

Supplementary Material 2 – Resistance Methods and Data

UK-VARSS 2023

Published November 2024



© Crown copyright 2024

You may re-use this information (excluding logos) free of charge in any format or medium, under the terms of the Open Government Licence v.3. To view this licence visit <u>www.nationalarchives.gov.uk/doc/open-government-licence/version/3/</u> or email <u>PSI@nationalarchives.gov.uk</u>.

Suggested citation: UK-VARSS (2023). *Veterinary Antibiotic Resistance and Sales Surveillance Report (UK-VARSS 2023)*. New Haw, Addlestone: Veterinary Medicines Directorate.

This publication is available <u>online</u>. Any enquiries or correspondence regarding this publication should be sent to us at: <u>postmaster@vmd.gov.uk</u>.

Published on 19th November 2024

Contents

Chapter 39
S1.1: Harmonised monitoring requirements7
Table S1.1.1: Summary of monitoring requirements in the UK from 2014 to 2023 bysampling year
S1.2: Harmonised monitoring methodology8
S1.3: PATH-SAFE methodology8
S1.4: Using selective media to detect resistant8
S1.5: Antibiotic Susceptibility Testing (AST)8
S1.6: Whole Genome Sequencing (WGS)9
S1.7: Polymerase chain reaction (PCR)9
S1.8: Interpretation10
S2.1: Harmonised monitoring results of susceptibility testing in <i>Escherichia coli</i>
Table S2.1.1: Susceptibility in <i>E. coli</i> interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy pigs at slaughter in the UK
Table S2.1.2: Distribution of ESBL and AmpC and CPE enzymes detected in <i>E. coli</i> isolated using selective agar from healthy pigs at slaughter in the UK in 2023
Table S2.1.3: Decreased susceptibility interpreted using EUCAST ECOFFs unless otherwise indicated in ESBL-/AmpC-producing <i>E. coli</i> isolated using selective agar from caecal samples from healthy pigs at slaughter in the UK in 2023
Table S2.1.4: Distribution of carbapenemase enzymes detected in <i>E. coli</i> isolated using selective agar from healthy pigs at slaughter in the UK in 2023
Table S2.1.5: Decreased susceptibility interpreted using EUCAST ECOFFs unless otherwise indicated in carbapenemase-producing <i>E. coli</i> isolated using selective agar from caecal samples from healthy pigs at slaughter in the UK for 2023
Table S2.1.6: Decreased susceptibility interpreted using EUCAST ECOFFs unless otherwise indicated in colistin resistant <i>E. coli</i> isolated using selective agar from caecal samples from healthy pigs at slaughter in GB for 2023

S2.2: Harmonised monitoring results of susceptibility testing in *Enterococcus* spp. 22

Table S2.2.1: Susceptibility in *E. faecalis* interpreted using both EUCAST a) ECOFFs Table S2.2.2: Susceptibility in *E. faecium* interpreted using both EUCAST a) ECOFFs and b) CBPs from caecal samples from healthy pigs at slaughter in the GB23 Table S2.3.1: Susceptibility in Salmonella spp. interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy pigs S2.4: Harmonised monitoring results of susceptibility testing in Campylobacter spp. 27 Table S2.4.1: Susceptibility in *C. coli* interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy pigs at slaughter in Table S2.5.1: Susceptibility in *E. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy beef cattle at Table S2.5.2: Susceptibility in *E. faecalis* interpreted using both EUCAST a) ECOFFs Table S2.5.3: Susceptibility in *E. faecium* interpreted using both EUCAST a) ECOFFs Table S2.5.4: Susceptibility in *C. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy beef cattle at Table S2.5.5: Susceptibility in *C. jejuni* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy beef cattle at Table S2.6.1: Susceptibility in *E. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy sheep at slaughter Table S2.6.2: Distribution of ESBL/AmpC and CPE enzymes detected in *E. coli* isolated using selective agar from healthy sheep at slaughter in England and Wales in 2023. 34

	Table S2.6.3: Decreased susceptibility in ESBL-/AmpC-producing <i>E. coli</i> isolated usingselective agar from caecal samples from healthy sheep at slaughter in England andWales in 2023.35
	Table S2.6.4: Susceptibility in <i>E. faecalis</i> interpreted using both EUCAST a) ECOFFsand b) CBPs from caecal samples from healthy sheep at slaughter in England andWales36
	Table S2.6.5: Susceptibility in <i>E. faecium</i> interpreted using both EUCAST a) ECOFFsand b) CBPs from caecal samples from healthy sheep at slaughter in England andWales37
	Table S2.6.6: Susceptibility in Salmonella spp. interpreted using both EUCAST a)ECOFFs and b) CBPs unless otherwise stated from caecal samples from healthy sheepat slaughter in England and Wales
	Table S2.6.7: Susceptibility in <i>C. coli</i> interpreted using EUCAST a) ECOFFs and b)CBPs unless otherwise indicated from caecal samples from healthy sheep at slaughterin England and Wales39
	Table S2.6.8: Susceptibility in <i>C. jejuni</i> interpreted using EUCAST a) ECOFFs and b)CBPs unless otherwise indicated from caecal samples from healthy sheep at slaughterin England and Wales40
S	2.7 PATH-SAFE results of susceptibility testing from the bulk milk survey
	Table S2.7.1: Susceptibility in <i>E. coli</i> interpreted using EUCAST a) ECOFFs and b)CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle inGB41
	Table S2.7.2: Distribution of ESBL/AmpC and CPE enzymes detected in <i>E. coli</i> isolated using selective agar from bulk milk samples from healthy dairy cattle in GB in 2023.42
	Table S2.7.3: Decreased susceptibility in ESBL-/AmpC-producing <i>E. coli</i> isolated using selective agar from bulk milk samples from healthy dairy cattle in GB in 202343
	Table S2.7.4: Susceptibility in <i>E. faecalis</i> interpreted using both EUCAST a) ECOFFs and b) CBPs from bulk milk samples from healthy dairy cattle in GB
	Table S2.7.5: Susceptibility in <i>E. faecium</i> interpreted using both EUCAST a) ECOFFs
	and b) CBPs from bulk milk samples from healthy dairy cattle in GB45
	and b) CBPs from bulk milk samples from healthy dairy cattle in GB
	Table S2.7.6: Susceptibility in <i>Klebsiella spp.</i> interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy

Table S2.7.8: Susceptibility in methicillin-resistant <i>S. aureus</i> (MRSA) interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle in GB49
Table S2.7.9: Susceptibility in <i>Streptococcus spp.</i> interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle in GB
Chapter 453
S3.1: Methodology susceptibility testing53
S3.1.1 Core data53
Table S3.1.1.1: Disc diffusion breakpoints, corresponding MIC breakpoints and breakpoints under review for the main bacteria covered in the core data of this report in a) England and Wales, b) Northern Ireland and c) Scotland
S3.1.2 Private Laboratory Initiative61
S3.1.3 MIC testing of veterinary pathogens62
Table S3.1.3.1: MIC breakpoints used for the interpretation of antibacterial susceptibility for veterinary pathogens from cattle, pigs, chickens and sheep
S4.1: Clinical surveillance data for isolates of zoonotic pathogens from all species66
Table S4.1.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all <i>E. coli</i> from pigs, chickens, turkeys, cattle and sheep (combined) in England and Wales, Northern Ireland and Scotland in 2023
Table S4.1.2: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all <i>Salmonella</i> from cattle, pigs, sheep, chickens and turkeys (combined) in England and Wales, Northern Ireland and Scotland in 2023
Table S4.1.3: Findings of LA-MRSA by government laboratories for England and Wales in 2023
S4.2: Clinical surveillance data for isolates from pigs68
Table S4.2.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all <i>E. coli</i> from pigs (all ages) in England and Wales, Northern Ireland and Scotland in 2023
Table S4.2.2: Resistance (and interpreted using clinical breakpoints) in <i>E. coli</i> from pigs in a) England and Wales, b) Northern Ireland and c) Scotland for 2023, split by age category

Table S4.2.4: Resistance (tested by broth microdilution and MIC values interpreted usingclinical breakpoints) of *Brachyspira hyodysenteriae* from infections of pigs in Englandand Wales in 202370

Table S4.2.7: Resistance (tested by broth microdilution and MIC values interpreted using
clinical breakpoints) of *Streptococcus suis* from infections of pigs in England and Wales
in 202372

S4.3: Clinical surveillance data for isolates from poultry72

Table S4.3.2: Resistance (tested by disc diffusion and interpreted using breakpoints) inall *E. coli* from turkeys (all ages) in England and Wales, Northern Ireland and Scotland in202373

Table S4.3.3: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *Salmonella* from chickens and turkeys (all ages) in England and Wales and Scotland in 2023. In Northern Ireland only chickens were tested in 2023.74

Table S4.3.4: Resistance (tested by disc diffusion and interpreted using clinicalbreakpoints) of *Klebsiella pneumoniae* from respiratory infections of chickens in Englandand Wales in 202375

Table S4.3.5: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Erysipelothrix rhusiopathiae*, *Staphylococcus aureus* and *Staphylococcus xylosus* from infections of chickens in England and Wales in 2023..75

Table S4.4.2: Resistance (tested by disc diffusion and interpreted using clinicalbreakpoints) of Staphylococci and Streptococci from mastitis cases from England andWales in 202376	
Table S4.4.3: Resistance (tested by disc diffusion and interpreted clinical using breakpoints) of Klebsiella pneumoniae, Pseudomonas aeruginosa and Trueperella pyogenes from mastitis cases from England and Wales in 2023	
Table S4.4.4: Resistance (tested by disc diffusion and interpreted using clinicalbreakpoints) in all <i>E. coli</i> from cattle (all ages) in England and Wales, Northern Irelandand Scotland in 2023	
Table S4.4.5: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in <i>E. coli</i> from cattle in a) England and Wales, b) Northern Ireland and c) Scotland for 2023, split by age category	
Table S4.4.6: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all <i>Salmonella</i> from cattle (all ages) in England and Wales, Northern Ireland and Scotland in 2023	
Table S4.4.7 Resistance (tested by broth microdilution and MIC values interpreted using clinical breakpoints) of <i>Mannheimia haemolytica</i> and <i>Pasteurella multocida</i> from respiratory infections of cattle in England and Wales in 2023	-
Table S4.4.8: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of <i>Histophilus somni</i> from respiratory infections of cattle in England and Wales in 2023	
Table S4.4.9: Resistance (tested by disc diffusion and interpreted using clinicalbreakpoints) of Listeria monocytogenes and Staphylococcus xylosus from infections ofcattle in England and Wales in 2023	
S4.5: Clinical surveillance data for isolates from sheep	
Table S4.5.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all <i>E. coli</i> from sheep (all ages) in England and Wales, Northern Ireland and Scotland in 2023	
Table S4.5.2: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in <i>E. coli</i> from sheep in a) England and Wales, b) Northern Ireland and c) Scotland from 2023, split by age category	
Table S4.5.3: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all <i>Salmonella</i> from sheep (all ages) in England and Wales, Northern Ireland and Scotland in 2023	

and 84
ctions 85
85
86
86
87
d using nd 87

S1.1: Harmonised monitoring requirements

Table S1.1.1: Summary of monitoring requirements in the UK from 2014 to 2023 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
	Carcasses	Broilers and turkeys	x		x		x		x			
Salmonella spp.	NCP	Broilers, turkeys and layers	x		x		x		x		x	
	Carcasses	Pigs		x		x		x				
	Caeca	Figs								x		x
Escherichia coli	Caeca	Broilers and turkeys	x		x		x		x		x	
	Caeca	Pigs		x		x		x		x		x
ESBL-, AmpC- or		Broilers and turkeys			x		x		x		x	
carbapenemase- producing <i>E. coli</i>	Caeca	Pigs		x		x		x		x		x
Compulabootor coli	Caeca	Broilers and turkeys									x	
Campylobacter coli	Caeca	Pigs										x
Campylobacter jejuni	Caeca	Broilers and turkeys	x		x		x		x		x	
Enterococcus	Саеса	Broilers and turkeys									x	
faecalis	Caeca	Pigs										x
Enterococcus	Casas	Broilers and turkeys									x	
faecium	Caeca	Pigs										x



S1.2: Harmonised monitoring methodology

Samples of faecal content were taken from healthy pigs at slaughter by Food Standards Agency (FSA) personnel and sampled for *Escherichia coli*, enterococci *Salmonella* and *Campylobacter coli* in accordance with <u>EU Decision 2020/1729</u>. The sampling plan was randomised, stratified, and weighted by slaughter throughput. Samples were collected from the biggest slaughterhouses covering 81% of the UK pig throughput in 2023. One caecal sample was collected per epidemiological unit (slaughter batch).

All countries within the UK were included in the sampling frame and contributed isolates from each of *E. coli, Salmonella* and *C. coli*. Isolates of *Enterococcus faecium* and *E. faecalis* were not taken from Northern Ireland. Caecal samples were cultured for *E. coli, Enterococcus* spp., *Salmonella* and *C. coli* using appropriate media.

