox 6.2, DANMAP 2016]. This maximum prevalence was calculated for the report using 95% confidence and assuming a perfect test by a probability formula to substantiate freedom from disease [Cameron and Baldock 1998, Prev. Vet. Med].

Link to calculation example: http://epitools.ausvet.com.au/content.php?page=HerdSens5&Sens=1&SampleSize=100&Population=&TargetSeH=0.95.

9.8 Data on antimicrobial consumption in humans

9.8.1 Data registration

All antimicrobial consumption in Denmark has since 1997 been reported to DANMAP once a year through the Register of Medicinal Product Statistics at the Danish Health Data Authority. Until 2012, data from hospitals on certain infusion substances was obtained directly from the hospital pharmacies. Since 2013, all data from hospitals are reported to and delivered by The Register of Medicinal Product Statistics at SSI.

Reportings on human antimicrobial consumption in Denmark exist from before 1997. These were performed through the Association of Medicine Importers (Medicinimportørforeningen, MEDIF) and the Association of Danish Medicinal Factories (Foreningen af danske Medicinfabrikker, MEFA) based on whole sales data to the pharmacies. This reporting became less reliable over time, since there was an increasing amount of parallel imported drugs from the late 1980´s, which were not covered by this registration.

In the primary sector, all antibacterial agents for human use are prescription-only medicines. Sales are reported through the pharmacies by a code relating to the defined package. The information from the code includes information on the active drug, the brand name of the product, formulation, size and number of packages. The sale also reports the age, gender and regional residence of the patient. Since 2004, the sales registration has included the indication code as well. Still, for the treatment of infectious diseases the clinical indications given were often quite unspecific, such as "against infection". Since 2016, the use of more specific indication codes has become more feasible through the implementation of the `common medicine card" (fælles medicinkortet, FMK), mandatory to be used by all medical doctors. In 2018, indication codes were available for 93% of prescriptions, but specific indication codes still accounted for only 69%.

For hospitals, reporting is based on deliverances from the hospital pharmacies to the different clinical departments and includes all generic products that are supplied through general trade agreements between different medical suppliers and Amgros, a private company under agreement with the regions, responsible for harmonisation of prices and for ensuring deliverances to all hospitals. Amgros works closely together with the Regions´ Joint Procurement. Detailed information is given on the different drugs delivered on ATC5 level. For surveillance purposes, it has to be assumed that the amount of delivered antimicrobials is similar to the consumption at the different departments. In reality, antimicrobials may be exchanged between different specialties and departments belonging to the same trust, which makes precise calculations of the consumption on specialty level difficult. In DANMAP, reporting

of data on hospital consumption is therefore kept at a national or regional level. Data on hospital level can be supplied upon request.

In case of production failures and shortages in deliverance of specific products, the hospitals have to apply for special deliverances through the Danish Medicines Agency. These special deliverances are reported separately to DANMAP through the Danish Health Authority. An example is the shortages in deliverance of piperacillin with tazobactam and pivemecillinam as well as mecillinam in 2017. The shortage in piperacillin with tazobactam had significant impact on the amount used, while the shortage in (piv)mecillinam could not be clearly tracked in changes in consumption. For 2018, no significant shortages or special deliverances were reported.

Data on treatment at patient level is available at very few of the hospitals and has so far been used in local quality assurance only but has not been available to the national surveillance system. Thus a national account of the prudence of use of antimicrobials at hospitals has so far not been possible.

9.8.2 Method

Primarily somatic hospitals were included in the DANMAP reporting. Data from private hospitals and clinics, psychiatric hospitals, specialised non-acute care clinics, rehabilitation centres and hospices were excluded from DANMAP in most calculations since their activity and functions are not comparable to public, acute care hospitals and therefor may skew the data. Their consumption accounts for approximately 3% of the antimicrobial consumption at hospitals.

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2017 update of the Anatomical Therapeutic Chemical (ATC) classification, in primary health care and in hospitals as well as consumption of per oral and rectal preparations of metronidazole (P01AB01) and oral preparations of vancomycin (A07AA09). As recommended by the World Health Organization (WHO), consumption of antibacterial agents in primary healthcare is expressed as DIDs, i.e. the number of DDDs per 1,000 inhabitants per day (DDD/1,000 inhabitant-days).

