10

MATERIALS AND METHODS

10. Materials and methods

10.1 General information

For the DANMAP 2023 report, population sizes and geographical data were obtained from Statistics Denmark [www.dst.dk] and data on the number of 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 for analyses of AMR trends. An overview of all antimicrobial agents registered for humans and animals in Denmark is presented in Table 2.4.

10.2 Data on antimicrobial consumption in animals

10.2.1 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. This monopoly was suspended in April 2007, and since then private companies have been able to obtain license to sell prescribed veterinary medicinal products for animals, if they adhere to the same guidelines that apply to pharmacies. A pharmacy or licensed company either sells the medicine to veterinarians for use in their practice or for resale to farmers or sells the medicine directly to the animal owner upon presentation of a prescription.

Data on all sales of veterinary prescription medicine from pharmacies, private companies, feed mills and veterinarians are sent electronically to a central database, VetStat, which is hosted by the Danish Veterinary and Food Administration. 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 their invoice system. Electronic registration of the sales at pharmacies is linked to the billing process and stock accounts at the pharmacy. This ensures a very detailed data of high quality. Data are transferred daily from pharmacies to The Register of Medicinal Product Statistics at The Danish Health Authority and to VetStat.

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

VetStat contains detailed information about source (veterinarian/pharmacy/feed mill) 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 sector [www.whocc.no]. The data presented in DANMAP 2023 were extracted from VetStat on 1 July 2024.

10.2.2 Methods

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

Furthermore, a more detailed comparison of antimicrobial use is performed, taking into account 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, and sows (including piglets and boars) and exclude antimicrobials dispensed as tablets, products for topical use, intramammaries and gynaecologicals.

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 principles 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 2023, only the live biomass for pigs and cattle 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]. The size of the breeding animals (sows and boars) 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-gender categories.

Treatment proportion - DAPD

The treatment proportion is a statistical measure for AMU in animal populations, calculated as the annual number of DADDs administered in the population, divided by the estimated total population live biomass. For a single animal, the mg active compound (e.g. the number of DADDs) given in a daily treatment depends on the body weight. The treatment proportions, therefore, also represents the proportion of animals treated daily with an average maintenance- dose of a particular antimicrobial agent. These 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 DID, defined daily dose per 1,000 inhabitants per day (DID), used in pharmaco-epidemiology for the human sector, see Section 10.8.2.

In 2023, DAPD calculations were carried out for pigs and cattle.

For example, the antimicrobial use per pig produced is calculated as:

$$DAPD = \frac{DADD_{sows} + DADD_{weaners} + DADD_{finishers}}{\Sigma biomassdays}$$

Where DADDs, DADDw, and DADDf are amounts of antimicrobial agents used in finishers, weaners, and sows (including piglets and boars).

10.3 Collection of bacterial isolates from animals and meat

In DANMAP, samples originate both from the EU harmonised monitoring of antimicrobial resistance in zoonotic and commensal bacteria, and the national *Salmonella* surveillance programs. Since 2014, most isolates available for DANMAP have been collected in accordance with the EU harmonised monitoring, according to Decision 2013/652/EU. This Decision was repealed by Decision 2020/1729/EU, applied from 1 January 2021. With the aim to ensure continuity in assessing future trends in antimicrobial resistance, the new Implementing Decision includes adaptations of food categories to be sampled, sampling design to be followed, bacterial species to be tested and the analytical methods to be used.

EU harmonized monitoring from 2021 to 2027 shall cover *Salmonella* spp., *Campylobacter coli*, *Campylobacter jejuni*, indicator commensal *Escherichia coli*, ESBL-, AmpC- or carbapenemase- producing *Salmonella* spp. and *E. coli*, and may cover *Enterococcus faecalis* and *Enterococcus faecium*. Previously, monitoring of *Campylobacter coli* was voluntary. For the monitoring of *Salmonella* in poultry, it is now possible to report only samples collected within the national control programme in poultry farms, while the monitoring of *Salmonella* in fattening pigs at slaughter is still required for most countries, including Denmark, due to the inexistence of an implemented national surveillance programme which has been approved at EU level.

Additionally to monitoring of fresh meat at retail, the present EU legislation requires monitoring of indicator *E. coli* and ESBL-, AmpC- and CP-producing *E. coli* on fresh imported meat sampled at border control posts, and the fresh meat categories to be monitored include turkey, both at retail and at the border.