S1.3: PATH-SAFE methodology

Samples of faecal content were taken from healthy sheep and beef cattle at slaughter by Food Standards Agency (FSA) and Food Standards Scotland (FSS) personnel and sampled for *Escherichia coli*, enterococci, *Campylobacter coli*, *C. jejuni* and *Salmonella* (for sheep only). For dairy cattle, samples of raw bulk milk were provided for testing by the National Milk Records (NMR). Raw bulk milk was sampled for *E. coli*, enterococci, streptococci, *Staphylococcus aureus* and *Klebsiella* spp. For these surveys, sheep caecal samples were provided from England and Wales. Beef cattle caecal samples and dairy cattle raw bulk milk samples were sourced from across Great Britain (GB).

For sheep and beef cattle the sampling plan was randomised, stratified, and weighted by slaughter throughput. As these surveys relied upon voluntary participation from abattoirs in a limited time period, samples were collected from slaughterhouses covering 27% of slaughterhouse throughput for beef and 28% for sheep. For bulk milk, 70% of milk produced on dairy cattle farms in the UK is processed by <u>nine companies</u>. In 2022, NMR arranged consent from one of these companies, who provided access to the dairy cattle farms spread across GB to act as the source population. This represented ~28% of the target population of dairy cattle farms actively contributing to GB milk production when sampling commenced.

None of the PATH-SAFE surveys were conducted over a full calendar year, due to timeline and funding constraints within the programme. Beef cattle and sheep were sampled over a 9-month period, whilst sampling for dairy cattle was conducted over 10 months.

S1.4: Using selective media to detect resistant

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 multiply, making them easier to detect.



Caecal and bulk milk samples were cultured for ESBL- and AmpC- producing *E. coli* following standard <u>procedures</u>. This included a pre-enrichment step followed by inoculation of samples onto MacConkey agar plates supplemented with 1 mg/L cefotaxime. 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 \leq 8 mg/L; and susceptibility to meropenem MIC \leq 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 \leq 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; showing synergy with cefotaxime and clavulanate and/or ceftazidime; no synergy may be a mg/L; and susceptibility to meropenem MIC \leq 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; showing synergy with cefotaxime and clavulanate and/or ceftazidime and clavulanate; reduced susceptibility to cefoxitin MIC > 8 mg/L; and susceptibility to meropenem MIC \leq 0.12 mg/L.

Carbapenemase-producing *E. coli* were also cultured for from caecal and bulk milk samples following standard procedures, as above for ESBL- and AmpC- producing *E. coli*. Following pre-enrichment the samples were inoculated onto chromID OXA-48® and chromID CARBA® agars. An *E. coli* with a carbapenemase-phenotype was defined as: having an MIC of >0.12 mg/L to meropenem.

Pig caecal samples were additionally cultured onto agar containing colistin and a subset of samples cultured onto agar containing amikacin.

Methicillin-resistant *Staphylococcus aureus* (MRSA) were cultured for from bulk milk samples. Following pre-enrichment the samples were inoculated onto Brilliance MRSA 2 agar.

S1.5: Antibiotic Susceptibility Testing (AST)

AST was carried out by the national reference laboratories (NRLs) using European Committee on Antimicrobial Susceptibility Testing (EUCAST) methodology. Single typical colonies were selected for speciation and susceptibility testing. Standardised broth microdilution was used to determine the minimum inhibitory concentration (MIC) against a panel of antibiotics as listed in <u>Decision (EU) 2020/1729</u> and <u>EFSA</u> guidelines, or where not available a panel of antibiotics following joint APHA/VMD <u>recommendations</u>. Tables of antibiotic panels and their corresponding cut-off values can be seen in **Tables S1.7.1 (a) to (f)** and **Tables S1.7.2 (a) to (f)**.

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

S1.6: Whole Genome Sequencing (WGS)

WGS and *in silico* bioinformatic tools were used to detect the antibiotic resistance determinants present in the isolates with ESBL, AmpC or carbapenem phenotypes



detected from pigs and sheep, and bulk milk samples. Similar isolates from beef cattle are undergoing WGS in 2024.

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 <u>this</u> and <u>this</u> paper). The sequences of *E. coli* isolates which expressed the ampC phenotype but were negative for all known AmpC-encoding genes were investigated for promoter mutations in *ampC*, using the APHA SeqFinder pipeline. These mutations are compatible with increased expression of chromosomal *E. coli ampC*.

S1.7: Polymerase chain reaction (PCR)

PCR was used to detect specific antibiotic resistance mechanisms in *E. coli* isolated from pigs using selective media: the *rmtB* gene present in amikacin-resistant isolates and selected *mcr* genes in colistin-resistant isolates. Amikacin-resistant isolates underwent inhouse real-time PCR using primers designed by <u>Doi and Arakawa</u>. Colistin-resistant isolates underwent PCR following standard <u>procedures</u>.

S1.8: 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. 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 01/09/2024) were used to interpret the MIC values. EUCAST cut-off values are regularly under review and updated as new values and drug/bacteria species combinations are determined. Where no EUCAST values were available, the EFSA recommended cut-off 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 2023.

For ease of comparison, both the ECOFF and corresponding EUCAST CBP values are presented in **Tables S1.8.1 (a) to (f)** and **Tables S1.8.2 (a) to (f)**.



Table S1.8.1: The ECOFF values applied when determining susceptibility of a) *E. coli* and *Salmonella*, b) *Campylobacter* spp., c) *Enterococcus* spp., d) *Klebsiella* spp., e) *Staphylococcus aureus* and f) *Streptococcus* spp. isolated from healthy pigs, beef cattle and sheep at slaughter, and from bulk milk samples. 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.

Antibiotic	<i>E. coli</i> (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 & clavulanic acid	>0.25*	N/A
Cefoxitin	>16*	N/A
Ceftazidime	>1*	>2*
Ceftazidime & clavulanic acid	>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*	-
Tetracycline	>8*	>8*
Tigecycline	>0.5*	>0.5**
Trimethoprim	>2*	>2**

a) E. coli and Salmonella



b) Campylobacter spp.

Antibiotic	<i>C. coli</i> (mg/L)	<i>C. jejuni</i> (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	<i>E. faecali</i> s (mg/L)	<i>E. faecium</i> (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*

d) Klebsiella spp.