The consumption in hospital healthcare is expressed as the number of DDDs per 100 occupied beds per day (DDD/100 occupied bed-days or DBD). Since reporting in DBD does not necessarily reflect changes in hospital activity and production, consumption at hospitals is also presented as DAD (the number of DDDs per 100 admissions) and crude DDD. Finally, the consumption of antibacterial agents at hospitals has also been calculated in DIDs, primarily for comparison with primary healthcare.

9.8.3 DDD

Defined daily dose is the assumed average maintenance dose per day for a drug used for its main indication in adults. DDDs provide a fixed unit of measurement independent of price and formulation, enabling the assessment of trends in drug consumption and to perform comparisons between population groups. The DDDs are defined and revised yearly by the WHO Collaborating Centre for Drug Statistics and Methodology (www.whocc.no/atcddd/ index database).

Per January 2019 the WHO updated the DDDs for seven main antimicrobial agents, based on recommendations from an expert working group in collaboration with the European Center for Disease Control (ECDC). New and former DDDs are presented in Table 9.4.

9.8.4 DBD

DDD/100 occupied bed-days. The number of occupied bed-days are calculated as the date of discharge minus the date of admission and rounded up to nearest 24 hours, e.g. one day. Every new admission to a new hospital department counts as a new bed-day. Number of admissions was extracted from the National Patient Registry at the National Board of Health [www.sst.dk].

Table 9.4 New DDDs assigned by WHO Collaborating Centre per January 2019 DANMAP 2018

ATC5 code	ATC level name	Previous DDD			New DDD		
		Weight	Unit	Route of administration	Weight	Unit	Route of administration
J01CA01	Ampicillin	2.0	g	Parenteral	6.0	g	Parenteral
J01CA04	Amoxicillin	1.0	g	Oral	1.5	g	Oral
J01CA04	Amoxicillin	1.0	g	Parenteral	3.0	g	Parenteral
J01CA17	Temocillin	2.0	g	Parenteral	4.0	g	Parenteral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.0	g	Oral	1.5	g	Oral
J01DE01	Cefepime	2.0	g	Parenteral	4.0	g	Parenteral
J01DH02	Meropenem	2.0	g	Parenteral	3.0	g	Parenteral
J01MA02	Ciprofloxacin	0.5	g	Parenteral	0.8	g	Parenteral
J01XB01	Colistin	3.0	MU	Parenteral	9.0	MU	Parenteral

9.9.5 DAD

DDD/100 admissions. One admission is registered whenever a patient is admitted to a specific ward (i.e. one patient can be registered as admitted multiple times if transferred between wards during the same hospital stay). The admissions were extracted from the National Patient Registry at the National Board of Health [www.sst.dk].

9.8.6 DaDDD

Danish adjusted daily dose. This unit was developed for DANMAP 2018 as an attempt to better picture the actual dosages used for antibiotic treatment in Denmark. DaDDD units were made by combining recommended dosages in Danish treatment guidelines with data from the prescription register, thus defining a Danish maintenance dose for each given drug. The work with DaDDD was initiated by an expert group under the Danish Regional Learning and Quality teams (LKT) developing measurable units for consumption at Danish hospitals. The units were developed for monitoring progress in a nationwide project on introducing antibiotic stewardship principles at emergency departments and internal medicine wards. The units covered only intravenously applied drugs. The DANMAP group further developed these units to also apply to drugs given orally. DaDDD, their counterparts DDD and the conversion factors are presented in Table 9.5 and Table 9.6 for the primary and hospital sector, respectively.

For further information regarding the LKT initiative please go to https://kvalitetsteams.dk/laerings-og-kvalitetsteams/lkt-rationelt-antibiotikaforbrug-paa-hospitaler, (only available in Danish). The LKT report with results from the project can be found at https://kvalitetsteams.dk/media/9049/lkt-ab_status_2018-06-01.pdf.

Table 9.5 Danish adjusted DDD for penicillins in the primary sector, 2019

ATC5 code	Antimicrobial agent	WHO DDDs in grams	Danish adjused DDDs in grams	Conversion factor	Primary indication
J01CA02	Pivampicillin	1.05	2.10	0.50	Urinary tract infection
J01CA04	Amoxicillin	1.50	1.50	1.00	Otitis media
J01CA08	Pivmecillinam	0.60	1.20	0.50	Urinary tract infection
J01CE02	Phenoxymethylpenicillin	2.00	1.90	1.05	Upper respiratory tract infection
J01CF01	Dicloxacillin	2.00	3.00	0.67	Skin- and soft tissue infection
J01CF05	Flucloxacillin	2.00	3.00	0.67	Skin- and soft tissue infection
J01CR02	Amoxicillin and beta-lactamase inhibitors	1.50	1.50	1.00	Upper respiratory tract infection