Decision 2020/1729/EU further allows the use of whole genome sequencing as an alternative method for the specific monitoring of ESBL-, AmpC- or CP-producing *E. coli* or for further testing of indicator *E. coli* and *Salmonella* showing resistance to cefotaxime, ceftazidime or meropenem.

The legislation continues to require mandatory sampling of broilers and fattening turkeys and meat thereof in even years (2022, 2024, 2026), and sampling of fattening pigs and cattle <1 year, and meat thereof in odd years (2021, 2023, 2025, 2027). In Denmark, fattening turkeys are not sampled at slaughter as part of the EU harmonised monitoring, because the national production of turkey meat is below 10.000 tonnes per year.

10.3.1 Animals

In 2023, most of the sampling for DANMAP was allocated to the mandatory sampling of caeca from fattening pigs and cattle <1 year at slaughter, and additional sampling of caeca from broilers was also carried out.

Caecal samples from healthy broilers, cattle (<1 year) and pigs were collected by meat inspection staff at the slaughterhouses. Samples were collected throughout the year, in major Danish slaughterhouses slaughtering conventionally produced chicken, pigs and cattle.

Sampling was stratified per slaughterhouse by allocating the number of samples from domestically produced animals per slaughterhouse, proportionally to the annual throughput of the slaughterhouse. For broiler flocks, ten intact caeca were pooled into one sample. For pigs and cattle, samples contained 30-100 g caecal material from a single animal.

All samples were processed by the Danish Veterinary and Food Administration's (DVFA) laboratory in Ringsted or by a DVFA-approved private laboratory. Samples from all three animal species were examined for indicator *E. coli, Campylobacter jejuni and Campylobacter coli.*

Furthermore, pig and cattle samples were also examined for ESBL/AmpC/carbapenemase-producing *E. coli*, and samples from pigs were examined for *Enterococcus faecalis and Enterococcus faecium* (Table 10.1).

Pathogenic bacteria from pigs reported in 2023 comprised *Actinobacillus pleuropneumoniae* (AST and WGS), *Bordetella bronchiseptica* (AST and WGS), *Clostridium perfringens* (WGS), *Erysipelothrix rhusiopathiae* (WGS), haemolytic and non-haemolytic *Escherichia coli* (AST and WGS), *Glaesserella parasuis* (WGS), *Klebsiella pneumoniae* (AST and WGS), *Salmonella enterica* (AST and WGS), *Staphylococcus hyicus* (AST and WGS) and *Streptococcus suis* (AST and WGS) isolates identified in clinical samples submitted by veterinarians to the Veterinary Laboratory, The Danish Agriculture and Food Council.

10.3.2 Meat

In 2023, ESBL/AmpC/carbapenemase-producing *E. coli* were isolated from packages of fresh, chilled pork and beef meat collected in Danish wholesale and retail outlets and at border control posts. These samples were collected throughout the year by DVFA officers (Table 10.1). Products with added saltwater or other types of marinade as well as minced meat were not included. Packages of meat were selected without preselecting by country of origin, as requested for the harmonised EU monitoring.

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.

Salmonella isolates from domestically produced pork and beef originated from the national control programme at the slaughterhouses (Table 10.1). Carcasses were swabbed in four designated areas (covering 4 x 100 cm^2) after min. 12 hours of chilling. All samples were processed at DVFA-approved Industry laboratories and isolates were sent to the DVFA laboratory.

10.4 Microbiological methods – isolates from animals and meat

10.4.1 *Salmonella*

Salmonella from pork not originating from the national *Salmonella* surveillance program were isolated at DVFA in accordance with the methods issued by the NMKL [NMKL No. 187, 2007] and in accordance with Annex D, ISO 6579-1 [ISO6579-1:2017]. Serotyping of those isolates was performed at DVFA 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.

Salmonella from carcasses originating from the national *Salmonella* surveillance program were isolated and serotyped according to the White-Kauffmann-Le Minor scheme at DVFA-approved Industry laboratories.

10.4.2 *Campylobacter*

Campylobacter from broiler and cattle caeca was isolated and identified according to the methods issued by the NMKL [NMKL No. 119, 2007] with modifications, with pre-enrichment in Bolton broth, and followed by species-determination by BAX® rtPCR assay (Hygiena, BAX® System PCR Assays for Campylobacter). Only one *Campylobacter* isolate per broiler flock or cattle or pig herd was selected for antimicrobial susceptibility testing.

