Veterinary Medicines Directorate

Supplementary Material 1 - methods

UK-VARSS 2021

Published November 2022

This publication is available at www.gov.uk/government/collections/veterinary-antimicrobial-resistance-and-sales-surveillance.

Suggested citation: UK-VARSS (2022). *Supplementary Material 1 (UK-VARSS 2021)*. New Haw, Addlestone: Veterinary Medicines Directorate.

Any enquiries or correspondence regarding this publication should be sent to us at: postmaster@vmd.gov.uk.

www.gov.uk/government/organisations/veterinary-medicines-directorate

Published on 8th November 2022

Contents

S1.1: Further details on the methodology

The European Commission has requested the European Medicines Agency (EMA) to take the lead in collating data collected on the use of antibiotic agents in animals in the European Union. The EMA has therefore developed a harmonised approach for the collection and reporting of data based on national sales figures. This is designed to be comparable with usage data of human antibiotics, to the extent possible.

Published European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) reports are available from: https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/european-surveillance-veterinary-antimicrobial-consumption-esvac.

UK-VARSS used a different method to calculate mg/kg (called milligram per Population Correction Unit (mg/PCU) for ESVAC purposes) compared to ESVAC until UK-VARSS 2015. Since 2015, the ESVAC mg/kg methodology has been adopted. For a full explanation please see VARSS Supplementary Material 2020.

For further details on how mg/PCU is calculated please see: https://www.gov.uk/government/publications/understanding-the-mgpcu-calculation-used-for-antibiotic-monitoring-in-food-producing-animals.

The data reported in Chapter 1 of the main report are presented according to the Anatomical Therapeutic Chemical Classification System for veterinary medicinal products (ATCvet) as shown in Table S1.5.2.

Table S1.1.1: Categories and ATCvet codes of antibiotic veterinary medicinal products included in the data

Veterinary antibiotic category	ATCvet codes
Antibiotics for intestinal use	QA07AA; QA07AB
Antibiotics for intrauterine use	QG01AA; QG01AE; QG01BA; QG01BE; QG51AA; QG51AG
Antibiotics for systemic use	QJ01
Antibiotics for intramammary use	QJ51
Antibiotics for antiprotozoal use (solely sulphonamides)	QP51AG

Table S1.1.2 shows the sales for other antibiotic products, which include topical preparations and those for sensory organs, for example aerosols, creams, gels, shampoos and ear and eye medications. These are not included in the ESVAC calculation.

Table S1.1.2: Active ingredient in tonnes of antibiotics sold for all animal species by 'other' routes of administration from 2014 to 2021

Administration Route	2014	2015	2016	2017	2018	2019	2020	2021
Other routes	2.3	1.9	2.4	2.4	2.5	2.6	2.1	2.6

S1.2: Weight of animal population at risk

When assessing antibiotic sales, it is important that the demographics of the animal population potentially exposed to treatment are also considered, (see Annex D of the main report for data limitations). For food-producing animals, this is achieved through use of the PCU, a technical unit of measurement (where 1 PCU = 1 kg of animal treated), which is calculated by multiplying a standardised average weight at time of treatment (which can be found in Table S1.2.1) with the associated annual animal/slaughter numbers. The calculation also considers animals exported from the UK for slaughter or imported to the UK for fattening. Full details on the methodology of calculation of the PCU can be found in the 2011 ESVAC report (which includes data from 2005 to 2009): https://www.ema.europa.eu/en/veterinary-regulatory/overview/antimicrobial-resistance/european-surveillance-veterinary-antimicrobial-consumption-esvac.

Table S1.2.1 shows the UK PCU value for food-producing animal species and horses. The standard formula used for calculation of the PCU for poultry does not include population figures for laying hens so the poultry PCU is an underestimate.

Table S1.2.1: PCU in 1,000 tonnes by food-producing animal species from 2014 to 2021

Please note that for horses, horse population data are obtained from the British Equestrian Trade Association survey which is run every 5 years.

Animal species	2014	2015	2016	2017	2018	2019	2020	2021
Sheep and goats	2824.9	2795.6	2845.3	2910.4	2832.7	2817.6	2743.9	2730.7
Cattle	1731.3	1743.0	1792.3	1785.2	1787.7	1774.7	1768.5	1716.3
Poultry	1041.7	1082.4	1150.9	1185.3	1233.0	1204.5	1250.9	1255.0
Pigs	744.6	769.7	788.9	766.4	781.0	795.5	795.5	808.7
Horses	395.2	377.6	377.6	377.6	377.6	338.8	338.8	338.8
Fish	177.0	193.1	187.3	117.3	203.6	168.8	217.5	204.5
Total PCU	**6914.7**	**6961.4**	**7142.4**	**7202.1**	**7215.7**	**7099.9**	**7115.2**	**7053.9**

For cats and dogs, the weight of the population at risk is calculated in a different way and shown in table S1.2.2. This is used for the dog and cat mg/kg and DDDVet/animal calculations. Population data was sourced from the Peoples Dispensary for Sick Animals

PAW Report survey data, and mean adult cat and dog weights provided by the Small Animal Veterinary Surveillance Network (SAVSNET).

Table S1.2.2: Weights, in 1,000 tonnes of a) dogs and b) cats from 2014 to 2021

a)

Dogs	2014	2015	2016	2017	2018	2019	2020	2021
Population (in 1,000s) heads	8,100	9,300	9,400	9,300	8,900	9,900	10,100	9,600
Mean weight (in kg)	19.4	19.1	18.8	18.5	18.3	18.2	18.1	18.3
Total weight of dogs (in 1,000 tonnes)	157.1.4	177.6	176.7	172.1	162.9	180.2	182.8	175.7

b)

Cats	2014	2015	2016	2017	2018	2019	2020	2021
Population (in 1,000s) heads	10,500	11,100	11,000	10,300	11,100	10,900	10,900	10,700
Mean weight in kg	4.4	4.4	4.5	4.5	4.5	4.5	4.5	4.5
Total weight of cats (in 1,000 tonnes)	46.2	48.8	49.5	46.4	50.0	49.1	49.1	48.2

