Veterinary Medicines Directorate

Supplementary Material 4 – Resistance Methods

UK-VARSS 2024

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Chapter S3 Harmonised Monitoring Supplementary Material

S3.1 Harmonised monitoring requirements

Table S4.1: Summary of monitoring requirements in the UK from 2014 to 2024 by sampling year. Year tested is indicated by an X.

Pathogen	Sample origin	Animal species	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024
Salmonella spp.	Carcasses	Broilers and turkeys	x		x		x		x				
	NCP	Broilers, turkeys and layers	x		x		x		x		x		x
	Carcasses	Pigs		x		x		x					
	Caeca									x		x	
Escherichia coli	Caeca	Broilers and turkeys	x		x		x		x		x		x
		Pigs		x		x		x		x		x	
ESBL-, AmpC- or carbapenemase-producing *E. coli*	Caeca	Broilers and turkeys			x		x		x		x		x
		Pigs		x		x		x		x		x	
Campylobacter coli	Caeca	Broilers and turkeys									x		x
		Pigs										x	

Campylobacter jejuni	Caeca	Broilers and turkeys	x		x		x		x		x		x
Enterococcus faecalis	Caeca	Broilers and turkeys									x		x
		Pigs										x	
Enterococcus faecium	Caeca	Broilers and turkeys									x		x
		Pigs										x	

S3.2 Harmonised monitoring methodology

Samples of faecal content were taken from healthy broilers and turkeys at slaughter by Food Standards Agency (FSA) personnel and sampled for indicator *Escherichia coli*, Enterococci, and *Campylobacter* in accordance with EU Decision 2020/1729. The sampling plan was randomised, stratified, and weighted by slaughter throughput. Samples were collected from the biggest slaughterhouses in the UK, jointly covering 62% of healthy broilers throughput and 82% of fattening turkey throughput in 2024. For broilers, ten caecal samples were collected per epidemiological unit and pooled before testing. For turkeys, one caecal sample was collected per epidemiological unit (flock) sampled.

Boot/dust swabs were collected for the isolation of *Salmonella* in accordance with the National Control Programme (NCP) for layers, broilers and turkeys. Swabs were taken from all flocks included in the NCPs and all isolated *Salmonella* were tested, unless there were 170 isolates or more, in which case a randomised sample of the isolates obtained from those swabs was further analysed.

All countries within the UK were included in the sampling frame and contributed isolates from each of *E. coli, Salmonella, Campylobacter jejuni*, *Campylobacter coli*, *Enterococcus faecium* and *Enterococcus faecalis.* For the first time, *Enterococcus* species isolates were recovered from Northern Ireland samples.

Caecal samples were cultured for *E. coli, Campylobacter* spp. and *Enterococcus* species. using appropriate media. *Salmonella* isolates are not cultured from these caecal samples and were instead received by the NRLs for serotyping and susceptibility testing. *E. coli* was isolated using the EU-RL method using MacConkey agar. *Campylobacter* species were isolated using the EU-RL method employing modified charcoal-cefoperazone-deoxycholate (MCCDA) agar and Butzler agar, without pre-enrichment. Matrix-assisted laser desorption/ionisation-time of flight, MALDI-ToF, was used to confirm identification. A single typical colony, of each target organism from each sample, was selected for speciation and susceptibility testing.

S3.3 Using selective media to detect specific resistances

Additional, more sensitive, testing was conducted using selective media. This inhibits the growth of susceptible *E. coli* in a sample but allows the resistant bacteria to grow, making them easier to detect. Caecal samples were cultured for ESBL- and AmpC- producing *E. coli* following standard procedures. This included a pre-enrichment step followed by inoculation of samples onto MacConkey agar plates supplemented with 1 mg/L cefotaxime. Carbapenemase-producing *E. coli* were cultured as above for ESBL- and AmpC- producing *E. coli*. Following pre-enrichment the samples were inoculated onto chromID OXA-48® and chromID CARBA® agars. In addition, samples were cultured for the presence of colistin resistant *E. coli*, using MacConkey agar plates supplemented with 1 mg/L colistin.