Table 10.1 Legislative and voluntary sampling plans under national control programmes and EU harmonised monitoring that contributed with isolates to DANMAP 2023 DANMAP 2023

Bacteria	Origin of isolates	Legislative reporting frequency (2020/1729/EU)	Number of tested and positive samples in 2023
Campylobacter spp.	Caecal samples from broilers[a]	Even years	168 flocks (64 positive)
	Caecal samples from cattle <1 yr[a]	Odd years	225 animals (201 positive)
	Caecal samples from fattening pigs[a]	Odd years	296 animals (160 positive)
Enterococcus spp.	Caecal samples from fattening pigs[b]	Odd years	492 animals (169 positive)
Indicator *E. coli*	Caecal samples from broilers	Even years	129 flocks (125 positive)
	Caecal samples from fattening pigs	Odd years	176 animals (173 positive)
	Caecal samples from cattle <1 yr	Odd years	172 animals (169 positive)
Specific monitoring of ESBL/AmpC and carbapenemase-producing *E. coli*	Caecal samples from fattening pigs	Odd years	294 animals (54 positive)[c]
	Caecal samples from cattle <1 yr	Odd years	309 animals (13 positive)[c]
	Fresh pork meat at BCPs	Odd years	3 units (0 positive)
	Fresh beef meat at BCPs	Odd years	6 units (0 positive)
	Fresh pork meat at retail (Danish)	Odd years	209 units (5 positive)[c]
	Fresh pork meat at retail (Imported)	Odd years	110 units (9 positive)[c]
	Fresh beef meat at retail (Danish)	Odd years	120 units (1 positive)[c]
	Fresh beef meat at retail (Imported)	Odd years	192 units (8 positive)[c]
	WGS data for collected ESBL/AmpC isolates	Odd years	90 isolates[d]
Salmonella spp.	Caecal samples from fattening pigs	Odd years	772 animals (85 positive)[f]
	Caecal samples from cattle <1 yr	Odd years	301 animals (3 positive)[f]
	Carcase swabs from fattening pigs[e]	Odd years	3448 units (67 positive)[f]
	Carcase swabs from cattle <1 yr[e]	Odd years	883 units (3 positive)[f]

a) Broilers: *C. jejuni* (n=44), *C. coli* (n=12), unspecified (n=5) ; Cattle <1 yr: *C. jejuni* (n=175), *C. coli* (n=8), unspecified (n=13); Fattening pigs: *C. jejuni* (n=1), *C. coli* (n=128), unspecified (n=24)
b) Fattening pigs: *E. faecalis* (n=87), *E. faecium* (n=85)
c) Positive for ESBL/AmpC-producing *E. coli* and negative for carbapenemase-producing *E. coli*
d) 90 isolates from the positive samples were sequenced (54 from fattening pigs, 13 from cattle <1 yr, 5 from Danish pork meat, 9 from imported pork meat, 1 from Danish beef meat and 8 from imported beef meat)
e) Carcass swab samples are part of the national *Salmonella* surveillance program and are classified in DANMAP as meat of domestic origin. Samples collected at slaughterhouses slaughtering more than 30,000 pigs or 7,500 cattle are analysed in pools of 5 individual samples. The total number of animals tested and the total number of positives refer to individual pooled samples
f) Fattening pigs: *S.* Derby (n=42), *S.* 4,[5],12:i:- (n=31), *S.* Typhimurium (n=6), other serotypes or unspecified (n=6); Cattle <1 yr: *S.* Dublin (n=2), *S.* Agona (n=1); Pork meat (carcass): *S.* Derby (n=23), *S.* 4,[5],12:i:- (n=18), *S.* Typhimurium (n=12), other serotypes or unspecified (n=14); Beef meat (carcass): *S.* Dublin (n=2), unspecified (n=1)

10.4.3 *Escherichia coli*

Indicator *E. coli* from broilers, pigs and cattle was isolated by direct spread 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. The specific isolation of ESBL/AmpC or carbapenemase-producing *E. coli* from pork and beef meat and caecal samples of pigs and cattle <1 year occurred within 96 h after sample collection, applying the current EURL-AR laboratory protocol [https://www.eurl-ar.eu/protocols.aspx]. Carbapenemase-producing *E. coli* screening was done with ChromID CARBA and ChromID OXA-48 plates. ESBL/AmpC-producing *E. coli* screening was done with MCA cefotaxime plates. All presumptive ESBL/AmpC or carbapenemase producing *E. coli* isolates were sequenced by WGS using the Illumina MiSeq platform (paired-end sequencing 2x250). Only one ESBL/AmpC-producing *E. coli* isolate per herd and meat sample was selected for antimicrobial susceptibility testing.

