9

MATERIALS AND METHODS

9. Materials and methods

9.1 General information

For the DANMAP 2019 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 were given license to sell prescribed veterinary medicinal products for animals, when following strict guidelines, identical to those applied to pharmacies. 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.

In 2019, 96% of all antimicrobial agents were purchased through pharmacies and the drug trading companies, while 4% were purchased from the feed mills. These numbers did not include prescribed zinc oxide from the feeding mills for the pigs. For cattle, 85% of antimicrobial agents used in 2019 were purchased from pharmacies, compared to only 6% in 2004. 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 The Danish Health Authority and to VetStat. However, VetStat does not have any validation on data entry and slight typing errors from veterinarians 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 2019 were extracted from VetStat on 3 March 2020.

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 and relevant codes for dispensation. For example, when calculating the antimicrobial use for systemic treatment in pigs, we select consumption data where the age groups

are defined as finishers, weaners, sows or boars and 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 as mg active compound per kg live animal for each antimicrobial agent, administration route and animal species. 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 - live biomass

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. For DANMAP 2019, only the live biomass for pigs, cattle and mink were updated.

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 2019, productivity data from 2018 were used to estimate the biomasses for pigs, since the 2019 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 was not accounted for.

Cattle production: The live biomass of the cattle population is estimated from census data [Statistics Denmark] 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.

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].

Treatment proportion - DAPD

The treatment proportion is a statistical measure for antimicrobial use in animal populations, calculated as the annual number of DADDs administered in the population divided by the estimated total population live biomass (in tonnes). For a single animal, the mg active compound (e.g. the number of DADDs) given in a daily treatment depends on the body weight, therefore, does treatment proportions also represent the proportion of animals treated daily with an average maintenance dose of a particular antimicrobial agent, and are reported as Defined animal daily dose per 1,000 animals per day (DAPD). For example, 10 DAPDs indicate that an estimated 1% of the population, on average, receives a certain treatment on a given day. In principle, the metric DAPD is parallel to the metric used in pharmaco-epidemiology for the human sector, defined daily dose per 1,000 inhabitants per day (DID), see section 9.8.2.

In 2019, 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 (DAPDadj). 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:

$$DAPD_{adj} = \frac{DADD_{sows} + DADD_{weaners} + (1+Q)*DADD_{finishers}}{\Sigma biomassdays_{all} + N_{export}*biomassdays_{adj}}$$

Σbiomassdays_all is the sum of estimated biomass-days for each age group of pigs, N_export is the number of weaning pigs exported, and biomassdays_adj is the assumed number of lost biomass-days per exported 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 2019, most of the sampling for DANMAP was allocated to the mandatory sampling of caeca from cattle and pigs, but additional sampling of broilers was also carried out.

Meat inspection staff or abattoir personnel at the slaughterhouses collected caecal samples from healthy broilers, cattle (<1 year) and pigs. For broilers, the samples were collected in August and September at the two major Danish slaughterhouses slaughtering conventionally produced chicken. For pigs and cattle, the samples were collected throughout 2019 at the ten major slaughterhouses in Denmark. The slaughterhouses included in the monitoring handled at least 75% of the total number of broilers, cattle and pigs slaughtered in Denmark during 2019.

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. Samples from all three animal species were examined for indicator *E. coli*, broiler and cattle samples were also examined for *Campylobacter jejuni*. ESBL/AmpC/carbapenemase-producing *E. coli* was isolated from the cattle and pig samples, whereas *Salmonella* and enterococci was isolated from pigs samples only (Table 9.1).

All Danish flocks of layers, broilers and turkeys are tested for *Salmonella* on-farm as part of the national *Salmonella* control programme. Due to the low prevalence of *Salmonella* in the Danish poultry production [Annual Report on Zoonoses in Denmark, 2019] these data are not included in DANMAP 2019.

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) [Decision 2013/652/EU].

In 2019, ESBL/AmpC/carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled meat from broiler, cattle and pigs 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 as well as minced meat were excluded. Packages of beef and pork were selected at retail without pre-selecting based on the country of origin as requested for the harmonised EU monitoring. In 2019. Additional sampling of broiler meat was carried out the main supermarket chains central storage facilities. 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 meat (minimum of 200 g) was collected and all samples were processed at the DVFA laboratory. Some of the samples were also examined for *Campylobacter jejuni* (imported broiler meat) and *Salmonella* (imported pork and beef, Table 9.1).