Antibiotic	<i>K. oxytoca</i> (mg/L)	<i>K. pneumonia</i> e (mg/L)
Amikacin	>8*	>8*
Ampicillin	N/A	N/A
Cefotaxime	>0.125*	>0.25*
Ceftazidime	>0.5*	>1*
Chloramphenicol	N/A	N/A
Ciprofloxacin	>0.06*	>0.125*
Colistin	>2*	>2*
Gentamicin	>2*	>2*
Meropenem	>0.125*	>0.125*
Tetracycline	>4*	>8*
Tigecycline	>1*	>2*
Trimethoprim	N/A	>2**



e) Staphylococcus aureus

Antibiotic	<i>S. aureus</i> (mg/L)
Cefoxitin	>4*
Chloramphenicol	>16*
Ciprofloxacin	>2*
Clindamycin	>0.25*
Erythromycin	>1*
Fusidate	>0.5*
Gentamicin	>2*
Kanamycin	>8***
Linezolid	>4*
Mupirocin	>1*
Penicillin	>0.125*
Rifampin	>0.03*
Streptomycin	>16*
Sulfamethoxazole	>125**
Quinupristin/dalfopristin	>1*
Tetracycline	>1*
Tiamulin	>2**
Trimethoprim	>2*
Vancomycin	>2*

f) Streptococcus spp.

Antibiotic	S. dysgalactiae (mg/L)	S. uberis (mg/L)
Doxycycline	>0.5***	>0.5*
Enrofloxacin	>2*	>2*
Erythromycin	>0.125*	>0.25*
Florfenicol	>4*	>4*
Lincomycin	>1*	>0.5*
Penicillin	N/A	>0.125***
Tetracycline	N/A	>1*
Trimethoprim/sulfamethoxazole	>0.25*	>1*
Tylosin	>1***	>4***

Key:

* EUCAST ECOFF

** EFSA-recommended ECOFF

*** EUCAST tentative ECOFF



Table S1.8.2: The EUCAST clinical breakpoint (CBP) values applied when determining susceptibility of a) *E. coli* and *Salmonella*, b) *Campylobacter* spp., c) *Enterococcus* spp., d) *Klebsiella* spp., e) *Staphylococcus aureus* and f) *Streptococcus* spp. isolated from healthy pigs, beef cattle and sheep at slaughter, and from bulk milk samples. 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	<i>E. coli</i> (mg/L)	Salmonella (mg/L)
Amikacin	>8	>16
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	<i>C. coli</i> (mg/L)	<i>C. jejuni</i> (mg/L)
Chloramphenicol	-	-
Ciprofloxacin	>0.5	>0.5
Ertapenem	-	-
Erythromycin	>8	>4
Gentamicin	-	-
Tetracycline	>2	>2

14

c) Enterococcus spp.

Antibiotic	<i>E. faecalis</i> (mg/L)	<i>E. faecium</i> (mg/L)
Ampicillin	>8	>8
Chloramphenicol	-	-
Ciprofloxacin	>4	>4
Daptomycin	-	-
Erythromycin	-	-
Gentamicin	-	-
Linezolid	>4	>4
Quinupristin/dalfopristin	-	>1
Teicoplanin	>2	>2
Tetracycline	-	-
Tigecycline	>0.25	>0.25
Vancomycin	>4	>4

d) Klebsiella spp.

Antibiotic	<i>K. oxytoca</i> (mg/L)	<i>K. pneumoniae</i> (mg/L)
Amikacin	>8	>8
Ampicillin	>8	>8
Cefotaxime	>2	>2
Ceftazidime	>4	>4
Chloramphenicol	>16	>16
Ciprofloxacin	>0.5	>0.5
Colistin	>2	>2
Gentamicin	>2	>2
Meropenem	>8	>8
Tetracycline	-	-
Tigecycline	-	-
Trimethoprim	>4	>4

e) Staphylococcus aureus

Antibiotic	<i>S. aureus</i> (mg/L)
Cefoxitin	>4
Chloramphenicol	-
Ciprofloxacin	>2
Clindamycin	>0.25
Erythromycin	>1
Fusidate	>1
Gentamicin	>2
Kanamycin	-
Linezolid	>4
Mupirocin	-
Penicillin	>0.125
Rifampin	>0.06
Streptomycin	-
Sulfamethoxazole	-
Quinupristin/dalfopristin	>1
Tetracycline	>1
Tiamulin	-
Trimethoprim	>4
Vancomycin	>2

f) Streptococcus spp.

Antibiotic	S. dysgalactiae (mg/L)	S. uberis (mg/L)
Doxycycline	>1	>1
Enrofloxacin	-	-
Erythromycin	>0.25	>0.25
Florfenicol	-	-
Lincomycin	-	-
Penicillin	>0.25	>0.25
Tetracycline	>1	>1
Trimethoprim/sulfamethoxazole	>2	>2
Tylosin	-	-

S2.1: Harmonised monitoring results of susceptibility testing in *Escherichia coli*

Please note, cefotaxime, ceftazidime, ciprofloxacin, colistin and nalidixic acid are included in the Antimicrobial Advice *ad hoc* Expert Group (<u>AMEG</u>) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. For individuals using screen readers, please note that cells read out as blank denote that no isolates were tested, or that no data is available.

Table S2.1.1: Susceptibility in *E. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy pigs at slaughter in the UK. This table shows the number and percentage of isolates with higher resistance levels than expected to background levels for that species for 2015, 2017, 2019, 2021 and 2023.

Antibiotic	2015 (n=150)	2017 (n=186)	2019 (n=208)	2021 (n=237)	2023 (n=170)
Amikacin	-	-	-	0	0
Ampicillin	57 (38.0)	57 (30.6)	75 (36.1)	79 (33.3)	55 (32.4)
Azithromycin*	2 (1.3)	0	1 (0.5)	2 (0.8)	2 (1.2)
Cefotaxime	0	0	5 (2.4)	3 (1.3)	2 (1.2)
Ceftazidime	0	0	5 (2.4)	3 (1.3)	2 (1.2)
Chloramphenicol	47 (31.3)	38 (20.4)	34 (16.3)	44 (18.6)	19 (11.2)
Ciprofloxacin	4 (2.7)	5 (2.7)	7 (3.4)	11 (4.6)	8 (4.7)
Colistin	0	0	0	0	0
Gentamicin	11 (7.3)	7 (3.8)	3 (1.4)	5 (2.1)	4 (2.4)
Meropenem	0	0	0	0	0
Nalidixic acid	2 (1.3)	4 (2.2)	2 (1.0)	4 (1.7)	3 (1.8)
Sulfamethoxazole	87 (58.0)	88 (47.3)	89 (42.8)	96 (40.5)	51 (30.0)
Tetracycline	108 (72.0)	110 (59.1)	122 (58.7)	125 (52.7)	73 (42.9)
Tigecycline	1 (0.7)	1 (0.5)	1 (0.5)	0	0
Trimethoprim	73 (48.7)	68 (36.6)	83 (39.9)	89 (37.6)	44 (25.9)