10.4.4 Enterococci

Indicator enterococci were isolated from pig caeca by adding 2 ml buffered peptone water to the content of a cotton swab, after which 100 µl were inoculated onto Slanetz agar and incubated for 48 h at 41,5 °C. Presumptive *E. faecium/E. faecalis* were identified by real-time PCR assay. When present, only one *E. faecalis* isolate per herd was selected for antimicrobial susceptibility testing.

10.5 Susceptibility testing – isolates from animals and meat

Antimicrobial susceptibility testing of *Salmonella*, *Campylobacter* and *E. coli* was carried out by Minimum Inhibitory Concentration (MIC) determination, using broth microdilution by Sensititre (Trek Diagnostic Systems Ltd.). Inoculation and incubation procedures were performed in accordance with the CLSI guidelines [Clinical and Laboratory Standards Institute, USA] and the European standard [ISO 20776-1:2020]. The isolates were tested for antimicrobial susceptibility in accordance with the Decision 2020/1729/EU about the EU harmonised monitoring of antimicrobial resistance.

The quality control strains used were: *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *C. jejuni* ATCC 33560 and *P. aeruginosa* ATCC 27853. Isolates from animals and meat were tested for antimicrobial susceptibility at the DVFA laboratory in Ringsted, which is accredited by DANAK (the national body for accreditation).

Antimicrobial susceptibility testing of pathogenic bacteria from pigs was performed at the Veterinary Laboratory, The Danish Agriculture and Food Council. In brief, MICs were determined by broth microdilution using customised Sensititre panels according to CLSI standards. The analysis is accredited by DANAK.

10.6 Whole genome sequencing – isolates from animals and meat

In addition to *Salmonella* serotyping performed by sequencing, whole genome sequencing (WGS) and in silico bioinformatics 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 using the ResFinder application v. 4.4.2 and the ResFinder database v. 2.2.1.

WGS of pathogenic bacteria from pigs was performed on Illumina platforms at Statens Serum Institut. Acquired resistance genes and point mutations were detected by mapping sequence reads against the ResFinder 4.1 database [Bortolaia *et al.* 2020. J. Antimicrob. Chemother 75(12):3491-3500) using the k-mer alignment (KMA) tool 1.3 (Clausen *et al.* 2018. BMC Bioinformatics 19(1):397], setting both length match and similarity match to 0.9 and excluding hits with less than 10X coverage.

10.7 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 sent 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.

For the samples processed at the Veterinary Laboratory, The Danish Agriculture and Food Council, sampling details and laboratory results were stored in the information management system used at the Veterinary Laboratory. Following internal validation and anonymisation, data were sent to DK-VET (Excel sheets). At DK-VET, data were harmonised and one isolate per epidemiological unit was selected for reporting.

Table 10.2 Interpretation criteriae for MIC-testing by EUCAST- and EFSA-provided epidemiological cut-off values (ECOFFs)

DANMAP 2023

Antimicrobial agent	*Salmonella*	*E. coli*	*E. faecalis*	*C. jejuni*	*C. coli*
	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml	ECOFF µg/ml
Amikacin	>4	>8	Not tested	Not tested	Not tested
Ampicillin	>8	>8	>4	Not tested	Not tested
Azithromycin	>16	>16 [a]	Not tested	Not tested	Not tested
Cefepime	>0.125 [a]	>0.125 [a]	Not tested	Not tested	Not tested
Cefotaxime	>0.5	>0.25	Not tested	Not tested	Not tested
Cefotaxime-clavulanic acid	>0.5 [a]	>0.25	Not tested	Not tested	Not tested
Cefoxitin	>8	>8	Not tested	Not tested	Not tested
Ceftazidime	>2	>0.5	Not tested	Not tested	Not tested
Ceftazidime-clavulanic acid	>2 [a]	>0.5	Not tested	Not tested	Not tested
Chloramphenicol	>16	>16	>32	>16 [d]	>16 [d]
Ciprofloxacin	>0.064	>0.064	>4	>0.5	>0.5
Colistin	>2 [a] [b]	>2	Not tested	Not tested	Not tested
Daptomycin	Not tested	Not tested	>4	Not tested	Not tested
Ertapenem	>0.064 [a]	>0.064 [a]	Not tested	>0.5 [a] [d]	>0.5 [a] [d]
Erythromycin	Not tested	Not tested	>4	>4	>8
Gentamicin	>2	>2	>64	>2 [a]	>2 [a]
Imipenem	>1	>0.5	Not tested	Not tested	Not tested
Linezolid	Not tested	Not tested	>4	Not tested	Not tested
Meropenem	>0.125 [a]	>0.125	Not tested	Not tested	Not tested
Nalidixic acid	>8	>8	Not tested	Not tested	Not tested
Quinopristin-dalfopristin	Not tested	Not tested	>1 [a] [c]	Not tested	Not tested
Sulfamethoxazole	>256 [a]	>64 [a]	Not tested	Not tested	Not tested
Teicoplanin	Not tested	Not tested	>2	Not tested	Not tested
Temocillin	>16 [a]	>16	Not tested	Not tested	Not tested
Tetracycline	>8	>8	>4	>1	>2
Tigecycline	>0.5 [a]	>0.5	>0.25	Not tested	Not tested
Trimethoprim	>2	>2	Not tested	Not tested	Not tested
Vancomycin	Not tested	Not tested	>4	Not tested	Not tested