Table S1.2.3: Average weight at time of treatment in kg used to calculate the PCU for food-producing animals

Please note that for the category imported/exported poultry for slaughter, it's assumed this is broilers. Also, for the category slaughter sheep and goats, it's assumed this is lambs.

a) Cattle

Animal category	Average weight at treatment (kg)	Source
Slaughter cows	425	Montforts (1999)
Slaughter heifers	200	EMA
Slaughter bullocks and bulls	425	Montforts (1999)
Slaughter calves and young cattle	140	Montforts (1999); EMA
Imported/exported cattle for slaughter	425	Montforts (1999)
Imported/exported cattle for fattening	140	Montforts (1999)
Livestock dairy cows	425	Montforts (1999); EMA

b) Pigs

Animal category	Average weight at treatment (kg)	Source
Slaughter pigs	65	Montforts (1999)
Imported/exported pigs for slaughter	65	Montforts (1999)
Imported/exported pigs for fattening	25	M. Goll (Eurostat, personal comm.)
Livestock sows	240	Montforts (1999)

c) Poultry

Animal category	Average weight at treatment (kg)	Source
Slaughter broilers	1	Montforts (1999); EMA
Slaughter turkeys	6.5	Montforts (1999); EMA
Imported/exported poultry for slaughter	1	Montforts (1999); EMA

d) Sheep and goats

Animal category	Average weight at treatment (kg)	Source
Slaughter sheep and goats	20	Montforts (1999)
Imported/exported sheep and goats for slaughter[4]	20	Montforts (1999)
Livestock sheep	75	Montforts (1999)

e) Horses

Animal category	Average weight at treatment (kg)	Source
Living horses	400	Montforts (1999); EMA

Please note that for fish, data from Eurostat is given in 1,000 tonnes slaughtered fish as live weight.

S1.3: Daily dose figures (DDDVet) used for calculating DDDVet/animal in dogs and cats

Table S1.3.1: length of activity, average daily dose rate and DDDVet figures (in mg/kg) used for calculating the DDDVet/animal metric for dogs and cats

a) Dogs

Ingredient	Formulation	Length of activity	Average daily dose rate	DDDVet (mg/kg)
Amoxicillin*	Tablets/ Oral Solution	1.0	20.0	20.0
Ampicillin	Tablets	1.0	20.0	20.0
Cephalexin	Tablets	1.0	30.0	30.0
Cefovecin	Injection	14.0	8.0	0.6
Clindamycin	Tablets/ Oral Solution	1.0	11.0	11.0
Doxycycline	Tablets	1.0	10.0	10.0
Enrofloxacin	Tablets/ Injection	1.0	5.0	5.0
Marbofloxacin	Tablets/ Injection	1.0	2.0	2.0
Metronidazole	Tablets	1.0	50.0	50.0
Metronidazole-spiramycin	Tablets	1.0	35.9	35.9
Oxytetracycline	Tablets	1.0	50	50
Pradofloxacin	Tablets	1.0	3.0	3.0
Trimethoprim-sulphadiazine	Tablets/ Injection	1.0	30.0	30.0

* Includes those in combination with clavulanic acid, although clavulanic acid is not counted as an active ingredient

b) Cats

Ingredient	Formulation	Length of activity	Average daily dose rate	DDD (mg/kg)
Amoxicillin*	Tablets/ Oral Solution	1.0	20.0	20.0
Cephalexin	Tablets	1.0	30.0	30.0
Cefovecin	Injection	14.0	8.0	0.6
Clindamycin	Tablets/ Oral Solution	1.0	11.0	11.0
Doxycycline	Tablets	1.0	10.0	10.0
Enrofloxacin	Tablets/ Injection	1.0	5.0	5.0
Marbofloxacin	Tablets/ Injection	1.0	2.0	2.0
Metronidazole	Tablets/ Oral Solution	1.0	50.0	50.0
Metronidazole-spiramycin	Tablets	1.0	35.9	35.9
Pradofloxacin	Tablets	1.0	3.0	3.0
Pradofloxacin	Oral Solution	1.0	5.0	5.0
Trimethoprim-sulphadiazine	Tablets/ Injection	1.0	30.0	30.0

* Includes those in combination with clavulanic acid – although clavulanic acid is not counted as an active ingredient)

S1.4: Antibiotic active ingredients authorised for use in animals

Table S1.4.1: Antibiotic active ingredient organised by class, authorised species and administration route

a) Tetracyclines

Active ingredient	Authorised species	Administration route
Chlortetracycline	Cattle, pigs, sheep, chickens, turkeys, ducks	Cutaneous spray, oral/water, premix
Doxycycline	Cattle,Pigs, chickens, turkeys, cats, dogs, pigeons	Tablet, oral/water, premix
Oxytetracycline	Cattle, pigs, sheep, chickens, salmon, trout, dogs, cats, horses	Tablet, injectable, premix, oral/water, cutaneous spray
Tetracycline	Cattle, pigs, chickens	oral/water, Intrauterine

b) Trimethoprim/sulphonamides

Active ingredient	Authorised species	Administration route
Sulfadiazine	Cattle, pigs, chickens, turkeys, cats, dogs, horses	Tablet, oral/water, injectable, premix, intramammary suspension
Sulfadimethoxine	Pigeons	Oral/water
Sulfadimidine	Cattle, pigs, sheep	Injectable
Sulfadoxine	Cattle, horses	Injectable
Sulfamethoxazole	Pigs, chickens, pigeon, bearded dragon	Oral/water
Trimethoprim	Cattle, pigs, chickens, turkeys, cats, dogs, horses, pigeon, bearded dragon	Tablet, oral/water, premix, intramammary suspension

c) Beta-lactams: first generation cephalosporins

Active ingredient	Authorised species	Administration route
Cefalexin	Cattle, cats, dogs	Tablet, injectable, intramammary suspension
Cefalonium	Cattle	Intramammary suspension
Cefapirin	Cattle	Intramammary suspension, intrauterine suspension