S3.4 Antibiotic Susceptibility Testing (AST)

AST was carried out by the national reference laboratories (NRLs) using European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology. Standardised broth microdilution was used to determine the minimum inhibitory concentration (MIC) against a panel of antibiotics as listed in Decision (EU) 2020/1729 and EFSA guidelines, or where not available a panel of antibiotics following joint APHA/VMD recommendations. Tables of antibiotic panels including cut-off values can be seen in **Table S4.2** and **Table S4.3** below.

S3.5 AST Interpretation

Epidemiological cut-off values (ECOFFs) were used to assess the susceptibility of the bacterial isolates to the antibiotics tested. ECOFFs represent the point at which bacteria have developed a higher level of resistance to an antibiotic than the background level of resistance that exists naturally for that bacterial species. Bacteria are classed as being wild type (WT) if they have not acquired or developed a resistance mechanism, and non-wild type (NWT) if they have acquired or developed resistance mechanisms. For the purposes of this report all WT bacteria are called sensitive, and NWT bacteria are called resistant. ECOFFs are more sensitive than clinical breakpoints (CBPs) for detecting emerging resistance issues. A 'decreased susceptibility' or 'resistant' result based on ECOFFs does not necessarily imply a level of resistance that would correspond to clinical treatment failure.

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology for ECOFFs was used in this report. Where possible EUCAST ECOFFs (as published on 01/04/2025) were used to interpret the MIC values. EUCAST ECOFFs are regularly under review and updated as new values and drug/bacteria species combinations are determined. Where no EUCAST ECOFF values were available, the EFSA recommended ECOFF values were used. Where neither defined EUCAST nor EFSA ECOFF values were available, tentative EUCAST ECOFF values were applied. Historical data presented in chapter 3 of the report has been updated to reflect cut-off values used in 2024.

For ease of comparison, both the ECOFF and corresponding EUCAST CBP values are presented in **Tables S4.2 (a) to (c)** and **Tables S4.3 (a) to (c)**.

The beta-lactamase phenotype was determined by MIC and interpreted using the following criteria:

- An *E. coli* with an **ESBL phenotype** was defined as: having an MIC of >1 mg/L to cefotaxime and/or ceftazidime; showing synergy with cefotaxime and clavulanate and/or ceftazidime and clavulanate; susceptibility to cefoxitin MIC ≤ 8 mg/L; and susceptibility to meropenem MIC ≤ 0.12 mg/L.
- An *E. coli* with an **AmpC phenotype** was defined as: having an MIC of >1 mg/L to cefotaxime and/or ceftazidime; no synergy with cefotaxime and clavulanate and/or

ceftazidime and clavulanate; reduced susceptibility to cefoxitin MIC > 8 mg/L; and susceptibility to meropenem MIC ≤ 0.12 mg/L.

- An *E. coli* expressing both an **ESBL and an AmpC phenotype** was defined as: having an MIC of >1 mg/L to cefotaxime and/or ceftazidime; showing synergy with cefotaxime and clavulanate and/or ceftazidime and clavulanate; reduced susceptibility to cefoxitin MIC > 8 mg/L; and susceptibility to meropenem MIC ≤ 0.12 mg/L.
- An *E. coli* with a carbapenemase-phenotype was defined as: having an MIC of >0.12 mg/L to meropenem.

Multidrug resistance (MDR) is defined as resistance to three or more antibiotic classes.

Table S4.2: The ECOFF values applied when determining susceptibility of a) *E. coli* and *Salmonella*, b) *Campylobacter* spp. and c) *Enterococcus* spp., isolated from healthy broilers and turkeys at slaughter. Values are expressed in mg/L.