EUCAST epidemiological cut-off values (ECOFFs) and ECOFFs provided by EFSA for EU harmonized reporting

a) ECOFF as provided by EFSA [EFSA Supporting publication 2023:EN-7826]

b) For colistin, a tentative ECOFF of 16 µg/ml for *Salmonella* Dublin is established by EUCAST. The same ECOFF is used in DANMAP to interpret results of *Salmonella* Enteritidis. Both serotypes belong to the O-group (O:1, 9,12), which has been associated with increased MIC for colistin [https://www.doi.org/10.1089/fpd.2011.1015]

c) For quinopristin-dalfopristin, ECOFF only applies for *E. faecium*. ECOFF >1 for *E. faecalis* (intrinsically resistant to quinopristin-dalfopristin) is used only for the purpose of EU harmonized reporting

d) In 2021, chloramphenicol and ertapenem were introduced in the test panel for *Campylobacter* spp.

Table 10.3 Definitions of antimicrobial classes for calculation of multidrug-resistance in *Salmonella* and indicator *E. coli*
DANMAP 2023

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
Polymyxins	Colistin
Aminoglycosides	Gentamicin and/or amikacin
Carbapenems	Meropenem
Sulfonamides	Sulfamethoxazole
Tetracyclines	Tetracycline
Glycylcyclines	Tigecycline
Trimethoprim	Trimethoprim

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; The aminoglycoside antimicrobial amikacin has been introduced in the test panel in 2021

10.7.1 Interpretation of MIC values

MIC values were retained as continuous variables, from which binary variables (resistant/sensitive) were created using the relevant cut-off. Since 2007, MIC results have been interpreted using EUCAST epidemiological cut-off (ECOFF) values, with a few exceptions, as described in Table 10.2. An isolate is considered multidrug-resistant if resistant to three or more of the antimicrobial classes defined in Table 10.3 and fully sensitive if susceptible to all antimicrobial agents included in the test panel.

For pathogenic bacteria from pigs, MIC values were interpreted with ECOFFs (1st choice) or tentative ECOFFs (2nd choice) established by EUCAST. When ECOFFs were unavailable, interpretation was based on CLSI-approved animal-specific or human clinical breakpoints (3rd and 4th choice, respectively) (available at https://www.vetssi.dk/).

10.7.2 ESBL/AmpC phenotypes

Classification of CP-, ESBL- or AmpC-producing phenotypes was done according to the scheme provided by EFSA. [EFSA 2023. EFSA Journal 21(3):7867].

1. ESBL phenotype if cefotaxime and/or 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);
2. AmpC phenotype if cefotaxime and/or 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);
3. ESBL-AmpC phenotype if cefotaxime and/or 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. CP phenotype if meropenem MIC >0.12 µg/ml;
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.

10.7.3 Statistical tests

Significance tests of differences between proportions of resistant isolates were calculated using Chi-square, or Fisher's Exact Tests as appropriate depending on sample size. Significance tests for trends in rates of resistance were performed by applying the Cochran-Armitage test, using the DescTools R package version 0.99.45. One-sided tests were chosen because of preliminary expected trend directions. A significance level of 0.05 was considered in all significance tests.

Analyses were done using R statistical software version 4.4.1 [R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org].