d) Beta-lactams: third generation cephalosporins

Active ingredient	Authorised species	Administration route
Cefoperazone	Cattle	Intramammary suspension
Cefovecin	Cats, dogs	Injectable
Ceftiofur	Cattle, pigs, horses	Injectable

e) Beta-lactams: fourth generation cephalosporins

Active ingredient	Authorised species	Administration route
Cefquinome	Cattle, pigs, horses	Injectable, intramammary suspension/ointment

f) Beta-lactams: penicillins

Active ingredient	Authorised species	Administration route
Amoxicillin	Cattle, pigs, sheep, chickens, turkeys, ducks, salmon, cats, dogs, pigeons	Injectable, tablet, oral/water, premix, intramammary suspension, top dressing
Ampicillin	Cattle, pigs, sheep, cats, dogs	Injectable, tablet, intramammary suspension
Benzylpenicillin	Cattle, pigs, sheep, chickens, cats, dogs, horses	Injectable, oral/water, intramammary suspension
Cloxacillin	Cattle, sheep, cats, dogs, horses	Intramammary suspension, eye ointment
Phenoxymethylpenicillin	Pigs, chickens	Premix, oral/water

g) Aminoglycosides

Active ingredient	Authorised species	Administration route
Apramycin	Cattle, pigs, chickens, Rabbits	Premix, oral/water
Dihydrostreptomycin	Cattle, pigs, sheep, cats, dogs, horses	Injectable, intramammary suspension
Framycetin	Cattle, cats, dogs	Injectable, intramammary suspension, ear drops
Gentamicin	Cats, dogs, horses, rabbits	Injectable, eye drops, ear drops, gel
Kanamycin	Cattle	Intramammary suspension
Neomycin	Cattle, pigs, sheep, cats, dogs, horses	Injectable, oral/water, intramammary suspension, ear drops
Paromomycin	Cattle, pigs, goats, sheep	Oral/water
Spectinomycin	Cattle, pigs, sheep, chickens	Injectable, premix, oral/water
Streptomycin	Cattle, sheep, cats, dogs, horses	Injectable, oral/water, intramammary suspension

h) Fluoroquinolones

Active ingredient	Authorised species	Administration route
Danofloxacin	Cattle, pigs	Injectable
Enrofloxacin	Cattle, pigs, sheep, chickens, turkeys, goats, cats, dogs, rabbits, reptiles, ornamental birds, rodents	Injectable, tablet, oral/water
Marbofloxacin	Cattle, pigs, cats, dogs	Tablet, injectable, ear drops
Orbifloxacin	Dogs	Ear drops, oral/water
Pradofloxacin	Cats, dogs	Tablet

i) Macrolides

Active ingredient	Authorised species	Administration route
Erythromycin	Chickens	Oral/water
Gamithromycin	Cattle, pigs, sheep	Injectable
Spiramycin	Dogs, cats	Injectable, tablet
Tildipirosin	Cattle, pigs	Injectable
Tilmicosin	Cattle, pigs, sheep, chickens, turkeys, rabbits	Injectable, premix, oral/water
Tulathromycin	Cattle, pigs, sheep	Injectable
Tylosin	Cattle, pigs, chickens, turkeys	Oral/water, premix, injectable
Tylvalosin	Pigs, chickens, turkeys, game birds	Oral/water, premix

j) Other: amphenicols

Active ingredient	Authorised species	Administration route
Florfenicol	Cattle, pigs, sheep, salmon	Injectable, oral/water, premix, ear gel

k) Other: lincomycins

Active ingredient	Authorised species	Administration route
Lincomycin	Cattle, pigs, chicken, cats, dogs	Oral/water, premix, injectable, intramammary solution
Clindamycin	Cats, dogs	Tablet, oral/water
Pirlimycin	Cattle	Intramammary solution

l) Other: pleuromutilins

Active ingredient	Authorised species	Administration route
Tiamulin	Pigs, chickens, turkeys, rabbits	Oral/water, premix, injectable

m) Other: polymyxins

Active ingredient	Authorised species	Administration route
Colistin	Cattle, pigs, sheep, chickens	Oral/water
Polymyxin B	Cats, dogs	Ear drops, cutaneous suspension

n) Other: other antibiotics

Active ingredient	Authorised species	Administration route
Fusidic acid	Cats, dogs, rabbits	Ear drops, gel
Novobiocin	Cattle	Intramammary suspension

Certain active ingredients included in the results in chapters 3 and 4 are not authorised for use in food-producing animals. These antibiotics (listed below) are however included in the test panels to monitor emergence or risk of resistance to those antibiotics in bacteria in

people or because no breakpoints are available for the antibiotic for which testing ideally should be taking place.

Table S1.4.2: Antibiotics not authorised for use in food-producing animals

Antibiotic class	Active ingredient
Aminoglycosides	Amikacin
Amphenicols	Chloramphenicol
Beta-lactams: 3rd generation cephalosporins	Cefotaxime
Beta-lactams: 3rd generation cephalosporins	Cefpodoxime
Beta-lactams: 3rd generation cephalosporins	Ceftazidime
Beta-lactams: 4th generation cephalosporins	Cefepime
Beta-lactams: Carbapenems	Ertapenem
Beta-lactams: Carbapenems	Imipenem
Beta-lactams: Carbapenems	Meropenem
Macrolides	Azithromycin
Fluoroquinolones	Ciprofloxacin
Quinolones	Nalidixic acid
Other anti-infectives and antiseptics	Furazolidone

S1.5: Cascade prescribing

The Cascade is a legislative provision in the Veterinary Medicines Regulations that allows a veterinary surgeon to prescribe unauthorised medicines that would not otherwise be permitted, for example, imported medicines or a medicine licensed for another animal species or human use. The principle of the Cascade is that, if there is no suitable veterinary medicine authorised in the UK to treat a condition, the veterinary surgeon responsible for the animal may in particular circumstances (for example to avoid causing unacceptable suffering) treat with an unauthorised medicine. Food-producing animals may only be treated under the Cascade with medicines whose pharmacologically active substances are listed in the Table of Allowed Substances in Commission Regulation EU No. 37/2010.