For individuals using screen readers, please note that cells read out as blank denote that no data is available.

a) *E. coli* and *Salmonella*

Antibiotic	*E. coli* (mg/L)	*Salmonella* (mg/L)
Amikacin	>8*	>4***
Ampicillin	>8*	>4*
Azithromycin	>16**	>16*
Cefepime	>0.125*	N/A
Cefotaxime	>0.25*	>0.5**
Cefotaxime/clavulanate	>0.25*	N/A
Cefoxitin	>16*	N/A
Ceftazidime	>1*	>2*
Ceftazidime/clavulanate	>1*	N/A
Chloramphenicol	>16*	>16*
Ciprofloxacin	>0.06*	>0.06*
Colistin	>2*	>2**
Ertapenem	>0.06**	N/A
Gentamicin	>2*	>2*
Imipenem	>0.5*	N/A
Meropenem	>0.06*	>0.125**
Nalidixic acid	>8*	>8*
Sulfamethoxazole	>64**	>256**
Temocillin	>16*	N/A
Tetracycline	>8*	>8*
Tigecycline	>0.5*	>0.5**
Trimethoprim	>2*	>2**

b) *Campylobacter* spp.

Antibiotic	*C. coli* (mg/L)	*C. jejuni* (mg/L)
Chloramphenicol	>16*	>16*
Ciprofloxacin	>0.5*	>0.5*
Ertapenem	>0.5**	>0.5**
Erythromycin	>8*	>4*
Gentamicin	>2*	>2*
Tetracycline	>2*	>1*

c) *Enterococcus* spp.

Antibiotic	*E. faecalis* (mg/L)	*E. faecium* (mg/L)
Ampicillin	>4*	>4*
Chloramphenicol	>32*	>32*
Ciprofloxacin	>4*	>8*
Daptomycin	>4*	>8*
Erythromycin	>4*	>4*
Gentamicin	>64*	>32*
Linezolid	>4*	>4*
Quinupristin/dalfopristin	N/A	>2*
Teicoplanin	>2*	>2*
Tetracycline	>4*	>4*
Tigecycline	>0.25*	>0.25*
Vancomycin	>4*	>4*

Key:

* EUCAST ECOFF
** EFSA-recommended ECOFF
*** EUCAST tentative ECOFF

Table S4.3: The EUCAST clinical breakpoint (CBP) values applied when determining susceptibility of a) *E. coli* and *Salmonella*, b) *Campylobacter* spp., and c) *Enterococcus* spp., isolated from healthy broilers and turkeys at slaughter. Values are expressed in mg/L.

For individuals using screen readers, please note that cells read out as blank denote that no data is available.

a) *E. coli* and *Salmonella*

Antibiotic	*E. coli* (mg/L)	*Salmonella* (mg/L)
Amikacin	>8	>8
Ampicillin	>8	>8
Azithromycin	-	-
Cefotaxime	>2	>2
Ceftazidime	>4	>4
Chloramphenicol	>16	>16
Ciprofloxacin	>0.5	>0.06
Colistin	>2	>2
Gentamicin	>2	>2
Meropenem	>8	>8
Nalidixic acid	-	-
Sulfamethoxazole	-	-
Tetracycline	-	-
Tigecycline	>0.5	-
Trimethoprim	>4	>4

b) *Campylobacter* spp.

Antibiotic	*C. coli* (mg/L)	*C. jejuni* (mg/L)
Chloramphenicol	-	-
Ciprofloxacin	>0.5	>0.5
Ertapenem	-	-
Erythromycin	>8	>4
Gentamicin	-	-
Tetracycline	>2	>2

c) *Enterococcus* spp.

Antibiotic	*E. faecalis* (mg/L)	*E. faecium* (mg/L)
Ampicillin	>4	>4
Chloramphenicol	-	-
Ciprofloxacin	>4	>4
Daptomycin	-	-
Erythromycin	-	-
Gentamicin	-	-
Linezolid	>4	>4
Quinupristin/dalfopristin	-	>1
Teicoplanin	>2	>2
Tetracycline	-	-
Tigecycline	>0.5	>0.5
Vancomycin	>4	>4

S3.6 Polymerase chain reaction (PCR)

PCR was used to detect specific antibiotic resistance mechanisms in *E. coli* isolated from broilers and turkeys using selective media for selected *mcr* genes in colistin-resistant isolates. Colistin-resistant isolates underwent PCR following standard procedures.