10.8 Data on antimicrobial consumption in humans

10.8.1 Data registration

Annual data on antimicrobial consumption in Denmark has been provided to DANMAP by the Register of Medicinal Product Statistics at the Danish Health Data Authority every year since 1997. Since 2020, DANMAP also reports monthly antimicrobial consumption data to allow analysis of the impact of the Covid-19 pandemic on antimicrobial consumption in humans since 2020.

Until 2012, data from hospitals on certain infusion substances such as cephalosporins, carbapenems and trimethoprim were obtained by DANMAP directly from hospital pharmacies. Since 2013, all data from hospitals are reported to and provided to DANMAP by The Register of Medicinal Product Statistics at the Danish Health Data Authority.

Reports of human antimicrobial consumption in Denmark existed already before 1997. These were prepared by 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 pharmacies. These reports became less reliable over time since there was an increasing amount of parallel imported drugs from the late 1980s, which were not covered by MEDIF/MEFA.

In the primary sector, all antibacterial agents for human use are prescription-only medicines. Sales are reported by pharmacies using a code relating to the defined package. The code includes information on the active drug, the brand name of the product, formulation, size and number of packages. The report also includes age, gender and regional residence of the patient. Since 2004, the sales registration has included a code for indication of the prescription as well. However, clinical indications provided for the treatment of infectious diseases were often quite unspecific ("against infection"). Since 2016, the use of more specific indication codes has increased following the implementation of electronic prescribing via the "common medicine card" (fælles medicinkortet, FMK), a digital pharmacy platform which is mandatory to be used by all medical doctors. In 2023, indication codes were available for 94% of prescriptions, but specific indication codes still only accounted for 75%.

For hospitals, reporting is based on deliveries 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 five Danish Regions. Amgros is responsible for harmonisation of prices and for ensuring deliveries to all hospitals and 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 hospital making precise calculations of the consumption on specialty level difficult. In DANMAP, reporting of data on hospital consumption is therefore kept at national and regional level. In case of production failures and shortages in delivery of specific products, the hospitals have to apply for special delivery through the Danish Medicines Agency (Figure A5.2 in web annex). These special deliveries are reported separately to DANMAP through the hospital pharmacies. An example is the shortages in delivery 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 2023, 126.490 DDD (3%) of the total antimicrobial consumption were special deliveries. Data on consumption at patient level are available at some hospitals and have so far been used in local quality assurance only but have not been available to DANMAP.

10.8.2 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 3% of the antimicrobial consumption at hospitals in Denmark.

The present report includes data on the consumption of "antibacterials for systemic use", or group J01, of the 2023 update of the Anatomical Therapeutic Chemical (ATC) classification, in primary healthcare and in hospitals as well as consumption of 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.

10.8.3 DDD

Defined daily dose is the assumed average maintenance dose per day for a drug used for it's 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 [https://www.whocc.no/atc_ddd_index/].

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) (Table 10.5). From DANMAP 2018, the new DDD values were applied and all tables and figures were updated ten years back.

10.8.4 DBD

DDD/100 occupied bed-days. The number of occupied bed-days are calculated as the exact duration of a hospital stay in hours divided by 24 hours. 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 are still being validated and may be adjusted in the future. This has to be kept in mind when data are interpreted.

10.8.5 DAD

DDD/100 admissions. One admission is registered whenever a patient is admitted to a specific hospital for ≥12 hours. If a patient is transferred between wards within 4 hours, it will not count as a new admission. 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 are still being validated and may be adjusted in the future. This has to be kept in mind when data are interpreted.

10.8.6 Antimicrobial consumption for elderly living in long care facilities

Data from the Care Home Register were combined with data from the Danish Civil Registration System (CPR) and with data from the Register of Medicinal Product Statistics in order to determine the antimicrobial consumption for elderly people living in care homes and for elderly people living in their own homes.

10.9 *Salmonella* and *Campylobacter* isolates from humans

10.9.1 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.

10.9.2 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.

10.9.3 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.

Table 10.5 New DDDs assigned by WHO Collaborating Centre per January 2019 DANMAP 2023

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

10.9.4 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.