a) ECOFFs

์ 17

Antibiotic	2015 (n=150)	2017 (n=186)	2019 (n=208)	2021 (n=237)	2023 (n=170)
Amikacin	-	-	-	0	0
Ampicillin	57 (38.0)	57 (30.6)	75 (36.1)	79 (33.3)	55 (32.4)
Azithromycin	-	-	-	-	-
Cefotaxime	0	0	3 (1.4)	3 (1.3)	2 (1.2)
Ceftazidime	0	0	2 (1.0)	2 (0.8)	0
Chloramphenicol	48 (32.0)	43 (23.1)	38 (18.3)	60 (25.3)	19 (11.2)
Ciprofloxacin	1 (0.7)	3 (1.6)	1 (0.5)	3 (1.3)	0
Colistin	0	0	0	0	0
Gentamicin	10 (6.7)	7 (3.8)	3 (1.4)	5 (2.1)	4 (2.4)
Meropenem	0	0	0	0	0
Nalidixic acid	-	-	-	-	-
Sulfamethoxazole	-	-	-	-	-
Tetracycline	-	-	-	-	-
Tigecycline	1 (0.7)	1 (0.5)	1 (0.5)	0	0
Trimethoprim	73 (48.7)	67 (36.0)	83 (39.9)	89 (37.6)	44 (25.9)

Table S2.1.2: Distribution of ESBL and AmpC and CPE enzymes detected in *E. coli* isolated using selective agar from healthy pigs at slaughter in the UK in 2023. Note - if more than one isolate was of an unknown sequence type (ST), it has been assumed that they belonged to different STs.

Enzyme	Number of isolates	Proportion of isolates (n=76) (%)	Proportion of caecal samples (n=336) (%)	Number of unique STs	Sequence type (ST)
AmpC promoter	35	46.1	10.4	12	23, 75, 88, 156, 212, 348, 603, 641, 2628, 15008, unknown, Novel 4
CMY-2	3	3.9	0.9	3	38, 542, 8977
CTX-M-1	18	23.7	5.4	11	20, 23, 57, 88,101, 117,156,685,2536, Novel 1, Novel 3
CTX-M-14	3	3.9	0.9	3	369, 410, 5909
CTX-M-15	10	13.2	3.0	6	58, 117, 616, 1408, 1722, unknown
CTX-M-55	1	1.3	0.3	1	224
DHA-1	2	2.6	0.6	2	2165, Novel 2
SHV-12	1	1.3	0.3	1	58
TEM-52b	3	3.9	0.9	1	58

Table S2.1.3: Decreased susceptibility interpreted using EUCAST ECOFFs unless otherwise indicated in ESBL-/AmpC-producing *E. coli* isolated using selective agar from caecal samples from healthy pigs at slaughter in the UK in 2023.

Antibiotic	Number of isolates with MIC>ECOFF	Proportion of isolates (%) (n=76)	Total proportion from caecal samples (%) (n=336)
Amikacin	0	0	0
Ampicillin	76	100	22.6
Azithromycin*	5	6.6	1.5
Cefepime	40	52.6	11.9
Cefotaxime	76	100	22.6
Cefotaxime & clavulanic acid	41	53.9	12.2
Cefoxitin	35	46.1	10.4
Ceftazidime	58	76.3	17.3
Ceftazidime & clavulanic acid	38	50	11.3
Chloramphenicol	9	11.8	2.7
Ciprofloxacin	19	25.0	5.7
Colistin	0	0	0
Ertapenem*	3	3.9	0.9
Gentamicin	5	6.6	1.5
Imipenem	0	0	0
Meropenem	0	0	0
Nalidixic acid	2	2.6	0.6
Sulfamethoxazole	53	69.7	15.8
Temocillin	0	0	0
Tetracycline	29	38.2	8.6
Tigecycline	0	0	0
Trimethoprim	41	53.9	12.2



Table S2.1.4: Distribution of carbapenemase enzymes detected in *E. coli* isolated using selective agar from healthy pigs at slaughter in the UK in 2023. Note - if more than one isolate was of an unknown sequence type (ST), it has been assumed that they belonged to different STs.

Enzyme	Number of isolates	Proportion of isolates (n=1) (%)	Proportion of caecal samples (n=336) (%)	Number of unique STs	Sequence type (ST)
OXA-48	1	100	0.3	1	38

Table S2.1.5: Decreased susceptibility interpreted using EUCAST ECOFFs unless otherwise indicated in carbapenemase-producing *E. coli* isolated using selective agar from caecal samples from healthy pigs at slaughter in the UK for 2023.

Antibiotic	Number of isolates with MIC>ECOFF	Proportion of isolates (%) (n=1)	Total proportion from caecal samples (%) (n=336)
Amikacin	0	0	0
Ampicillin	1	100	0.3
Azithromycin*	1	100	0.3
Cefepime	1	100	0.3
Cefotaxime	1	100	0.3
Cefotaxime & clavulanic acid	1	100	0.3
Cefoxitin	0	0	0
Ceftazidime	0	0	0
Ceftazidime & clavulanic acid	0	0	0
Chloramphenicol	0	0	0
Ciprofloxacin	1	100	0.3
Colistin	0	0	0
Ertapenem*	1	100	0
Gentamicin	0	0	0
Imipenem	1	100	0.3
Meropenem	1	100	0.3
Nalidixic acid	1	100	0.3
Sulfamethoxazole	1	100	0.3
Temocillin	1	100	0.3
Tetracycline	1	100	0.3
Tigecycline	0	0	0
Trimethoprim	1	100	0.3



Table S2.1.6: Decreased susceptibility interpreted using EUCAST ECOFFs unless otherwise indicated in colistin resistant *E. coli* isolated using selective agar from caecal samples from healthy pigs at slaughter in GB for 2023.

Antibiotic	Number of isolates with MIC>ECOFF	Proportion of isolates (%) (n=1)	Total proportion from caecal samples (%) (n=304)
Amikacin	0	0	0
Ampicillin	1	100	0.3
Azithromycin*	0	0	0
Cefepime	0	0	0
Cefotaxime	0	0	0
Cefotaxime & clavulanic acid	0	0	0
Cefoxitin	0	0	0
Ceftazidime	0	0	0
Ceftazidime & clavulanic acid	0	0	0
Chloramphenicol	0	0	0
Ciprofloxacin	0	0	0
Colistin	1	100	0.3
Ertapenem*	0	0	0
Gentamicin	0	0	0
Imipenem	0	0	0
Meropenem	0	0	0
Nalidixic acid	0	0	0
Sulfamethoxazole	0	0	0
Temocillin	0	0	0
Tetracycline	0	0	0
Tigecycline	0	0	0
Trimethoprim	0	0	0



S2.2: Harmonised monitoring results of susceptibility testing in *Enterococcus* spp.

Please note, ciprofloxacin, teicoplanin and vancomycin are included in the Antimicrobial Advice *ad hoc* Expert Group (<u>AMEG</u>) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. For individuals using screen readers, please note that cells read out as blank denote that no isolates were tested, or that no data is available.