The *Salmonella* isolates from domestically produced broiler meat, beef and pork originate from the national control programme at the slaughterhouses (Table 9.1). For beef and pork, carcases are swabbed in four designated areas (the jaw, breast, back and ham) after min. 12 hours of chilling (covering 10x10cm). For broiler meat, 300 neck-skin samples (1 g) are collected and pooled after slaughter into subsamples of 60 grams. All samples were processed at Industry laboratories. *Salmonella* isolates from cattle and pig carcases were send to the DVFA laboratory, and *Salmonella* isolates from the neck-skin sampling were send to the DTU laboratory.

Campylobacter from domestically produced broiler meat for DANMAP originate from sampling of leg-skins at slaughterhouses receiving either conventionally or organic/free-range broiler flocks. The numbers of samples collected depend on the slaughterhouse capacity and all samples were processed at the DVFA laboratory.

Salmonella from broiler meat and beef are not included in DANMAP 2019 due to low numbers of isolates available from the national surveillance programmes [Annual Report on Zoonoses in Denmark, 2019].

Table 9.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contribute isolates to DANMAP 2019
DANMAP 2019

Bacteria	Origin of isolates	Legislative reporting frequency (2013/652/EU)	Number of tested and positive samples in 2019
Campylobacter spp.	Caecal samples from broilers[a]	Even years	174 flocks (78 positive)
	Caecal samples from cattle <1 yr		142 animals (122 positive)
	Leg skins from conventional broilers[b]		1,248 units (407 positive)
	Leg skins from organic/free-range broilers[b]		123 units (84 positive)
	Fresh broiler meat - ready for retail (imports)[c]		54 units (30 positive)
Salmonella spp.	On-farm samples from laying hens (production flocks)	Even years	411 flocks (8 positive)
	On-farm samples from broilers (production flocks)	Even years	4,012 flocks (12 positive)
	Caecal samples from fattening pigs[d]		798 animals (124 positive)
	Neck skin samples from broilers	Even years	254 units (0 positive)
	Carcase swabs from fattening pigs[e]	Odd years	10,743 animals (1.2% positive)
	Carcase swabs from cattle <1 year[e]	Odd years	4,125 animals (0.1% positive)
	Fresh pork - ready for retail (imports)		141 units (24 positive)
	Fresh beef - ready for retail (imports)		280 units (0 positive)
Enterococcus spp.	Caecal samples from fattening pigs[d]		799 animals (195 positive)
Indicator *E. coli*	Caecal samples from broilers	Even years	168 flocks (159 positive)
	Caecal samples from fattening pigs	Odd years	195 animals (192 positive)
	Caecal samples from cattle <1 yr	Odd years	186 animals (174 positive)
Specific monitoring of ESBL/AmpC and carbapenemase-producing *E. coli*	Caecal samples from cattle <1 yr	Odd years	306 animals (25 positive)
	Caecal samples from fattening pigs	Odd years	330 animals (90 positive)
	Fresh broiler meat - ready for retail (domestic origin)[c]	Even years	257 units (14 positive)
	Fresh broiler meat - ready for retail (imports)[c]	Even years	44 units (15 positive)
	Fresh beef at retail (domestic origin)	Odd years	123 units (3 positive)
	Fresh beef at retail (imports)	Odd years	196 units (9 positive)
	Fresh pork at retail (domestic origin)	Odd years	317 units (9 positive)
	Fresh pork at retail (imports)	Odd years	36 units (15 positive)
	WGS data for collected ESBL/AmpC isolates		164 isolates

Note: 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 samples
a) Ceacum samples from broilers were collected during August and September, where the *Campylobacter* prevalence in Danish broilers is highest
b) Collected from carcases at the end of the slaughterline and classified as broiler meat
c) For 2019, broiler meat - ready for retail include both conventional and organic/free-range products
d) Among the 798 fattening pigs tested, the monitoring included animals from 31 of 765 farms more than once
e) Carcase swabs collected at slaughterhouses 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 positive sample in each pool are taken into consideration

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 and broiler meat 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 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 cattle herd, pig herd, and meat sample was selected (no isolates of carbapenemase-producing *E. coli* was detected).

9.4.4 Indicator enterococci

Indicator enterococci from pigs was isolated from an adequate amount of caecal material suspended in 2 ml buffered peptone water, and inoculated onto Slanetz Barrtley agar incubated at 41,5°C for 2 days. Three colonies resembling *E. faecium*, or *E. faecalis* if no suspect *E. faecium* were present, were identified by a real-time PCR assay. Only one enterococci per herd, was selected for antimicrobial resistance testing (selecting an *E. faecium* if possible).