S3.7 Whole Genome Sequencing (WGS)

WGS and *in silico* bioinformatic tools were used to detect the antibiotic resistance determinants present in the isolates recovered from MacConkey agar supplemented with 1mg/L cefotaxime, chromID OXA-48® and chromID CARBA® agars. These isolated exhibited ESBL, AmpC or carbapenem phenotypes obtained from broiler and turkey samples. 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 and Abricate pipeline (please see this and this paper). The AMR gene database includes acquired resistance genes and chromosomal genes, such as *ampC* promotor, where specific mutations lead to antibiotic resistance. The sequence type (ST) of the isolates were determined, from the WGS data, using multi-locus sequence typing (MLST) tool mlst.

Chapter S4 Methodology Susceptibility Testing for Clinical Surveillance

S4.1 Disc diffusion for England and Wales (APHA)

The method used for assessing the susceptibility to antibiotics is, unless otherwise stated in the report, the disc diffusion method formerly recommended by the British Society for Antimicrobial Chemotherapy (BSAC). 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.

Tests were performed (unless otherwise stated) by disc diffusion on Iso-Sensitest Agar (Oxoid) with appropriate media supplementation where necessary for fastidious organisms. The disc antibiotic concentrations used were as stated in Table S4.4, and a semi-confluent inoculum was used.

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 S4.4** which also provides the MIC corresponding to that zone diameter breakpoint, where this is known or has been estimated from APHA data. Breakpoints used to interpret the results from the antimicrobial susceptibility testing are reviewed on a regular basis. Data presented in the report and the supplementary material are retrospectively updated when required to reflect any changes to the interpretative criteria and to ensure consistency and comparability of the data.

Published breakpoints are not available for all animal species or for all of the bacterial/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 bacterial/antibiotic combinations. However, where most isolates of a particular bacterial species are either highly resistant or fully susceptible to an antibiotic, breakpoint issues may affect only a low number of isolates.

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.).

Isolates which were tested using the disc diffusion method have been described as having limited treatment options if they were found to be resistant to four or more individual antibiotics. Please note, cefalexin, cefotaxime, ceftazidime, cefpodoxime, ceftiofur, ciprofloxacin, nalidixic acid, colistin and enrofloxacin are included in the Antimicrobial Advice *ad hoc* Expert Group (AMEG) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. There are several antibiotic classes - such as carbapenems - that have been designated by the World Health Organisation (WHO) as human-only, and therefore are not categorised as HP-CIAs. Because of their importance to human medicines, these results are presented alongside HP-CIAs on the second graph.

Table S4.4: Disc diffusion breakpoints, corresponding MIC breakpoints and breakpoints under review for the main bacteria covered in the core data of this report in a) England and Wales, b) Northern Ireland and c) Scotland.

a) England and Wales

Please note that for 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.

Antibiotic	Disc charge (µg)	*Escherichia coli*, Enterobacteriaceae	*Salmonella*	*Staphylococci*	*Streptococci*
Amikacin (AK)	30	R ≤18 mm* R ≥16 mg/L*	R ≤18 mm* R ≥16 mg/L*	N/A	N/A
Amoxicillin/clavulanate (AMC)	20/10	R ≤14 mm* R >8 mg/L*	R ≤14 mm* R > 8mg/L*	N/A	N/A
Amoxicillin/clavulanate	2/1	N/A	N/A	R ≤17 mm* R >1 mg/L*	R ≤13 mm***
Ampicillin (AMP)	10	R ≤14 mm* R >8 mg/L*	R ≤14 mm* R >8 mg/L*	R ≤13 mm***	R ≤13 mm***
Apramycin (APR)	15	R ≤13 mm** R ≥32 mg/L**	R ≤13 mm** R ≥32 mg/L**	N/A	N/A
Cefalexin	30	R ≤15 mm* R >16 mg/L*	N/A	R ≤13 mm***	R ≤24 mm* R >2 mg/L*
Cefotaxime (CTX)	30	R ≤29 mm* R ≥2 mg/L*	R ≤29 mm* R ≥2 mg/L*	N/A	N/A