Table S2.2.1: Susceptibility in *E. faecalis* interpreted using both EUCAST a) ECOFFs and b) CBPs from caecal samples from healthy pigs at slaughter in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

Antibiotic	2023 (n=56)
Ampicillin	0
Chloramphenicol	12 (21.4)
Ciprofloxacin	0
Daptomycin	0
Erythromycin	23 (41.1)
Gentamicin	7 (12.5)
Linezolid	1 (1.8)
Teicoplanin	0
Tetracycline	35 (62.5)
Tigecycline	0
Vancomycin	0

a) ECOFF



Antibiotic	2023 (n=56)
Ampicillin	0
Chloramphenicol	-
Ciprofloxacin	0
Daptomycin	-
Erythromycin	-
Gentamicin	-
Linezolid	1 (1.8)
Teicoplanin	0
Tetracycline	-
Tigecycline	0
Vancomycin	0

Table S2.2.2: Susceptibility in *E. faecium* interpreted using both EUCAST a) ECOFFs and b) CBPs from caecal samples from healthy pigs at slaughter in the GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=176)
Ampicillin	36 (20.5)
Chloramphenicol	8 (4.5)
Ciprofloxacin	0
Daptomycin	0
Erythromycin	50 (28.4)
Gentamicin	1 (0.6)
Linezolid	6 (3.4)
Quinupristin/dalfopristin	122 (69.3)
Teicoplanin	0
Tetracycline	116 (65.9)
Tigecycline	0
Vancomycin	0



Antibiotic	2023 (n=176)
Ampicillin	0
Chloramphenicol	-
Ciprofloxacin	4 (2.3)
Daptomycin	-
Erythromycin	-
Gentamicin	-
Linezolid	6 (3.4)
Quinupristin/dalfopristin	142 (80.7)
Teicoplanin	0
Tetracycline	-
Tigecycline	0
Vancomycin	0



S2.3: Harmonised monitoring results of susceptibility testing in *Salmonella* spp.

Please note, cefotaxime, ceftazidime, ciprofloxacin, colistin and nalidixic acid are included in the Antimicrobial Advice *ad hoc* Expert Group (<u>AMEG</u>) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. For individuals using screen readers, please note that cells read out as blank denotes that no isolates were tested, or that no data is available.

Table S2.3.1: Susceptibility in *Salmonella* spp. interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy pigs at slaughter UK. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2021 and 2023.

Antibiotic	2021 (n=117)	2023 (n=97)
Amikacin**	0	0
Ampicillin	53 (45.3)	37 (38.1)
Azithromycin	0	0
Cefotaxime	0	0
Ceftazidime	0	0
Chloramphenicol	23 (19.7)	12 (12.4)
Ciprofloxacin	7 (6.0)	4 (4.1)
Colistin*	0	0
Gentamicin	9 (7.7)	2 (2.1)
Meropenem*	0	0
Nalidixic acid	6 (5.1)	1 (1.0)
Sulfamethoxazole*	60 (51.3)	51 (52.6)
Tetracycline	63 (53.8)	39 (40.2)
Tigecycline*	13 (11.1)	4 (4.1)
Trimethoprim*	30 (25.6)	19 (19.6)

a) ECOFF

* Interpreted using an EFSA-recommended ECOFF

** Interpreted using a tentative EUCAST ECOFF



Antibiotic	2021 (n=117)	2023 (n=97)
Amikacin	0	0
Ampicillin	53 (45.3)	37 (38.1)
Azithromycin	-	-
Cefotaxime	0	0
Ceftazidime	0	0
Chloramphenicol	27 (23.1)	12 (12.4)
Ciprofloxacin	7 (6.0)	4 (4.1)
Colistin	0	0
Gentamicin	9 (7.7)	2 (2.1)
Meropenem	0	0
Nalidixic acid	-	-
Sulfamethoxazole	-	-
Tetracycline	-	-
Tigecycline	-	-
Trimethoprim	30 (25.6)	19 (19.6)



S2.4: Harmonised monitoring results of susceptibility testing in *Campylobacter* spp.

Please note, ciprofloxacin is included in the Antimicrobial Advice *ad hoc* Expert Group (<u>AMEG</u>) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. For individuals using screen readers, please note that cells read out as blank denotes that no isolates were tested, or that no data is available.

Table S2.4.1: Susceptibility in *C. coli* interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy pigs at slaughter in the UK. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=201)
Chloramphenicol	0
Ciprofloxacin	40 (19.9)
Ertapenem*	5 (2.5)
Erythromycin	15 (7.5)
Gentamicin	1 (0.5)
Tetracyclines	144 (71.6)

* Interpreted using an EFSA-recommended ECOFF

b) CBP

Antibiotic	2023 (n=201)
Chloramphenicol	-
Ciprofloxacin	40 (19.9)
Ertapenem	-
Erythromycin	15 (7.5)
Gentamicin	-
Tetracyclines	144 (71.6)

27

S2.5 PATH-SAFE results of susceptibility testing from the beef cattle survey

Please note, cefotaxime, ceftazidime, ciprofloxacin, colistin and nalidixic acid are included in the Antimicrobial Advice ad hoc Expert Group (<u>AMEG</u>) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. For individuals using screen readers, please note that cells read out as blank denote that no isolates were tested, or that no data is available.

Table S2.5.1: Susceptibility in *E. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy beef cattle at slaughter in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

Antibiotic	2023 (n=234)
Amikacin	0
Ampicillin	3 (1.3)
Azithromycin*	0
Cefotaxime	1 (0.4)
Ceftazidime	1 (0.4)
Chloramphenicol	5 (2.1)
Ciprofloxacin	0
Colistin	0
Gentamicin	1 (0.4)
Meropenem	0
Nalidixic acid	0
Sulfamethoxazole	9 (3.8)
Tetracycline	21 (9.0)
Tigecycline	0
Trimethoprim	4 (1.7)

a) ECOFFs



Antibiotic	2023 (n=234)
Amikacin	0
Ampicillin	3 (1.3)
Azithromycin	-
Cefotaxime	0
Ceftazidime	0
Chloramphenicol	5 (2.1)
Ciprofloxacin	0
Colistin	0
Gentamicin	1 (0.4)
Meropenem	0
Nalidixic acid	-
Sulfamethoxazole	-
Tetracycline	-
Tigecycline	0
Trimethoprim	4 (1.7)