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).

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 and the bioinformatics analysis was conducted at DTU National Food Institute from Centre for Genomic Epidemiology [www.genomicepidemiology.org; https://cge.cbs.dtu.dk//services/all.php]. ResFinder 4.0 was used for detection of antimicrobial resistance genes including 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]. ST types were defined using MLST Finder 2.0 [Larsen et al. 2012. J Clin Mico- biol. 50(4):1355].

9.7 Data handling – isolates from animals and meat

For the samples processed at the DVFA laboratory, sampling details and laboratory results were stored in the DVFA Laboratory system. Following validation by DVFA, data were send to DTU National Food Institute (Excel sheets). At the DTU National Food Institute, 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 includes: 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 2019 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 www.danmap.org. Each of the tables provides information on the number of isolates, the applied interpretation of MIC-values and the estimated level of resistance and confidence intervals calculated as 95% binomial proportions presenting Wilson intervals.

An isolate 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.

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

DANMAP 2019

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 isolate 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

Table 9.2 Interpretation criteriae for MIC-testing by EUCAST epidemiological cut-off values and the corresponding EUCAST clinical breakpoints
DANMAP 2019

Antimicrobial agent	*Salmonella*		*E. coli*		*Enterococcus*		*C. jejuni*		*S. aureus*
	ECOFF µg/ml	Clinical breakpoint µg/ml	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	>4	>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	>32				
Ciprofloxacin	>0.064	>0.064	>0.064	>0.5	>4		>0.5	>0.5	
Clindamycin									>0.5[c]
Colistin	>2[b]	>2	>2	>2					
Daptomycin					>4/8[d]				>1
Ertapenem	>0.064	>1	>0.064	>1					
Erythromycin					>4		>4	>4	>2
Fusidic acid									>1
Gentamicin	>2	>4	>2	>4	>32		>2		>1
Imipenem	>1	>8	>0.5	>8					
Kanamycin									>16
Linezolid					>4	>4			>4
Meropenem	>0.125	>8	>0.125	>8					
Mupirocin									>2
Nalidixic acid	>16		>16				>16		
Norfloxacin									>4
Penicillin									>0.125
Quinupristin/ dalfopristin					>4[d]				
Rifampicin									>0.5
Streptomycin							>4		
Sulfamethoxazole/ trimethoprim									>4
Sulfonamide	>256[a]		>64[a]						
Teicoplanin					>2	>2			
Temocillin	>32		>16						
Tetracycline	>8		>8		>4	>0.5	>1	>2	>2
Tigecycline	>1[e]	>2	>1[f]	>2	>0.25	>0.25			
Trimethoprim	>2	>4	>2	>4					
Vancomycin					>4	>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) No current EUCAST ECOFF is available. For colistin, an ECOFF >2 was applied for *S.* Typhimurium and other serotypes, except for *S.* Enteritidis and *S.* Dublin where an ECOFF >8 was applied according to investigations presented in DANMAP 2011
c) Inducible clindamycin resistance is included
d) For daptomycin, an ECOFF >8 was applied for *E. faecium* [EUCAST, 30 December 2018]. *E. faecalis* are assumed inherent resistant to streptogramins, and ECOFF only applies to *E. faecium*. For *E. faecium*, the EUCAST ECOFF (>1) was not applied for quinopristin/dalfopristin (tradename synercid) according to investigations presented in DANMAP 2006
e) For human isolates, the tigecycline, ECOFF >2 was applied to all serovars
f) The most recent EUCAST ECOFF >0.5 was not applied for *E. coli*

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 Chi-square, or Fisher's Exact Tests as appropriate.

Significance tests for trends in rates of resistance were performed by applying the Cochran-Armitage test. One-sided tests were chosen because of preliminary expected trend directions and a p-value of <0.05 is generally considered significant.

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 at epitools.ausvet.com.au.

Analysis were done using SAS®Software, SAS Enterprise Guide 8.2 or Sergeant, ESG, 2018. Epitools Epidemiological Calculators. Ausvet. Available at: http://epitools.ausvet.com.au.

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 the Danish Health Data Authority.

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 2019, indication codes were available for 93% of prescriptions, but specific indication codes still accounted for only 72%.