The data used in this report do not include da on sales of imported or human antibiotics used in animals in accordance with the prescribing Cascade, as currently there is no mechanism by which such information can be obtained. The understanding is that use of human products in food-producing animal species is not extensive, due to issues with longer withdrawal periods when using such products. The VMD continues to explore methods that can accurately incorporate information on the amounts of antibiotics imported into or exported out of the UK, as well as methods that can accurately incorporate sales of antibiotics licensed for humans that are sold for animal use under the Cascade prescribing system.

S3.1: Methodology - Antibiotic Susceptibility Testing (AST)

Caecal contents from pigs were sampled for indicator *Escherichia coli* and *Salmonella* in accordance with EU Decision 2020/1729. Stratification and randomisation were performed in accordance with Decision 2020/1729 and EFSA guidelines. All countries within the UK were included in the sampling frame. In accordance with EFSA's guidelines, each eligible slaughter batch of pigs (the 'epidemiological unit') was eligible to contribute one randomly selected *E. coli* and *Salmonella* isolate and thereby avoid clustering. In 2021 the epidemiological unit was the slaughter batch of fattening pigs, rather than the fattening pig herd, which had been used in preceding years. Indicator *E. coli* and *Salmonella* were isolated from caecal contents using MacConkey agar. An isolate was randomly selected and sub-cultured for further testing. Standard biochemical tests were used to identify *E. coli*.

AST was carried out by the national reference laboratories (NRLs). Standardised broth microdilution was used to determine the minimum inhibitory concentration (MIC) against a panel of antibiotics in accordance with Decision 2020/1729 and EFSA guidelines.

The following antimicrobials were tested for *Salmonella* isolates (the ECOFF applied is stated in brackets): ampicillin (>8), azithromycin (>16), cefotaxime (>0.5), ceftazidime (>2), chloramphenicol (>16), ciprofloxacin (>0.064), colistin (>2), gentamicin (>2), meropenem (>0.125), nalidixic acid (>8), sulfamethoxazole (>256), tetracyclines (>8), tigecycline (>1), trimethoprim (>2), amikacin (>4). Further testing of the supplementary panel of antimicrobials (Table 5 in Decision 2020/1729/EU) was not performed since there were no isolates detected which were microbiologically resistant to cefotaxime, ceftazidime or meropenem.

The following antimicrobials were tested for indicator *E. coli* isolates (the ECOFF applied is stated in brackets): ampicillin (>8), amikacin (>8), azithromycin (>16), cefotaxime (>0.25), ceftazidime (>0.5), chloramphenicol (>16), ciprofloxacin (>0.064), colistin (>2), gentamicin (>2), meropenem (>0.125), nalidixic acid (>8), sulfamethoxazole (>64), tetracyclines (>8), tigecycline (>0.5), trimethoprim (>2). Further testing of the supplementary panel of antimicrobials (in accordance with Table 4 in Decision 2020/1729) was then performed on isolates resistant to cefotaxime or ceftazidime or meropenem using cefepime (>0.125), cefotaxime (>0.25), cefotaxime + clavulanate (NA), cefoxitin (>8), ceftazidime (>0.5), ceftazidime plus clavulanate (NA), ertapenem (NA), imipenem (>0.5), meropenem (>0.125) and temocillin (>16).

Please note the ECOFFS have been revised for nalidixic acid, temocillin and tigecycline and an ECOFF is not stipulated for ertapenem in Decision 2020/1729.

In addition, caecal samples were cultured for ESBL-/AmpC-/carbapenemase-producing *E. coli* following the selective procedures outlined in Decision 2020/1729. This included a pre-

enrichment step followed by inoculation of samples onto MacConkey agar plates supplemented with 1 mg/L cefotaxime for isolation of ESBL- or AmpC-producing *E. coli* and chromID OXA-48® and chromID CARBA® agars for isolation of carbapenemase-producing *E. coli*. An *E. coli* with an ESBL phenotype was defined as showing synergy with cefotaxime and clavulanate and/or ceftazidime and clavulanate. An *E. coli* with an AmpC phenotype was defined as showing decreased susceptibility to cefoxitin, cefotaxime and ceftazidime.

Whole genome sequencing (WGS) and *in silico* bioinformatic tools were used to detect the antibiotic resistance determinants present in the isolates with ESBL- or AmpC-phenotypes. The isolates were sequenced using the Illumina NextSeq platform followed by quality control steps and mapping of the raw reads to a database of antibiotic resistance genes, using the APHA SeqFinder pipeline (please see this and this paper). The sequence of *E. coli* isolates negative for all known ESBL-, AmpC- and carbapenemase-encoding genes were investigated for promoter mutations in *ampC*, which is compatible with increased expression of the chromosomal *E. coli ampC*, using the APHA SeqFinder pipeline.

S4.1: Methodology susceptibility testing

S4.1.1 Core data

The susceptibility tests described in UK-VARSS (excluding the MIC testing of veterinary pathogens and the Private Laboratory Initiative) were performed using the method formerly recommended by the British Society for Antimicrobial Chemotherapy (BSAC, www.bsac.org.uk).

Tests were performed (unless otherwise stated) by disc diffusion on Isosensitest Agar (Oxoid) with appropriate media supplementation where necessary for fastidious organisms. The disc antibiotic concentrations used were as stated in Table S4.1.1.1, and a semi-confluent inoculum was used.

The method used for assessing the susceptibility to antibiotics is, unless otherwise stated in the report, the disc diffusion method described by BSAC (www.bsac.org.uk). This assumes that the level of antibiotic achieved at the site of infection in the animal is similar to that achieved in a human treated with the same antibiotic. This assumption may not always be correct: different concentrations may be achieved at the site of infection in animals as a consequence of different dosing regimens or pharmacokinetics in different animal species.