Note: Solely per oral administration routes

Table 9.6 Danish adjusted DDD for main antimicrobials in the hospital sector, 2019

ATC5 code	Antimicrobial agent	WHO DDDs in gram	Danish adjusted DDDs	Conversion factor	Route of administration
J01CA01	Ampicillin	6.00	8.00	0.75	Parenteral
J01CA02	Pivampicillin	1.05	2.10	0.50	Oral
J01CA04	Amoxicillin	1.50	1.50	1.00	Oral
J01CA08	Pivmecillinam	0.60	1.20	0.50	Oral
J01CE01	Benzylpenicillin	3.60	4.80	0.75	Parenteral
J01CE02	Phenoxymethylpenicillin	2.00	2.67	0.75	Oral
J01CF01	Dicloxacillin	2.00	4.00	0.50	Oral
J01CF05	Flucloxacillin	2.0	4.0	0.5	Oral
J01CR02	Amoxicillin and beta-lactamase inhibitor	1.50	1.50	1.00	Oral
J01CR05	Piperacillin and beta-lactamase inhibitor	14.00	11.97	1.17	Parenteral
J01DC02	Cefuroxim	3.00	4.48	0.67	Parenteral
J01DH02	Meropenem	3.00	3.00	1.00	Parenteral
J01FA09	Clarithromycin	0.50	1.00	0.50	Oral
J01FA10	Azithromycin	0.30	1.00	0.30	Oral
J01GB03	Gentamicin	0.24	0.35	0.69	Parenteral
J01MA02	Ciprofloxacin	0.80	0.80	1.00	Parenteral

Figures based on DaDDD can be found in chapter 5, antimicrobials in humans, Figure 5.4b and Figure 5.13b, presenting data from primary sector and hospital sector, respectively.

9.9 *Salmonella* and *Campylobacter* in humans

9.9.1 Data source

Antimicrobial susceptibility was performed on human clinical isolates submitted to Statens Serum Institut (SSI). *Salmonella* isolates were submitted from all clinical microbiology laboratories in Denmark and *Campylobacter* isolates were submitted from clinical microbiology laboratories representing the island of Zealand excluding the capital region, Funen, and Northern Jutland. As in previous years, SSI collected information on travel history from the patients. Cases were categorised as "domestically acquired" if the patients had not travelled abroad within the week prior to the onset of disease.

9.9.2 Microbiological methods

Salmonella isolates were analysed by whole genome sequencing and the serotypes were derived from the DNA sequences. In a few cases, the DNA information was supplemented with slide agglutination according to the Kauffman-White Scheme. *Campylobacter* species identification was performed by the use of MALDI-TOFF.

9.9.3 Susceptibility testing

Antimicrobial susceptibility testing of *Salmonella* and *Campylobacter* was performed as Minimum Inhibitory Concentration (MIC) determination using the Sensititre broth microdilution system (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006.

9.9.4 Data handling

Data on *Salmonella* and *Campylobacter* infections are stored in the Danish Registry of Enteric Pathogens (SQL database) maintained by SSI. This register includes only one isolate per patient within a window of six months and includes data on susceptibility testing of gastrointestinal pathogens.

9.10 *E. coli, K. pneumoniae, P. aeruginosa, Acinetobacter* spp., *E. faecium* and *E. faecalis* in human patients

9.10.1 Data source

The surveillance of invasive isolates of *Escherichia coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterococcus faecium, Pseudomonas aeruginosa* and *Acinetobacter* species and urine isolates of *E. coli* and *K. pneumoniae* was based on data from routine diagnostics at the 10 departments of clinical microbiology (DCMs) in Denmark. For 2018, all these data were extracted directly from the Danish Microbiology Database (MiBa) (https://miba.ssi.dk/Service/English.aspx). Before 2018, data were reported from the individual DCMs to SSI. A description of MiBa and the usage and validation of MiBa-data is given in Textbox 8.1.

9.10.2 Microbiological methods

All microbiological analyses including species identification, susceptibility testing and interpretation of test results, were performed by the DCMs. Since November 2015, all Danish DCMs used the EUCAST terminology with the EUCAST breakpoints and the EUCAST methods for roughly all species. Few exceptions exist at some DCMs were local rules were applied on the susceptibility interpretations in specific cases - e.g. susceptibility to mecillinam in invasive cases.