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. Amgros is responsible for harmonisation of prices and for ensuring deliverances to all hospitals and work 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 (Figure A5.2 in web annex). These special deliverances are reported separately to DANMAP through the hospital pharmacies. An example is the shortages in deliver-

ance of piperacillin with tazobactam and pivmecillinam 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. In 2019, 188,609 DDD (0.6% of total antimicrobial consumption) was consumed at the hospitals through special deliverances. The need of special deliverances might have been caused by shortages in deliverances.

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, somatic hospitals and therefor may skew the data. Their consumption accounts for approximately 4% of the antimicrobial consumption at hospitals.

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2019 update of the Anatomical Therapeutic Chemical (ATC) classification, in primary healthcare and in hospitals as well as consumption of per oral and rectal preparations of metronidazole (P01AB01) and for hospitals 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.

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. From DANMAP 2018, the new DDD values were applied and all tables and figures were updated ten years back.

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 bed-days was extracted from the National Patient Registry at the Danish Health Data Authority [www.sundhedsdatastyrelsen.dk].

The National Patient Registry was upgraded in 2019, and data in the new registry are not comparable to data in the previous registry. Unification of data is ongoing but not completed. Thus the number of bed-days in 2019 is estimated by applying the average decrease observed in 2009-2018.

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

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

9.8.5 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 Danish Health Data Authority [www.sundhedsdatastyrelsen.dk].

The National Patient Registry was upgraded in 2019, and data in the new registry are not comparable to data in the previous registry. Unification of data is ongoing but not completed. Thus the number of admissions in 2019 is estimated by applying the average increase observed in 2009-2018.

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 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 in 2017-2019, please go to www.kvalitetsteams.dk > Lærings- og kvalitetsteams > Rationelt antibiotikaforbrug på hospitaler (only available in Danish). The LKT report with results from the project can be found at www.kvalitetsteams.dk.

Table 9.5 Danish adjusted DDD for penicillins in the primary sector, 2019 DANMAP 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 and lower respiratory tract infection

Note: Solely per oral administration routes

Table 9.6 Danish adjusted DDD for main antimicrobials in the hospital sector, 2019 DANMAP 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 5.5b and Figure 5.14b, 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) that is 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 2019, all these data were extracted directly from the Danish Microbiology Database (MiBa) [https://miba.ssi.dk]. 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 in DANMAP 2018 [https://www.danmap.org].

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. In 2019 EUCAST introduced the Area of Technical Uncertainty (ATU) for some combinations of species and agents reflecting problematic areas regarding variations and uncertainty of susceptibility categorisation. Piperacillin-tazobactam, ciprofloxacin and amoxicillin-clavulanic acid (in systemic breakpoints) and Enterobacterales are examples where ATUs were applied. ATUs can be handled differently by the DCMs and may influence interpretation results and was commented on when necessary in the affected sections.

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. 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 and five years. 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. The resulting p-values are reported supplemented 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 the Antimicrobial Resistance Reference Laboratory, Statens Serum Institut.

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 quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket. org/genomicepidemiology/resfinder] 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]. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types (*E. coli* scheme).

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 the Antimicrobial Resistance Reference Laboratory, Statens Serum Institut. Since September 5th 2018, CPO has had mandatory notification in Denmark. For outbreak investigation data from The National Patient Register (LPR), information gathered at the hospitals, information of residence from the Danish Civil Registration System (CPR) and telephone interviews was conducted.

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. More than one isolate from the same patient were included, only if the isolates belonged to different bacterial species and/or if isolates within the same species harboured different carbapenemases.

9.12.3 Data handling

The quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder], 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*). For outbreak investigation identified clonal clusters were linked with patient data like time and place of hospitalisation, place of residence and telephone interviews. Isolates from two or more persons (cases) sharing the same unique genotype was defined as an outbreak. In verified outbreaks, an epidemiological link could be established between two or more cases in the cluster, e.g. the patients had been at the same hospital ward at the same time or they live in the same geographical location such as a nursing home. When no epidemiological link could be establish between cases with the same unique genotype, the outbreak was classified as a possible outbreak. A possible outbreak can be reclassified as a verified outbreak, if new cases or information is providing an epidemiological link between two or more of the cases. Outbreak investigations of a cluster of cases is closed when no new cases has appeared within 6 months, but can be reopened, if new cases is detected in the surveillance of CPO.

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 the Antimicrobial Resistance Reference Laboratory, Statens Serum Institut.