Use of the susceptibility testing method formerly employed in human medicine in the UK in many hospitals and clinical medical establishments, enabled and facilitated direct comparison of veterinary susceptibility results with medical susceptibility results collected using similar methods. Direct comparison with the susceptibility results reported in other countries can be difficult because of differences in methodology and breakpoints. However, BSAC clinical breakpoints were harmonised and completely aligned with those of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) which are commonly adopted across Europe. Thus, although different disc diffusion methods are employed in the BSAC and EUCAST procedures, the result obtained by either method should be the same because susceptibility is determined in both methods according to the same breakpoint.

Isolates were classed as either sensitive or resistant; intermediate isolates under the BSAC guidelines are considered resistant. The disc diffusion breakpoints used are given in Table S4.1.1.1 which also provides the MIC corresponding to that zone diameter breakpoint where this is known or has been estimated from APHA data on file.

Published breakpoints are not available for all animal species and for all of the bacterial organism/antibiotic combinations which may require testing. In these cases, a uniform cut-off point of 13mm zone size diameter has been used to discriminate between sensitive and resistant strains; an intermediate category of susceptibility has not been recorded. This breakpoint is the historical APHA veterinary breakpoint and although it has been used for a considerable number of years, published validation data are not available for a number of

organism/antibiotic combinations. However, where most isolates of a particular organism are either highly resistant or fully susceptible to an antibiotic, breakpoint issues may affect only a low number of isolates.

Breakpoints used to interpret the results from the antimicrobial susceptibility testing are reviewed on a regular basis. Data presented in this report and the supplementary material are retrospectively updated when required to reflect any changes to the interpretative criteria and ensure consistency and comparability of the data.

Susceptibility was determined for certain antibiotics not authorised for use in any food-producing animal species (for example, cefpodoxime) or not authorised for particular animal species (for example, tetracycline in sheep). This is to provide a full picture of resistance emergence and/or as a surrogate (for example, tetracycline, chlortetracycline and oxytetracycline are all equivalent for resistance testing purposes.).

Multiple antibacterial resistance, or multi-drug resistance (MDR), where referred to in the core data, is defined in this report as resistance to any of three or more separate antibiotics which were tested for a particular isolate. There is no internationally agreed definition of multiple resistance, and the term has been used differently in different studies. The panels of antimicrobials which may be tested at a particular APHA laboratory can also show slight variation, dependent on the circumstances of the case and the requirements of the veterinary surgeon administering treatment. The multiple resistance figures should therefore be regarded as subject to a degree of variation.

Please note that the methodology for susceptibility testing used by the SRUC is detailed in the Scottish One Health Antimicrobial Use and Antimicrobial Resistance (SONAAR) report.

Please note that throughout this section, cefalexin, cefotaxime, ceftazidime, cefpodoxime, ceftiofur, ciprofloxacin, colistin and enrofloxacin are all HP-CIAs. It should also be noted that within this section, a hyphen indicates that no isolates were tested, or that no data is available. For individuals using screen readers, please note that cells read out as blank, indicates no isolates were tested, or that no data is available.

Table S4.1.1.1: Disc diffusion breakpoints, corresponding MIC breakpoints and breakpoints under review for the main bacteria covered in the core data of this report

a) England and Wales

Please note that the erythromycin the R ≤21 mm breakpoint is for beta-haemolytic streptococci and R ≤19 mm for other streptococci, for penicillin the R ≤19 mm breakpoint is for beta-haemolytic streptococci and R ≤16 mm for other streptococci and the tetracycline R ≤19 mm breakpoint is for beta-haemolytic streptococci and R ≤23 mm for other streptococci. Additionally, some *Haemophilus-Pasteurella-Actinobacillus*, or "HPA" organisms (for example *Actinobacillus pleuropneumoniae*) show a degree of intrinsic resistance to aminoglycosides. The historical veterinary breakpoint was used for *H. somni* and *A. pleuropneumoniae*.

Antibiotic	Disc charge (micrograms)	*Escherichia coli,* Enterobacteriaceae	*Salmonella*	*Staphylococci*	*Streptococci*	*Pasteurella, Mannheimia*
Amikacin (AK)	30	R ≤18 mm R ≥16 mg/l	R ≤18 mm R ≥16 mg/l	NA	NA	NA
Amoxicillin/clavulanate (AMC)	20/10	R ≤14 mm R >8 mg/l	R ≤14 mm R > 8mg/l	NA	NA	R ≤13 mm
Amoxicillin/clavulanate	2/1	NA	NA	R ≤17 mm R >1 mg/l	R ≤13 mm	NA
Ampicillin (AM)	10	R ≤14 mm R >8 mg/l	R ≤14 mm R >8 mg/l	R ≤13 mm	R ≤13 mm	R ≤29 mm R >1 mg/l
Apramycin (APR)	15	R ≤13 mm R ≥32 mg/l	R ≤13 mm R ≥32 mg/l	NA	NA	R ≤13 mm†
Cefalexin	30	R ≤15 mm R >16 mg/l	NA	R ≤13 mm	R ≤24 mm R >2 mg/l	R ≤13 mm

Antibiotic	Disc charge (micrograms)	*Escherichia coli*, Enterobacteriaceae	*Salmonella*	*Staphylococci*	*Streptococci*	*Pasteurella, Mannheimia*
Cefotaxime (CTX)	30	R ≤29 mm R ≥2 mg/l	R ≤29 mm R ≥2 mg/l	NA	NA	NA
Cefpodoxime	10	R ≤ 19 mm R >1 mg/l	NA	NA	NA	R ≤13 mm
Ceftazidime (CAZ)	30	R ≤ 26 mm R ≥2 mg/l	R ≤26 mm R ≥2 mg/l	NA	NA	NA
Chloramphenicol (C)	30	R ≤20 mm R >8 mg/l	R ≤20 mm R >8 mg/l	NA	NA	NA
Ciprofloxacin (CIP)	1	NA	R ≤16 mm R ≥1 mg/l	NA	NA	NA
Doxycycline	30	R ≤13 mm	NA	R ≤30 mm R ≥2 mg/l	NA	R ≤13 mm
Enrofloxacin	5	R ≤13 mm R ≥4 mg/l	NA	R ≤13 mm	R ≤13 mm	R ≤13 mm
Erythromycin	5	NA	NA	R ≤19 mm R ≥2 mg/l	R ≤21 mm R ≥0.5 mg/l	R ≤13 mm
Florfenicol	30	R ≤13 mm R >32 mg/l	NA	NA	R ≤13 mm	R ≤13 mm
Furazolidone (FR)	15	NA	≤13 mm	NA	NA	NA
Gentamicin (CN)	10	NA	R ≤19 mm R ≥4 mg/l	NA	NA	NA
Lincomycin	10	NA	NA	R ≤13 mm	R ≤13 mm	R ≤13 mm
Nalidixic acid (NA)	NA	NA	≤13 mm	NA	NA	NA
Neomycin (N)	10	R ≤13 mm R >8 mg/l	R ≤13 mm R >8 mg/l	NA	NA	NA