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

Antibiotic	Disc charge (µg)	*Escherichia coli*, Enterobacteriaceae	*Salmonella*	*Staphylococci*	*Streptococci*
Novobiocin	30	N/A	N/A	R ≤13 mm***	R ≤13 mm***
Penicillin	1IU	N/A	N/A	R ≤24 mm* R >0.12 mg/L*	R ≤16 mm* R ≤19 mm*▲ R >0.25 mg/L*
Spectinomycin	25	R ≤13 mm***	N/A	N/A	N/A
Streptomycin (S)	10	R ≤12 mm* R >8 mg/L*	R ≤13 mm R > ~8 mg/L	N/A	N/A
Sulfonamide compounds (S)	3/300	N/A	≤13 mm	N/A	N/A
Tetracycline (TE)	10	R ≤13 mm** R >8 mg/L**	R ≤13 mm R >8 mg/L	R ≤19 mm* R ≥2 mg/L*	R ≤23 mm* R ≤19 mm*▲ R ≥2 mg/L*
Trimethoprim/ sulfonamide (SXT)	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*
Tylosin	30	N/A	N/A	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

▲ Breakpoint for beta-haemolytic streptococci

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.
- *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.*

b) Northern Ireland

Antibiotic	Disc charge (µg)	Resistant (mm)	Intermediate (mm)	Susceptible (mm)
Amoxicillin (AMC)	30	≤13	14–17	≥18
Ampicillin (AMP)	10	≤13	14–16	≥17
Apramycin (APR)	15	N/A	N/A	N/A
Cefotaxime (CTX)	30	≤22	23–25	≥26
Ceftazidime (CAZ)	30	≤17	18–20	≥21
Chloramphenicol (C)	30	≤12	13–17	≥18
Ciprofloxacin (CIP)	5	≤15	16–20	≥21
Framycetin (FY)	100	N/A	N/A	N/A
Furazolidone (FR)	100	N/A	N/A	≥17
Gentamicin (CN)	10	≤12	13–14	≥15
Kanamycin (K)	30	≤13	14–17	≥18
Nalidixic acid (NA)	30	≤13	14–18	≥19
Spectinomycin (SH)	100	N/A	N/A	N/A
Streptomycin (S)	10	≤11	12–14	≥15
Sulfonamides (S)	3/300	≤12	13–16	≥17
Tetracycline (TE)	30	≤11	12–14	≥15
Trimethoprim (W)	5	≤10	11–15	≥16

(c) Scotland

Antibiotic	Disc charge (µg)	*Escherichia coli*, Enterobacteriaceae		*Salmonella*	
		Cattle and sheep (mm)	Pigs and poultry (mm)	Cattle and sheep (mm)	Pigs and poultry (mm)
Amoxicillin/clavulanate (AMC)	20/10	R ≤14 I ≤18	R ≤18	R ≤14 I ≤18	R ≤18
Ampicillin (AMP)	10	R ≤11 I ≤14	R ≤13	R ≤11 I ≤14	R ≤13
Apramycin (APR)	15	R ≤13 I ≤14	R ≤11 I ≤14	R ≤13 I ≤14	R ≤11 I ≤14
Cefotaxime (CTX)	30	R ≤17 I ≤19	N/A	N/A	N/A
Cefpodoxime (CPD)	10	R ≤ 19	N/A	R ≤ 19	N/A
Enrofloxacin (ENR)	5	R ≤16 I ≤20	R ≤16 I ≤22	R ≤16 I ≤20	R ≤16 I ≤22
Florfenicol (FFC)	30	R ≤12 I ≤17	R ≤18	R ≤12 I ≤17	R ≤18
Nalidixic acid (NA)	30	N/A	N/A	R ≤13	N/A
Neomycin (N)	10	R ≤19	R ≤14 I ≤16	R ≤19	R ≤14 I ≤16
Spectinomycin (SH)	25	R ≤14	-	R ≤14	-
	100	-	R ≤17 I ≤20	-	R ≤17 I ≤20
Streptomycin (S)	10	R ≤11 I ≤14	-	N/A	-
	25	-	R ≤10 I ≤14	-	R ≤10 I ≤14