9.13.2 Microbiological methods

All clinical VRE isolates 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 quality of the raw sequencing and assembly data was ensured using the analytical tool BIFROST [https://github.com/ssi-dk/bifrost/]. An in-house bacterial analysis pipeline, building on a weekly update of the ResFinder database [https://bitbucket.org/genomicepidemiology/resfinder], 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 it is mandatory to submit all invasive isolates of *S. pneumoniae* to SSI for serotyping and susceptibility testing. For cases, where isolates could not be submitted, identification and registration is conducted by use of the Danish Microbiology Database (MiBa).

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, SSI Diagnostica, Denmark) or Neufeld based Omni serum (SSI Diagnostica, Denmark). If challenging results occurred, MALDI-TOF, bile solubility test, or whole genome sequencing were performed to further confirm the correct species identification. For non-viable isolates, species identification was based on the detection of the *lytA* gene by the use of PCR.

Serotype identification of invasive *S. pneumoniae* was performed by the use of latex agglutination (ImmuLex™ Pneumotest Kit, SSI Diagnostica, Denmark) and serotype specific antisera by the Neufeld test (SSI Diagnostica, Denmark). For non-viable isolates, serotyping was often possible by the use of PCR.

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 Mueller-Hinton agar (Mueller-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.9.0). Isolates that were either resistant or susceptible increased exposure were categorised together as non-wild-type. For cases, where an isolate was not received at the reference laboratory, susceptibility data could often be found in MiBa.

9.14.4 Data handling

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 (BHS) 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). DNA from all isolates was extracted using DNeasy Blood & Tissue kit as described by the manufacturer (Qiagen, Valencia, CA). Fragment libraries were constructed using the Nextera XT DNA Library Preparation Kit (Illumina, San Diego, CA), followed by 150-bp paired-end sequencing on a NextSeq (Illumina) according to manufacturer's instructions. The sequencing reads were assembled using SKESA [https://github.com/ncbi/SKESA]. The isolates were species typed using Kraken [https://ccb.jhu.edu], and MLST typed using https://github.com/tseemann/mlst. The isolates were *emm* typed by performing a BLAST search to all published *emm* types by CDC [http://www.cdc.gov/streplab/protocol-emm-type.html].

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 Mueller-Hinton agar (Mueller-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 ETEST (Biomérieux), with either benzylpenicillin, erythromycin or clindamycin on Mueller-Hinton agar. The breakpoints used were as defined by the EUCAST (EUCAST Clinical Breakpoint Tables v. 9.0). Isolates that were either resistant or susceptible increased exposure were categorised together as resistant.

9.15.4 Data handling

A case of invasive BHS disease was defined as the isolation of BHS from a normally sterile site. A new case was defined as an invasive isolate with a different Lancefield group within 30 days from the first one or an invasive isolate of any Lancefield group more than 30 days after the first episode, or the isolation of a new type (T-type, *emm*-type or GBS type) if the group was identical on both occasions.

Only one isolate from each unique case of BHS infection was included in the DANMAP report.

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*, SSI Diagnostika, Denmark). Biotypes were determined by a series of biochemical reactions.

9.16.3 Susceptibility testing

Susceptibility-testing of the 2019 isolates was performed by betalactamase test and disc diffusion assays. The presence

of beta-lactamase encoding plasmids TEM-1 and ROB-1 were moreover found through whole-genome sequencing. Where isolates were not submitted, data from antimicrobial susceptibility testing at the departments of clinical microbiology were extracted from MiBa, when available.

9.16.4 Data handling

A case was defined as isolation of *H. influenzae* from normally sterile sites (e.g. blood, spinal fluid, pleura, joint). 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, most often urethra, cervix, rectum, throat, and rarely from other sites, e.g. eyes, joint fluid, and blood.

9.18.2 Microbiological methods

The bacteriological identification of the received isolates was performed by MALDI-TOF.

9.18.3 Susceptibility testing

For all isolates the MICs of azithromycin, ceftriaxon and ciprofloxacin were determined with ETEST (Biomérieux) on chocolate agar. The MICs of cefixime, gentamicin, and spectinomycin were determined for 118 consecutive isolates as part of an ECDC project on gonococcal antimicrobial resistance. The breakpoints used were those defined by EUCAST (EUCAST Clinical Breakpoint Tables v. 9.0). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

9.18.4 Data handling

Only one isolate from each unique case of gonorrhoea were included in the DANMAP report. Laboratory demonstration of gonococci in repetitive specimens was considered to represent a new case of gonorrhoea if the specimens were obtained with an interval of more than 21 days.