9.10.3 Data handling

Cases were identified in MiBa and susceptibility results extracted. Before 2018, cases were identified based on the reported data from the individual DCMs.

The case definition has been harmonised with the definition by the European Antimicrobial Resistance Surveillance Network (EARS-Net): The first sample, by date of sample collection, of each given bacterial species per unique patient per year of observation. Duplicates from the same patient, within the year of observation, were removed. Thereby only resistance data on the first isolate per patient per specimen per year were included. Resistance data from the individual DCMs were excluded if not tested or registered in MiBa routinely (minimum 75% of the specific species/antimicrobial agent combination). Samples were either invasive (including blood cultures or cerebrospinal fluid) or urinary samples from hospital patients or from primary healthcare patients.

9.10.4 Statistical test

Significance tests for trends in rates of resistance in human bacteria were performed by applying Cochran-Armitage test. The significance levels were calculated using the DescTools v0.99.19 package in R version 3.5.0. A p-value of < 0.05 was considered significant. Presented in this report are results from trend analysis for five years and ten years trends, respectively.

The test was applied to several bacterias resistance to a broad range of antimicrobials. One-sided tests were chosen because of preliminary expected trend directions. Cochran-Armitage test calculates probability in binomial proportions across one single, levelled variable. In this report, the test has been performed on susceptibility data from the past 10 years containing numbers of resistant and susceptible/intermediate cases, respectively. The resulting p-values are reported in chapter eight, supplied by an arrow indicating trend direction. Note that the significance levels serve to support the graphs and thus should be interpreted with caution.

9.11 ESBL-producing bacteria in human patients

9.11.1 Data source

Since 2014, the Danish DCMs have on a voluntary basis submitted 3rd generation cephalosporin resistant *Escherichia coli* isolates from bloodstream infections for verification and genotyping at SSI. Since January 1st 2018, 3rd generation cephalosporin resistant *Klebsiella pneumoniae* isolates from

bloodstream infections have been included as well (data not available at this point).

9.11.2 Microbiological methods of isolates from patients

Since 2014, whole genome sequencing (WGS) and *in silico* bioinformatics analysis have been applied for isolates predicted to carry ESBL and/or AmpC genes based on initial phenotypic tests, to characterize the genetic background of the ESBL and/or AmpC phenotypes. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.11.3 Data handling

The Bifrost QC and analysis pipeline (https://github.com/ssi-dk/bifrost) was used for the *in silico* detection of acquired ESBL genes, pAmpCs, carbapenemase genes and MLST from assembled WGS data. For isolates with no ESBL-, pAmpC-, or carbapenemase-encoding genes detected, the sequences were investigated for promotor mutations presumed to up-regulate chromosomal AmpC by the use of myDb-Finder version 1.2 (https://cge.cbs.dtu.dk/services/myDbFinder-1.2/). Further subtyping of isolates by core genome MLST (cgMLST) were performed using the SeqSphere+ software from Ridom GmbH (https://www.ridom.de/seqsphere/).

9.12 CPO in human patients

9.12.1 Data source

The Danish DCMs have on a voluntary basis submitted carbapenem resistant isolates for verification and genotyping at SSI. Since September 5th 2018, CPO has had mandatory notification in Denmark (https://www.retsinformation.dk/Forms/R0710.aspx?id=202889).

9.12.2 Microbiological methods

All submitted isolates (originating both from screening and clinical samples) predicted to carry a carbapenemase based on initial phenotypic tests were subjected to WGS. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates. More than one isolate from the same patient were included, if the isolates belonged to different bacterial species and/or if the isolates harboured different carbapenemases.

9.12.3 Data handling

The Bifrost QC and analysis pipeline (https://github.com/ssi-dk/bifrost) was used for the *in silico* detection of acquired CPO genes and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types where such schemes are available (*E. coli, Klebsiella pneumoniae* and *Acinetobacter baumannii*).

9.13 VRE in human patients

9.13.1 Data source

The Danish DCMs have on a voluntary basis, submitted VRE for species identification, genotyping and surveillance to SSI.

9.13.2 Microbiological methods

All clinical VRE isolates (i.e. excluding screening samples) have been whole-genome sequenced. Only one isolate from each patient was included if less than 12 months were between isolation of the two isolates.

9.13.3 Data handling

The Bifrost QC and analysis pipeline (https://github.com/ssi-dk/*bifrost)* was used for the *in silico* detection of genes related to vancomycin resistance in enterococci and MLST from assembled WGS data. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types.