Antibiotic	Disc charge (micrograms)	*Escherichia coli*, Enterobacteriaceae	*Salmonella*	*Staphylococci*	*Streptococci*	*Pasteurella, Mannheimia*
Neomycin	30	NA	NA	R ≤13 mm	R ≤13 mm	NA
Novobiocin	30	NA	NA	R ≤13 mm	R ≤13 mm	NA
Penicillin	1IU	NA	NA	R ≤24 mm R >0.12 mg/l	R ≤19 mm R >0.25 mg/l	NA
Spectinomycin	25	R ≤13 mm	NA	NA	NA	R ≤13 mm
Streptomycin (S)	10	R ≤12 mm R >8 mg/l	R ≤13 mm R > ~8 mg/l	NA	NA	R ≤13 mm
Sulphonamide compounds (SU)	300	NA	≤13 mm	NA	NA	NA
Tetracycline (T)	10	R ≤13 mm R >8 mg/l	R ≤13 mm R >8 mg/l	R ≤19 mm R ≥2 mg/l	R ≤19 mm R ≥2 mg/l	R ≤25 mm R >1 mg/l
Trimethoprim/ sulphonamide (TM)	25	R ≤15 mm R ≥4 mg/l	R ≤15 mm R ≥4 mg/l	R ≤16 mm R ≥4 mg/l	R ≤19 mm R ≥2 mg/l	R ≤13 mm
Tylosin	30	NA	NA	R ≤13 mm	R ≤13 mm	R ≤13 mm

Key:

- BSAC human clinical breakpoint
- APHA historical veterinary disc diffusion zone size breakpoint and MIC corresponding to that zone size breakpoint, derived from studies of zone size and MIC
- Animal Health and Veterinary Laboratories Agency (AHVLA) historical veterinary breakpoint (under ongoing review)

Notes:

- Where zone size disc diffusion data collected using the BSAC method and MIC data are both available then it is possible to draw regression lines and investigate the MIC which approximately corresponds to the historical veterinary breakpoint of 13 mm. This has been done for several compounds (highlighted in blue in the table above).

- BSAC state that all *Salmonella* isolates should be reported as resistant to gentamicin and amikacin; resistance traits are used for epidemiological purposes (correlation with particular resistance mechanisms) in this report.
- The 16 antibiotics with antibiotic code, for example, amikacin (AK), are the set used for *Salmonella* susceptibility testing.
- A breakpoint of resistance > 4 mg/l tiamulin has been suggested for MIC determination by agar dilution[1] this has also been quoted in a Dutch study of swine dysentery in pigs[2] whilst for broth microdilution the suggested clinical breakpoint is one dilution lower at > 2 mg/l tiamulin. An epidemiological cut-off value of wild type ≤ 0.25 has been suggested for broth dilution MIC determination of tiamulin versus *B. hyodysenteriae*.
- *S. aureus* isolates resistant to amoxicillin/clavulanate are currently screened for susceptibility to cefoxitin and by agglutination tests for altered penicillin binding protein in order to detect *mecA* and *mecC.*

[1] Rønne, H. and Szancer, J. (1990) In vitro susceptibility of Danish field isolates of Treponema hyodysenteriae to chemotherapeutics in swine dysentery (SD) therapy. Interpretation of MIC results based on the pharmacokinetic properties of the antibacterial agents. Proceedings of the 11th IPVS Congress. Lausanne, Switzerland, July 1 to 5, 1990. p 126

[2] Duinhof TF, Dierikx CM, Koene MGJ, van Bergen MAP, Mevius DJ, Veldman KT, van Beers-Schreurs HMG and de Winne RTJA. (2008). Multiresistentie bij Brachyspira hyodysenteriae-isolaten op een varkensvermeerderingsbedrijf in Nederland. Tijdschrift voor Diergeneeskunde 133:604-608.

b) Scotland

Antibiotic	Disc charge (micrograms)	*Escherichia coli*, Enterobacteriaceae	*Salmonella*
Amikacin (AK)	30	R ≤18 mm R ≥16 mg/l	R ≤18 mm R ≥16 mg/l
Amoxicillin/clavulanate (AMC)	20/10	R ≤14 mm I ≤17 mm	R ≤14 mm
Ampicillin (AM)	10	R ≤14 mm	R ≤14 mm
Apramycin (APR)	15	R ≤13 mm	R ≤13 mm
Cefalexin	30	R ≤15 mm R >16 mg/l	NA
Cefotaxime (CTX)	30	R ≤29 mm R ≥2 mg/l	R ≤29 mm R ≥2 mg/l
Cefpodoxime	10	R ≤ 19 mm	R ≤ 19 mm
Ceftazidime (CAZ)	30	R ≤ 26 mm R ≥2 mg/l	R ≤26 mm R ≥2 mg/l
Chloramphenicol (C)	30	R ≤20 mm R >8 mg/l	R ≤20 mm R >8 mg/l
Ciprofloxacin (CIP)	1	NA	R ≤16 mm R ≥1 mg/l
Doxycycline	30	R ≤13 mm	NA
Enrofloxacin	5	R ≤16 mm I ≤20 mm	R ≤16 mm I ≤20 mm
Erythromycin	5	NA	NA
Florfenicol	30	R ≤13 mm I >17mm	R ≤13 mm I >17mm
Furazolidone (FR)	15	NA	≤13 mm
Gentamicin (CN)	10	NA	R ≤19 mm R ≥4 mg/l
Lincomycin	10	NA	NA
Nalidixic acid (NA)	NA	≤13 mm	≤13 mm
Neomycin (N)	10	R ≤12 mm I ≤17 mm	R ≤12 mm
Neomycin	30	R ≤12 mm I ≤17 mm	R ≤12 mm
Novobiocin	30	NA	NA
Penicillin	1IU	NA	NA