10.10 *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter* species, *Enterococcus faecium* and *Enterococcus faecalis* isolates from humans

10.10.1 Data source

The surveillance of invasive isolates of *E. coli, K. pneumoniae, E. faecalis* and *faecium, P. aeruginosa* and A. spp. and urine isolates of *E. coli* and *K. pneumoniae* are all based on data from routine diagnostics at the ten Departments of Clinical Microbiology (DCMs) in Denmark. All data were extracted directly from the Danish Microbiology Database (MiBa) [https://miba.ssi.dk]. Before 2018, data were reported by the individual DCM to SSI. A description of MiBa and the usage and validation of MiBa-data is given in Textbox 8.1 in DANMAP 2018 [www.danmap.org].

10.10.2 Microbiological methods

All microbiological analyses including species identification, susceptibility testing and interpretation of test results, were performed by the DCM. Since November 2015, all Danish DCMs used the EUCAST terminology with the EUCAST clinical breakpoints and the EUCAST methods for roughly all species. Few exceptions exist at some DCMs where local rules were applied to 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 individual DCMs and may influence interpretation results. This was commented on when necessary in the affected sections.

To be included in resistance surveillance more than 75% of respective isolates need to be antimicrobial susceptibility tested for a given antibiotic, if not stated otherwise. Data of antimicrobial susceptibility testing was mainly performed by disc diffusion. The presented data consist of the registered interpretation results, performed by the respective DCM, based on the S-I-R system. In addition, zone diameters have also been registered since 2015 and will be commented upon in specific cases.

Urine specimen taken in primary health care are also being tested at DCMs except for some samples taken by GPs in the Capital Region of Denmark that are being tested at a private laboratory.

All enterococci isolates reported as VRE in MiBa (based on PCR results for *vanA/B* genes) were reported as vancomycin-resistant independent of the actual zone/MIC result. It was not distinguished between VRE and VVE (vancomycin-variable enterococci). High-level resistance to gentamicin was defined using EUCAST break points (MIC >128 mg/L and/or zone diameters <8 mm) for MIC and/or zone diameters reported in MiBa. Gentamicin MIC and/or zone diameters were routinely reported by three DCMs in 2020.

10.10.3 Data handling

Cases and susceptibility results were extracted from MiBa and analysed in Python 3.8.10.

The case definition has been harmonised with the definition used 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 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 patients at hospitals or primary healthcare settings.

10.11 ESBL-producing bacterial isolates from humans

10.11.1 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.

10.11.2 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.

10.11.3 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/src/master/] 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.food.dtu.dk/]. Possible clonal clusters were detected using the SeqSphere+ (Ridom) software to call cgMLST types (*E. coli* scheme).

10.12 CPO isolates from humans

10.12.1 Data source

Historically, Danish DCMs have submitted carbapenem-resistant isolates for verification and genotyping on a voluntary basis to the Antimicrobial Resistance Reference Laboratory at the Statens Serum Institut. Since 5 September 2018, notification of CPO has been mandatory in Denmark. For outbreak investi-gation Data from The National Patient Register (LPR), informa-tion gathered at the hospitals and information of residence from the Danish Civil Registration System (CPR) has been included in the analysis for this report.

10.12.2 Microbiological methods

All submitted isolates (originating both from screening and clinical samples) predicted to carry a carbapenemase based on initial phenotypic tests were analysed using WGS. More than one isolate from the same patient was only included in the dataset if the isolates belonged to different bacterial species and/or if isolates within the same species harboured different carbapenemases.

10.12.3 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 investigations, identified clonal clusters were linked with patient data like time and place of hospitalization and place of residence. Identification of isolates from two or more persons (cases) sharing the same unique genotype was defined as an outbreak. An outbreak was defined as a verified outbreak if 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 lived at 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 providing an epidemiological link between two or more of the cases becomes available. Both, possible and verified outbreaks, are registered in the CPO-outbreak database KURS (coordinated outbreak registration).

Outbreak investigations of a cluster of cases are closed when no new cases have been reported within 6 months after the last reported case, but can be reopened, if new cases are being detected.

10.13 VRE isolates from humans

10.13.1 Data source

Danish DCMs are submitting VRE for species identification, genotyping and surveillance on a voluntary basis to the Antimicrobial Resistance Reference Laboratory at Statens Serum Institut.

10.13.2 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.

10.13.3 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.

10.14 Invasive *Streptococcus pneumoniae* isolates from humans

10.14.1 Data source

Invasive pneumococcal disease is a notifiable disease in Denmark and it is mandatory to submit all invasive isolates of *S. pneumoniae* for serotyping and susceptibility testing to the Neisseria and Streptococci Reference Laboratory at Statens Serum Institut. For cases of invasive pneumococcal disease, where isolates from blood/spinal fluid could not be submitted, identification and registration of cases is conducted by extracting the required information from the Danish Microbiology Database (MiBa).