Antibiotic	Disc charge (µg)	*Escherichia coli*, Enterobacteriaceae		*Salmonella*	
		Cattle and sheep (mm)	Pigs and poultry (mm)	Cattle and sheep (mm)	Pigs and poultry (mm)
Tetracycline (TE)	10	R ≤19	-	R ≤19	-
	30	-	R ≤11 I ≤14	-	R ≤11 I ≤14
Trimethoprim/ sulfonamide (SXT)	25	R ≤15	R ≤10 I ≤13	R ≤15	R ≤10 I ≤13

S4.2 Minimum Inhibitory Concentration (MIC) testing of veterinary pathogens

The Animal and Plant Health Agency (APHA) are transitioning antibiotic sensitivity testing for clinical surveillance from disc diffusion to the more robust determination of minimum inhibitory concentration (MIC) using the broth microdilution method. Table 4.1 in main report summaries the AST method employed for each pathogen.

The samples came from diagnostic submissions to the APHA and its partner laboratories in 2024. 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.

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 CA-SFM when these are not available; if veterinary breakpoints were not available, human CBPs were used (see Table S4.5). For some veterinary antibiotic and organism combinations, there are no published breakpoints available and, in these cases, resistance cannot be interpreted from MIC distribution.

Table S4.5: MIC breakpoints used for the interpretation of antibacterial susceptibility for veterinary pathogens from cattle, pigs, chickens and sheep. Cattle breakpoints were 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 a breakpoint for bovine isolates was used.

Antibiotic	***Pasteurella multocida* (mg/L)**			***Mannheimia haemolytica* (mg/L)**		***Actinobacillus pleuropneumoniae* (mg/L)**	***Bibersteinia trehalosi* (mg/L)**
	Cattle	**Pigs**	**Sheep**	**Cattle**	**Sheep**	**Pigs**	**Sheep**
Amoxicillin/clavulanate	R > 16**	R > 16**	R > 16**	R > 16**	R > 16**	N/A	R > 16**
Ampicillin	R > 1***	R > 1***	R > 1***	R > 1***	R > 1***	R ≥ 2*	R > 1***
Ceftiofur	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 8*
Doxycycline	R >8**	R >8**	R > 8**	R >8**	R > 8**	R > 8**	R > 8**
Enrofloxacin	R ≥ 2*	R ≥ 1*	R ≥ 2*	R ≥ 2*	R ≥ 2*	R ≥ 1*	R ≥ 2*
Florfenicol	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 8*
Gamithromycin	R ≥ 16*	R ≥ 16*	R ≥ 16*	R ≥ 16*	R ≥ 16*	N/A	R ≥ 16*
Spectinomycin	R ≥ 128*	R ≥ 128*	R ≥ 128*	R ≥ 128*	R ≥ 128*	N/A	R ≥ 128*
Tetracycline	R ≥ 8*	R ≥ 2*	R ≥ 8*	R ≥ 8*	R ≥ 8*	R ≥ 2*	R ≥ 8*
Tiamulin	N/A	N/A	N/A	N/A	N/A	R ≥ 32*	N/A

Antibiotic	*Pasteurella multocida* (mg/L)			*Mannheimia haemolytica* (mg/L)		*Actinobacillus pleuropneumoniae* (mg/L)	*Bibersteinia trehalosi* (mg/L)
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*
Trimethoprim/ sulfonamide	R > 8**	R > 8**	R > 8**	R > 8**	R > 8**	R > 8**	R > 8**
Tulathromycin	R ≥ 64*	R ≥ 64*	R ≥ 64*	R ≥ 64*	R ≥ 64*	S ≤ 64*	R ≥ 64*

Key:

- * CLSI veterinary clinical breakpoint
- ** CA-SFM 2024 veterinary clinical breakpoint
- *** EUCAST human breakpoint

b) Other pathogens

Antibiotic	*Streptococcus suis* (mg/L) Pigs	*Brachyspira hyodysenteriae* (mg/L) Pigs
Ceftiofur	R > 8*	N/A
	S ≤ 2*	N/A
Doxycycline	R > 1***	R > 2****
	S ≤ 0.25***	N/A
Enrofloxacin	R > 2*	N/A
	S ≤ 0.5*	N/A
Erythromycin	R > 1*	N/A
	S ≤ 0.25*	N/A