Antibiotic	Disc charge (micrograms)	*Escherichia coli*, Enterobacteriaceae	*Salmonella*
Spectinomycin	25	R ≤13 mm	R ≤13 mm I ≤14 mm
Streptomycin (S)	10	R ≤12 mm R >8 mg/l	R ≤13 mm R > ~8 mg/l
Sulphonamide compounds (SU)	300	NA	≤13 mm
Tetracycline (T)	10	R ≤13 mm	R ≤13 mm I ≤14 mm
Trimethoprim/ sulphonamide (TM)	25	R ≤15 mm	R ≤15 mm
Tylosin	30	NA	NA

Table S4.1.1.2: Antibiotic disc concentrations used in Northern Ireland, defined by expected zone diameter in millimetres

Antibiotic	Disc	Resistant	Intermediate	Susceptible
Amoxicillin	AMC30	≤13	14–17	≥18
Ampicillin	AMP10	≤13	14–16	≥17
Apramycin	APR15	N/A	N/A	N/A
Cefotaxime	CTX30	≤22	23–25	≥26
Ceftazidime	CAZ30	≤17	18–20	≥21
Chloramphenicol	C30	≤12	13–17	≥18
Ciprofloxacin	CIP5	≤15	16–20	≥21
Framycetin	FY100	N/A	N/A	N/A
Furazolidone	FR100	N/A	N/A	≥17
Gentamicin	CN10	≤12	13–14	≥15
Kanamycin	K30	≤13	14–17	≥18
Nalidixic acid	NA30	≤13	14–18	≥19
Spectinomycin	SH100	N/A	N/A	N/A
Streptomycin	S10	≤11	12–14	≥15
Sulphonamides	S3.300	≤12	13–16	≥17
Tetracycline	TE30	≤11	12–14	≥15
Trimethoprim	W5	≤10	11–15	≥16

S4.1.2 MIC testing of veterinary pathogens

Summary susceptibility data is presented in the UK-VARSS report for bacterial respiratory pathogens of cattle, sheep, and pigs, as well as *S. suis* in pigs, *S. uberis* in cattle mastitis and avian *E. coli* isolates. These were isolated from diagnostic submissions to the Animal and Plant Health Agency (APHA) and its partner laboratories in 2021. The population of bacterial organisms described in this report has therefore originated, for the most part, from samples of field cases of clinical disease undergoing investigation by veterinary surgeons for diagnostic purposes. The figures thus reflect the AMR of respiratory bacterial pathogens of clinical veterinary significance recovered from farm animals in England and Wales. In some instances, the samples may originate from animals that have already been treated with antibiotics and therefore may have been under selective pressure.

Susceptibility testing was performed using broth microdilution to determine MIC values, on microtitre plates, with cation adjusted Mueller-Hinton broth. Appropriate media supplementation with Veterinary Fastidious Medium was performed for *A. pleuropneumoniae* (CLSI VET01S ED5:2020). Broth microdilution methods conforming to the International Standards Organisation provide a robust and reliable means of determining susceptibility and are commonly used in harmonised monitoring programmes.

For the purposes of presenting results in the main report, resistance has been interpreted using clinical breakpoints. Isolates have been classed as either sensitive or resistant using veterinary CBPs from CLSI in the first instance, or CASFM when these are not available; if veterinary breakpoints were not available, human CBPs were used (see Table S4.1.2.1). For some veterinary antibiotic and organism combinations, there are no published breakpoints available and in these cases, resistance cannot be interpreted from MIC distributions. EUCAST has also recently published ECOFFs and tentative ECOFFs (TECOFFs) for some of the organisms (see https://www.eucast.org/mic_distributions_and_ecoffs/), and where these have been available, results have also been interpreted by these means. The CBP relates to efficacy of treatment in each animal species, whereas the ECOFFs differentiate non-wild type from wild-type organisms, that is to say ECOFFs detect those bacteria which have any degree of increased resistance. The ECOFFs are therefore useful to demonstrate an emerging decline in susceptibility.

Multi-drug resistance (MDR) was considered to indicate resistance to three or more classes of antimicrobials. For the purposes of assessing MDR, the macrolides gamithromycin, tildipirosin, tilmicosin and tulathromycin were considered as a single class (because common or linked resistance mechanisms have been described to these compounds), as were tetracycline and doxycycline and ampicillin and amoxicillin/clavulanate (because hyper-expression of beta-lactamase can overcome the inhibitor clavulanate).

Table S4.1.2.1 MIC breakpoints used for the interpretation of antibacterial susceptibility for veterinary pathogens from cattle, pigs, chickens and sheep. Cattle breakpoints applied to sheep isolates unless indicated otherwise.

a) Respiratory pathogens

Please note, for amoxicillin/clavulanate, the clavulanate concentration is fixed at 2 mg/ml. For tilmicosin in cattle and sheep, a breakpoint for porcine isolates was used. For spectinomycin and gamithromycin in pigs, breakpoint for bovine isolates was used.