9.14 Invasive *Streptococcus pneumoniae* in humans

9.14.1 Data source

Invasive pneumococcal disease is a notifiable disease in Denmark, and all invasive isolates nationwide were sent to SSI for identification or confirmation as well as serotyping and susceptibility testing.

9.14.2 Microbiological methods

Identification or confirmation of the species *S. pneumoniae* was based on: visual evaluation of colonies, positive optochin test and test with either latex omni test (ImmuLex™ *S. pneumoniae* Omni, SSIDiagnostica, Denmark) or Neufeld based Omni serum (SSIDiagnostica, Denmark). If challenging results occurred, MALDI-TOF and bile solubility test were performed to further confirm the correct species identification.

Serotype identification of invasive *S. pneumoniae* were performed by using latex agglutination (ImmuLex™ Pneumotest Kit, SSI Diagnostica, Denmark) and serotype specific antisera by the Neufeld test (SSI Diagnostica, Denmark).

9.14.3 Susceptibility testing

Screening for penicillin- and erythromycin-resistant *S. pneumoniae* was performed with 1 µg oxacillin discs and 15 µg erythromycin discs (Oxoid, Roskilde, Denmark), respectively, on Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Denmark). Isolates, that were found non-susceptible by screening were further analysed for penicillin and erythromycin MICs by broth microdilution using the STP6F plate, Sensititre (Trek Diagnostic Systems, Thermo Scientific) as recommended by the manufacturer. All breakpoints used were as defined by EUCAST (Eucast Clinical Breakpoint Tables v.8.0). Both resistant and intermediate susceptible isolates were counted as non-susceptible.

9.14.4 Data handling

Data on susceptibility testing of isolates were stored as zone diameters and/or as MICs in a Microsoft® Access database linked to a SQL server at SSI. Only cases with isolates from blood or spinal fluid were included in the DANMAP report. Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.15 Invasive beta-haemolytic streptococci (group A, B, C and G streptococci) in humans

9.15.1 Data source

Isolates of beta-haemolytic streptococci from normally sterile sites (e.g., blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery) were submitted to SSI from the departments of clinical microbiology on a voluntary basis.

9.15.2 Microbiological methods

Identification of streptococcal group was performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Denmark).

9.15.3 Susceptibility testing

Screening for penicillin, erythromycin and clindamycin resistance was performed with 1 unit penicillin G discs, 15 µg erythromycin discs and 2 µg clindamycin discs (Oxoid, Denmark) on Müller-Hinton agar (Müller-Hinton plate, 5% blood, 20 mg beta-NAD, SSI Diagnostica, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with E-test (Biomérieux), with either benzylpenicillin, erythromycin or clindamycin on Müller-Hinton agar. The breakpoints used were as defined by the EUCAST (EUCAST Clinical Breakpoint Tables v. 8.0). Both resistant and intermediate susceptible isolates were categorised as resistant.

9.15.4 Data handling

Data on susceptibility testing of isolates were stored as inhibition zone diameters and if indicated also as MICs in a Microsoft® Access database linked to a SQL server at SSI. Only one isolate from each unique case of invasive beta-haemolytic streptococci were included in the DANMAP report. A new case was defined if there were 30 days or more between consecutive isolates.

9.16 Invasive *Haemophilus influenzae* in humans

9.16.1 Data source

Invasive infections with *Haemophilus influenzae* type b (Hib) is a notifiable disease in Denmark and all invasive isolates nationwide are sent to the reference laboratory at SSI. By tradition, invasive isolates of other serotypes have also been submitted on a voluntary basis, and thus a broader picture of invasive *H. influenzae* in Denmark can be obtained. All cases were identified through MiBa and registered in the surveillance database at SSI. Cases, where isolates were not submitted to the reference laboratory were registered as "unknown serotype".

9.16.2 Microbiological methods

At SSI the isolates were serotyped and biotyped. Identification or confirmation of the species *H. influenzae* was based on: visual evaluation of colonies, the satellitism test and biochemical reactions. Serotypes were determined by latex agglutination (ImmuLexTM H. influenzae, SSIDiagnostika, Denmark). Biotypes were determined by a series of biochemical reactions.

9.16.3 Susceptibility testing

Susceptibility-testing was not performed at the reference laboratory for the 2018 isolates. The presence of beta-lactamase encoding plasmids TEM-1 and ROB-1 were found through whole-genome sequencing. Data from antimicrobial susceptibility testing at the departments of clinical microbiology were extracted from MiBa.