Table S2.5.2: Susceptibility in *E. faecalis* interpreted using both EUCAST a) ECOFFs and b) CBPs from caecal samples from healthy beef cattle at slaughter in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=24)
Ampicillin	0
Chloramphenicol	0
Ciprofloxacin	0
Daptomycin	0
Erythromycin	1 (4.2)
Gentamicin	0
Linezolid	0
Teicoplanin	0
Tetracycline	4 (16.7)
Tigecycline	0
Vancomycin	0



Antibiotic	2023 (n=24)
Ampicillin	0
Chloramphenicol	-
Ciprofloxacin	0
Daptomycin	-
Erythromycin	-
Gentamicin	-
Linezolid	0
Teicoplanin	0
Tetracycline	-
Tigecycline	0
Vancomycin	0

Table S2.5.3: Susceptibility in *E. faecium* interpreted using both EUCAST a) ECOFFs and b) CBPs from caecal samples from healthy beef cattle at slaughter in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=185)
Ampicillin	1 (0.5)
Chloramphenicol	0
Ciprofloxacin	5 (2.7)
Daptomycin	1 (0.5)
Erythromycin	7 (3.8)
Gentamicin	0
Linezolid	1 (0.5)
Quinupristin/dalfopristin	61 (33.0)
Teicoplanin	0
Tetracycline	17 (9.2)
Tigecycline	0
Vancomycin	0

30

Antibiotic	2023 (n=185)
Ampicillin	0
Chloramphenicol	-
Ciprofloxacin	25 (13.5)
Daptomycin	-
Erythromycin	-
Gentamicin	-
Linezolid	1 (0.5)
Quinupristin/dalfopristin	93 (50.3)
Teicoplanin	0
Tetracycline	-
Tigecycline	0
Vancomycin	0

Table S2.5.4: Susceptibility in *C. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy beef cattle at slaughter in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=23)
Chloramphenicol	0
Ciprofloxacin	0
Ertapenem*	5 (21.7)
Erythromycin	0
Gentamicin	0
Tetracyclines	1 (4.3)



Antibiotic	2023 (n=23)
Chloramphenicol	-
Ciprofloxacin	0
Ertapenem	-
Erythromycin	0
Gentamicin	-
Tetracyclines	1 (4.3)

Table S2.5.5: Susceptibility in *C. jejuni* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy beef cattle at slaughter in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=114)
Chloramphenicol	0
Ciprofloxacin	18 (15.8)
Ertapenem*	1 (0.9)
Erythromycin	0
Gentamicin	1 (0.9)
Tetracyclines	13 (11.4)

* Interpreted using an EFSA-recommended ECOFF

b) CBP

Antibiotic	2023 (n=114)
Chloramphenicol	-
Ciprofloxacin	18 (15.8)
Ertapenem	-
Erythromycin	0
Gentamicin	-
Tetracyclines	13 (11.4)

32

S2.6 PATH-SAFE results of susceptibility testing from the sheep survey

Please note, cefotaxime, ceftazidime, ciprofloxacin, colistin and nalidixic acid are included in the Antimicrobial Advice *ad hoc* Expert Group (<u>AMEG</u>) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. For individuals using screen readers, please note that cells read out as blank denote that no isolates were tested, or that no data is available.

Table S2.6.1: Susceptibility in *E. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy sheep at slaughter in England and Wales. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

- Antibiotic 2023 (n=185) Amikacin 0 Ampicillin 8 (4.3) 1 (0.5) Azithromycin* Cefotaxime 0 Ceftazidime 0 Chloramphenicol 4 (2.2) Ciprofloxacin 1 (0.5) Colistin 2 (1.1) Gentamicin 0 0 Meropenem Nalidixic acid 0 Sulfamethoxazole 9 (4.9) Tetracycline 14 (7.6) Tigecycline 0 Trimethoprim 5 (2.7)
- a) ECOFFs



b) CBPs

Antibiotic	2023 (n=185)
Amikacin	0
Ampicillin	8 (4.3)
Azithromycin	-
Cefotaxime	0
Ceftazidime	0
Chloramphenicol	4 (2.2)
Ciprofloxacin	0
Colistin	2 (1.1)
Gentamicin	0
Meropenem	0
Nalidixic acid	-
Sulfamethoxazole	-
Tetracycline	-
Tigecycline	0
Trimethoprim	5 (2.7)

Table S2.6.2: Distribution of ESBL/AmpC and CPE enzymes detected in *E. coli* isolated using selective agar from healthy sheep at slaughter in England and Wales in 2023. Note - if more than one isolate was of an unknown sequence type (ST), it has been assumed that they belonged to different STs.

Enzyme	Number of isolates	Proportion of isolates (n=25) (%)	Proportion of caecal samples (n=291) (%)	Number of unique STs	Sequence type (ST)
AmpC	14	56.0	4.8	9	23, 56, 88, 155, 297,
promoter			_	_	661, 1304, 2175, 3090
CTX-M-15	7	28.0	2.4	1	515
CTX-M-55	1	4.0	0.3	1	1139
CTX-M-214	1	4.0	0.3	1	164
DHA-1	2	8.0	0.7	2	10, 301
TEM-192	1	4.0	0.3	1	301

34

Table S2.6.3: Decreased susceptibility in ESBL-/AmpC-producing *E. coli* isolated using selective agar from caecal samples from healthy sheep at slaughter in England and Wales in 2023.

Antibiotic	Number of isolates with MIC>ECOFF	Proportion of isolates (%) (n=25)	Total proportion from caecal samples (%) (n=291)
Amikacin	0	0	0
Ampicillin	25	100	8.6
Azithromycin*	3	12	1.0
Cefepime	11	44	3.8
Cefotaxime	25	100	8.6
Cefotaxime & clavulanic acid	16	64	5.5
Cefoxitin	11	44	3.8
Ceftazidime	23	92	7.9
Ceftazidime & clavulanic acid	13	52	4.5
Chloramphenicol	3	12	1.0
Ciprofloxacin	9	36	3.1
Colistin	0	0	0
Ertapenem*	0	0	0
Gentamicin	0	0	0
Imipenem	0	0	0
Meropenem	0	0	0
Nalidixic acid	0	0	0
Sulfamethoxazole	10	40	0
Temocillin	0	0	0
Tetracycline	10	40	3.4
Tigecycline	0	0	0
Trimethoprim	8	32	2.7

* Interpreted using an EFSA-recommended ECOFF



Table S2.6.4: Susceptibility in *E. faecalis* interpreted using both EUCAST a) ECOFFs and b) CBPs from caecal samples from healthy sheep at slaughter in England and Wales. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=52)
Ampicillin	0
Chloramphenicol	0
Ciprofloxacin	0
Daptomycin	0
Erythromycin	0
Gentamicin	0
Linezolid	0
Teicoplanin	0
Tetracycline	5 (9.6)
Tigecycline	0
Vancomycin	0