10.14.2 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 and Ply 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.

10.14.3 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, SSI Diagnostica, Denmark). Breakpoints were according to EUCAST Clinical Breakpoint Tables v. 11.0. 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.11.0). For cases, where an isolate was not received at the reference laboratory, susceptibility data could often be found in MiBa.

10.14.4 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.

10.15 Isolates of beta-haemolytic streptococci of groups A, B, C, and G from invasive infections in humans

10.15.1 Data source

All invasive isolates of beta-haemolytic streptococci (BHS) (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.

10.15.2 Microbiological methods

Identification of streptococcal group was performed by latex agglutination (Streptococcal Grouping Reagent, Oxoid, Denmark). Genomic DNA was extracted using an enzymatic pre-lysis step before automated purification on MagNA Pure 96 DNA Small Volume Kit (Roche Diagnostics). 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 (Il- lumina) 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.

For Group A Streptococcus (GAS), isolates were *emm* typed by performing a BLAST search to all published *emm* types by CDC [https://www.cdc.gov/streplab/protocol-emm-type.html]. For Group B Streptococcus (GBS), all isolates were serotyped by latex agglutination test and, if needed, confirmed using Lancefield tests. In addition, blasting of capsular sequencing was used for identification of genotypes. No additional identification tests were performed for isolates from Group C or G.

10.15.3 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, Den- mark) on Mueller-Hinton agar (Mueller-Hinton plate, 5% blood, SSI Diagnostica, Denmark). Isolates were also tested for inducible clindamycin resistance. For non-susceptible streptococci the MIC was determined with ETEST (Biomérieux), with erythromycin or clindamycin on Mueller-Hinton agar. The breakpoints used were as defined by the EUCAST (EUCAST v. 14.0 Breakpoint Tables).

Isolates that were either resistant or susceptible to increased exposure were categorised together as resistant.

10.15.4 Data handling

A case of invasive BHS disease was defined as the isolation of BHS from a normally sterile site (e.g., blood, cerebrospinal fluid, synovial fluid, pleural fluid, ascites, and tissue obtained during surgery). 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 (*emm*-type or GBS serotype) if the group was identical on both occasions.

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

10.16 Invasive *Haemophilus influenzae* isolates from humans

10.16.1 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".

10.16.2 Microbiological methods

At SSI, the received isolates were analysed by whole-genome sequencing, from which serotype and biotype were extracted.

10.16.3 Susceptibility testing

Susceptibility data for the 2023 isolates were retrieved from MiBa. In cases where a series of isolates from the same episode developed non-susceptibility over time, the most non-susceptible profile was used for the analysis in DANMAP. In addition, for isolates received at SSI, whole-genome sequencing data was analysed for the presence of beta-lactamase encoding plasmids TEM-1 and ROB-1 as well as for the presence of mutations in the *ftsI* gene that encodes for penicillin-binding protein 3 (PBP3).

10.16.4 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.

10.17 *Staphylococcus aureus* including MRSA isolates from humans

10.17.1 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.

10.17.2 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).

10.17.3 Susceptibility testing

Data on antimicrobial susceptibility was extracted from MiBa.

10.17.4 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.

10.18 Gonococcal isolates

10.18.1 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.

10.18.2 Microbiological methods

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

10.18.3 Susceptibility testing

For all isolates the MICs of azithromycin, ceftriaxon and ciprofloxacin were determined with ETEST (Biomérieux) on chocolate agar incubated at 35 °C in 5% CO_2. The breakpoints used were those defined by EUCAST (EUCAST v. 14.0 Breakpoint Tables). A cefinase disc technique was used to examine the isolates for beta-lactamase production.

The breakpoints for azithromycin were changed as of January 1, 2019 (EUCAST version 9.0) (S: MIC ≤1 mg/L; R: MIC >1 mg/L) and it was advised that azithromycin should only be used in conjunction with another efficient agent. Until January 1, 2019, S was defined by MIC ≤0.25 mg/L and R by MIC >0.5 mg/L.

In addition to the above, the MIC of cefixime was determined for 117 consecutive isolates as part of an ECDC project on gonococcal antimicrobial resistance (Euro-GASP). The breakpoints used were those defined by EUCAST (EUCAST v. 14.0 Breakpoint Tables).

10.18.4 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.