9.16.4 Data handling

Data on all invasive cases of *H. influenzae* were stored in a Microsoft® Access database linked to a SQL server at SSI. A case was defined as isolation of *H. influenzae* from normally sterile sites (e.g. blood, spinal fluid, pleura). Repeated samples within a 30 days window were classified as duplicates and were omitted from the analysis.

9.17 *Staphylococcus aureus* including MRSA in humans

9.17.1 Data source

Blood isolates were referred on a voluntary basis by all DCMs to the National reference laboratory for antimicrobial resistance at SSI. Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) is a notifiable condition in Denmark and therefore all MRSA isolates from all sample types were sent to the reference laboratory.

9.17.2 Microbiological methods

At SSI, all isolates were initially tested using a multiplex PCR detecting the *spa, mecA, hsd, scn* and *pvl* (LukF-PV) genes [Larsen et al. 2008. Clin Microbiol Infect. 14: 611-614; Stegger et al. 2012. Clin Microbiol Infect. 18: 395-400]. *Spa* was used as *S. aureus* specific marker and for subsequent typing by Sanger sequencing [Harmsen et al. 2003. J Clin Microbiol. 41: 5442-5448], *mecA* to determine MRSA status, and *scn* and *hsd* as markers for human adaptation and relation to CC398, respectively. All bacteremia cases and *mecA* negative presumed MRSA were tested for presence of the *mecC* gene.

spa-negative isolates were confirmed as *S. aureus* by MALDI-TOF. Based on the *spa* type and known association with MLST typing, each isolate was assigned to a clonal complex (CC).

9.17.3 Susceptibility testing

Antimicrobial susceptibility testing of *Staphylococcus aureus* was performed by Minimum Inhibitory Concentration (MIC) determination using a custom-made panel (DKSSP2, Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard ISO 20776-1:2006. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2013/652/EU about the EU harmonised monitoring of antimicrobial resistance.

Staphylococcus aureus ATCC 29213 was included as quality control for each batch of resistance determination.

9.17.4 Data handling

For blood isolates a case was defined as a patient with a positive blood culture. Subsequent isolates from the same patient was only included if the positive blood cultures were obtained at least one month apart (new episode).

For MRSA, data on the characteristics of the isolates and the clinical/epidemiological information were obtained from the Danish MRSA register at SSI (mandatory reportable). Patients were registered, regardless of colonisation or clinical infection, the first time they were diagnosed with MRSA or when a new subtype was demonstrated. Based on the reported information, MRSA cases were classified as colonisation/active screening (i.e. surveillance samples to detect nasal, throat, gut or skin colonisation), imported infection (i.e. acquired outside Denmark), infection acquired in a Danish hospital, defined as diagnosed >48 hours after hospitalisation with no sign of infection at admittance (HA-MRSA) or infection diagnosed outside hospitals (community onset).

MRSA cases with community onset were further classified according to risk factors during the previous 6 months as either health-care associated with community onset (HACO) or community-acquired (CA). Health-care associated risk factors included prior hospitalisations, stay in long-term care facilities and being a health-care worker. Community risk factors included known MRSA-positive household members or other close contacts. Due to the increasing numbers of cases belonging to CC398, this type was treated separately as both epidemiology and relevant exposure are different from other CA cases.

9.18 Gonococci in humans

9.18.1 Data source

Isolates of gonococci *(Neisseria gonorrhoeae)* were submitted from the departments of clinical microbiology to SSI on a voluntary basis. The isolates were obtained by culture of specimens from a variety of anatomical locations, e.g. urethra, cervix, rectum, throat, eyes, joint fluid, and blood.

9.18.2 Microbiological methods

The bacteriological identification of the submitted isolates were performed by MALDI-TOF.

9.18.3 Susceptibility testing

For all isolates the MIC was determined with E-test (Biomérieux) with azithromycin, ceftriaxon and ciprofloxacin on chocolate agar. The MIC of approximately 110 consecutive isolates was determined with cefixime, gentamicin, and spectinomycin. The breakpoints used were those defined by the EUCAST (EUCAST Clinical Breakpoint Tables v. 8.0). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

9.18.4 Data handling

The susceptibility data were stored as MIC values in a Microsoft® Access database linked to a SQL server at SSI. Only one isolate from each unique case of gonorrhoea were included in the DANMAP report. Laboratory demonstration of gonococci in repetitive specimens were considered to represent a new case of gonorrhoea if the specimens were obtained with an interval of more than 21 days.