Antibiotic	***Pasteurella multocida***			***Mannheimia haemolytica***		***Actinobacillus pleuropneumoniae***	***Bibersteinia trehalosi***
	Cattle	**Pigs**	**Sheep**	**Cattle**	**Sheep**	**Pigs**	**Sheep**
Ampicillin	R > 1	R ≥ 2 R > 1	R > 1	R > 1	R > 1	R ≥ 2	R > 1
Amoxicillin/clavulanate†	R > 16/2 R > 1	R > 16/2 R > 1	R > 16/2 R > 1	R > 16/2 R > 1	R > 16/2 R > 1	NA	R > 16/2 R > 1
Ceftiofur	R ≥ 8 R > 4	R ≥ 8 R > 4	R ≥ 8 R > 4	R ≥ 8 R > 4	R ≥ 8 R > 4	R ≥ 8	R ≥ 8 R > 4
Tetracycline	R ≥ 8 R > 8	R ≥ 2 R > 8	R ≥ 8 R > 8	R ≥ 8 R > 8	R ≥ 8 R > 8	R ≥ 2	R ≥ 8 R > 8
Doxycycline	R >8 R > 1	R >8 R > 1	R >8 R > 1	R >8	R >8	R >8	R >8
Spectinomycin	R ≥ 128	R ≥ 128	R ≥ 128	R ≥ 128	R ≥ 128	NA	R ≥ 128
Enrofloxacin	R ≥ 2 R > 2	R ≥ 1 R > 2	R ≥ 2 R > 2	R ≥ 2 R > 2	R ≥ 2 R > 2	R ≥ 1 R > 2	R ≥ 2 R > 2
Trimethoprim/ Sulphonamide	R >8 R > 0.25	R >8 R > 0.25	R >8 R > 0.25	R >8	R >8	R >8	R >8

Antibiotic	*Pasteurella multocida*			*Mannheimia haemolytica*		*Actinobacillus pleuropneumoniae*	*Bibersteinia trehalosi*
Florfenicol	R ≥ 8	R ≥ 8	R ≥ 8	R ≥ 8	R ≥ 8	R ≥ 8	R ≥ 8
	R > 4	R > 4	R > 4	R > 4	R > 4	R > 4	R > 4
Gamithromycin	R ≥ 16	R ≥ 16	R ≥ 16	R ≥ 16	R ≥ 16	NA	R ≥ 16
Tildipirosin	R ≥ 32	S < 4	R ≥ 32	R ≥ 16	R ≥ 16	S ≤ 16	R ≥ 16
Tilmicosin	R ≥ 32	R ≥ 32	R ≥ 32	R ≥ 32	R ≥ 32	R ≥ 32	R ≥ 32
	R > 16	R > 16	R > 16	R > 16	R > 16	R > 16	R > 16
Tulathromycin	R ≥ 64	R ≥ 64	R ≥ 64	R ≥ 64	R ≥ 64	S ≤ 64	R ≥ 64
Tiamulin	NA	NA	NA	NA	NA	R ≥ 32	NA

Key:

- CLSI veterinary clinical breakpoint
- CASFM veterinary clinical breakpoint
- EUCAST human breakpoint

b) Other pathogens

Antibiotic	*Escherichia coli*	*Streptococcus uberis*	*Streptococcus suis*
	Chickens	**Cattle**	**Pigs**
Penicillin	-	R > 1	R > 1
		S ≤ 0.25	S ≤ 0.25
Ampicillin	R > 8	-	-
Amoxicillin/ clavulanate	R > 32/16	-	-
	R > 16/8	-	-
Cephalexin	R > 16	-	-
	R > 32	-	-
Cefotaxime	R > 2	-	-
Ceftazidime	R > 4	-	-
Ceftiofur	R ≥ 8	R > 8	R > 8
	R > 4	S ≤ 2	S ≤ 2
Meropenem	NA	-	-
Tetracycline	R ≥ 16	R > 2	R > 2
	R > 8	S ≤ 0.25	S ≤ 0.5
Doxycycline	R >16	R > 1	R > 1
	R > 8	S ≤ 0.25	S ≤ 0.25
Colistin	R > 2	-	-
	R > 2	-	-
Spectinomycin	NA	-	-
Enrofloxacin	R ≥ 2	R > 2	R > 2
	R > 2	S ≤ 0.5	S ≤ 0.5
Trimethoprim/ Sulphonamide	R > 8	-	R > 2
	R > 4	-	S ≤ 1
Lincomycin	-	R > 8	R > 8
		S ≤ 2	S ≤ 2
Florfenicol	R ≥ 16	R > 8	R > 8
		S ≤ 2	S ≤ 2
Gamithromycin	R ≥ 16	-	-
Amikacin	NA	-	-
Apramycin	R > 16	-	-
Neomycin	R > 16	NA	-
Erythromycin	-	R > 1	R > 1
		S ≤ 0.25	S ≤ 0.25
Streptomycin	R > 16	-	-

Key:

CLSI veterinary clinical breakpoint

CASFM veterinary clinical breakpoint

EUCAST human breakpoint

\- Not tested

S4.1.3 Private Laboratory Initiative

The methods used to determine antimicrobial susceptibility, are based on those in CLSI Vet01 July 2013[3]. Tests were performed by disc diffusion on Mueller-Hinton agar (MHA) without supplements for *Enterobacteriaceae* and staphylococci, and Mueller-Hinton agar with blood (MH-F) for streptococci. The inoculum used gives confluent growth of bacterial colonies. Zone edges are read at the point of complete inhibition. A summary of the disc diffusion breakpoints applied by the Vale Veterinary Laboratory are found in Table S4.1.3.1 below.

Table 4.1.3.1: Disc diffusion breakpoints applied by Vale Veterinary Laboratories for the interpretation of resistance of bovine mastitis pathogens.

Antibiotic	*E. coli*	*S. dysgalactiae*	*S. aureus*	*S. uberis*
Amoxicillin/clavulanate	R < 19mm	NA	20mm	NA
Ampicillin	R < 14mm	24mm	13-17mm	24mm
Cefapirin	14-18mm	14-18mm	14-18mm	14-18mm
Cloxacillin	NA	18mm	18mm	18mm
Penicillin	NA	18mm	18mm	18mm
Trimethoprim/ sulphonamide	R < 13mm	R < 15mm	R < 14mm	R < 15mm
Oxytetracycline	11-15mm	NA	14-19mm	NA
Spectinomycin	20mm	NA	20mm	NA
Neomycin	R < 11mm	NA	R < 14mm	NA

[3] The Vale Veterinary Laboratory, personal communications, 2021