Antibiotic	*Streptococcus suis* (mg/L)	*Brachyspira hyodysenteriae* (mg/L)
Florfenicol	R > 8*	N/A
	S ≤ 2*	N/A
Lincomycin	R > 8**	R > 8****
	S ≤ 2**	N/A
Penicillin	R > 1*	N/A
	S ≤ 0.25*	N/A
Tetracycline	R > 2*	N/A
	S ≤ 0.5*	N/A
Tiamulin	N/A	R > 2****
Trimethoprim/sulfonamide	R > 2***	N/A
	S ≤ 1***	N/A
Tylosin	N/A	R > 8****
Tylvalosin	N/A	R > 8****

Key:

* CLSI veterinary clinical breakpoint
** CA-SFM 2024 veterinary clinical breakpoint
*** EUCAST human breakpoint
**** Suggested broth microdilution clinical breakpoints are considered to be one dilution lower than clinical breakpoints for agar dilution

S4.3 Disc Diffusion for Scotland

Please note that the methodology for susceptibility testing used by Scotland's Rural College Veterinary Services (SRUC) is detailed in the Scottish One Health Antimicrobial Use and Antimicrobial Resistance (SONAAR) report.

S4.4 Disc Diffusion for the Vale Veterinary Laboratory: key mastitis pathogens

The methods used to determine antimicrobial susceptibility, are based on those in CLSI Vet01 July 2013[1]. 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 S4.6 below.

Table S4.6: Disc diffusion breakpoints applied by Vale Veterinary Laboratories for the interpretation of resistance of bovine mastitis pathogens in millimetres.

Antibiotic	*Escherichia coli* (mm)	*Staphylococcus aureus* (mm)	*Streptococcus dysgalactiae* (mm)	*Streptococcus uberis* (mm)
Amoxicillin/clavulanate	R <19	R <20	N/A	N/A
Ampicillin	R <14	R <13 I <17	R <24	R <24
Cefapirin	R <14 I <18	R <14 I <18	R <14 I <18	R <14 I <18
Cloxacillin	N/A	R <18	R <18	R <18
Neomycin	R <11	R <14	N/A	N/A
Oxytetracycline	R <11 I <15	R <14 I <19	N/A	N/A
Penicillin	N/A	R <18	R <18	R <18
Spectinomycin	R <20	R <20	N/A	N/A
Trimethoprim/ sulfonamide	R <13	R <14	R <15	R <15

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

S4.5 MIC testing of trout pathogens by Cefas

For trout, Clinical and Laboratory Standards Institute (CLSI 2020) MIC protocols were used to test *Aeromonas salmonicida* and *Yersinia ruckeri*. Where available the CLSI epidemiological cut-off values were used. However, published cut-off values are not available for all combinations of bacteria and antibiotics. For these combinations, the normalised resistance interpretation (NRI) method was chosen to determine the wild type cut-off value (COwt), which does not necessarily imply clinical resistance.

Table S4.7: MIC breakpoints used for the interpretation of antibacterial susceptibility for trout pathogens.

Antibiotic	*A. salmonicida* (mg/L)	*Y. ruckeri* (mg/L)
Gentamicin	≥ 4*	≥ 4
Oxolinic acid	≥ 0.25	≥ 0.125
Sulfamethoxazole±	≥ 16*	≥ 128*
Ampicillin	≥ 4*	≥ 16
Ceftazidime	≥ 0.5*	≥ 0.25*
Trimethoprim / Sulfamethoxazole±	≥ 0.125*	≥ 0.25*
Meropenem	≥ 0.0625*	≥ 0.125*
Enrofloxacin	≥ 0.125*	≥ 0.03125
Florfenicol	≥ 8	≥ 16
Oxytetracycline	≥ 2	≥ 4

±dihydrofolate reductase inhibitors and combinations
* internal wild type cut-off value (CO_{wt}) using the normalized resistance interpretation (NRI) method described by Kronvall & Smith (2016).
- Cut-off values not available. Not enough data to generate CO_{wt}.