Antibiotic	2023 (n=52)
Ampicillin	0
Chloramphenicol	-
Ciprofloxacin	0
Daptomycin	-
Erythromycin	-
Gentamicin	-
Linezolid	0
Teicoplanin	0
Tetracycline	-
Tigecycline	0
Vancomycin	0

Table S2.6.5: Susceptibility in E. faecium interpreted using both EUCAST a) ECOFFs and b) CBPs from caecal samples from healthy sheep at slaughter in England and Wales. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

- Antibiotic 2023 (n=96) Ampicillin 1 (1.0) Chloramphenicol Ciprofloxacin Daptomycin 3 (3.1) Erythromycin Gentamicin Linezolid Quinupristin/dalfopristin 31 (32.3) Teicoplanin 16 (16.7) Tetracycline Tigecycline Vancomycin
- a) ECOFF

b) CBP

Antibiotic	2023 (n=96)
Ampicillin	1 (1.0)
Chloramphenicol	-
Ciprofloxacin	11 (11.5)
Daptomycin	-
Erythromycin	-
Gentamicin	-
Linezolid	0
Quinupristin/dalfopristin	41 (42.7)
Teicoplanin	0
Tetracycline	-
Tigecycline	0
Vancomycin	0



0

0 0

0

0

0

0 0 **Table S2.6.6:** Susceptibility in *Salmonella* spp. interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise stated from caecal samples from healthy sheep at slaughter in England and Wales. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

Antibiotic	2023 (n=108)
Amikacin**	0
Ampicillin	0
Azithromycin	0
Cefotaxime	0
Ceftazidime	0
Chloramphenicol	0
Ciprofloxacin	0
Colistin*	1 (0.9)
Gentamicin	0
Meropenem*	0
Nalidixic acid	0
Sulfamethoxazole*	0
Tetracycline	0
Tigecycline*	0
Trimethoprim*	0

a) ECOFF

* Interpreted using an EFSA-recommended ECOFF

** Interpreted using a tentative EUCAST ECOFF



b) CBP

Antibiotic	2023 (n=108)
Amikacin	0
Ampicillin	0
Azithromycin	-
Cefotaxime	0
Ceftazidime	0
Chloramphenicol	0
Ciprofloxacin	0
Colistin	1 (0.9)
Gentamicin	0
Meropenem	0
Nalidixic acid	-
Sulfamethoxazole	-
Tetracycline	-
Tigecycline	-
Trimethoprim	0

Table S2.6.7: Susceptibility in *C. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy sheep at slaughter in England and Wales. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=30)
Chloramphenicol	0
Ciprofloxacin	1 (3.3)
Ertapenem*	8 (26.7)
Erythromycin	1 (3.3)
Gentamicin	0
Tetracyclines	2 (6.7)

* Interpreted using an EFSA-recommended ECOFF



b) CBP

Antibiotic	2023 (n=30)	
Chloramphenicol	-	
Ciprofloxacin	1 (3.3)	
Ertapenem	-	
Erythromycin	1 (3.3)	
Gentamicin	-	
Tetracyclines	2 (6.7)	

Table S2.6.8: Susceptibility in *C. jejuni* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from caecal samples from healthy sheep at slaughter in England and Wales. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	2023 (n=162)	
Chloramphenicol	0	
Ciprofloxacin	14 (8.6)	
Ertapenem*	2 (1.2)	
Erythromycin	1 (0.6)	
Gentamicin	0	
Tetracyclines	6 (3.7)	

* Interpreted using an EFSA-recommended ECOFF

Antibiotic	2023 (n=162)	
Chloramphenicol	-	
Ciprofloxacin	14 (8.6)	
Ertapenem	-	
Erythromycin	1 (0.6)	
Gentamicin	-	
Tetracyclines	6 (3.7)	

40

S2.7 PATH-SAFE results of susceptibility testing from the bulk milk survey

Please note, cefotaxime, ceftazidime, ciprofloxacin, colistin, nalidixic acid and vancomycin are included in the Antimicrobial Advice *ad hoc* Expert Group (<u>AMEG</u>) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. For individuals using screen readers, please note that cells read out as blank denote that no isolates were tested, or that no data is available.

Table S2.7.1: Susceptibility in *E. coli* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

Antibiotic	2023 (n=503)
Amikacin	0
Ampicillin	54 (10.7)
Azithromycin*	1 (0.2)
Cefotaxime	1 (0.2)
Ceftazidime	1 (0.2)
Chloramphenicol	14 (2.8)
Ciprofloxacin	3 (0.6)
Colistin	5 (1.0)
Gentamicin	0
Meropenem	0
Nalidixic acid	3 (0.6)
Sulfamethoxazole	44 (8.7)
Tetracycline	50 (9.9)
Tigecycline	0
Trimethoprim	32 (6.4)

a) ECOFFs

* Interpreted using an EFSA-recommended ECOFF



b) CBPs

Antibiotic	2023 (n=503)
Amikacin	0
Ampicillin	54 (10.7)
Azithromycin	-
Cefotaxime	0
Ceftazidime	0
Chloramphenicol	14 (2.8)
Ciprofloxacin	3 (0.6)
Colistin	5 (1.0)
Gentamicin	0
Meropenem	0
Nalidixic acid	-
Sulfamethoxazole	-
Tetracycline	-
Tigecycline	0
Trimethoprim	32 (6.4)

Table S2.7.2: Distribution of ESBL/AmpC and CPE enzymes detected in *E. coli* isolated using selective agar from bulk milk samples from healthy dairy cattle in GB in 2023. Note - if more than one isolate was of an unknown sequence type (ST), it has been assumed that they belonged to different STs.

Enzyme	Number of isolates	Proportion of isolates (n=6) (%)	Proportion of caecal samples (n=1055) (%)	Number of unique STs	Sequence type (ST)
AmpC promoter	3	50.0	0.3	3	58, 88, 1126
CTX-M-15	1	16.7	0.1	1	362
CTX-M-32	1	16.7	0.1	1	4624
CTX-M-39	1	16.7	0.1	1	442

42

Table S2.7.3: Decreased susceptibility in ESBL-/AmpC-producing *E. coli* isolated using selective agar from bulk milk samples from healthy dairy cattle in GB in 2023.

Antibiotic	Number of isolates with MIC>ECOFF	Proportion of isolates (%) (n=6)	Total proportion from caecal samples (%) (n=1055)
Amikacin	0	0	0
Ampicillin	6	100	0.6
Azithromycin*	1	16.7	0.1
Cefepime	3	50	0.3
Cefotaxime	6	100	0.6
Cefotaxime & clavulanic acid	3	50	0.3
Cefoxitin	2	33.3	0.2
Ceftazidime	5	83.3	0.5
Ceftazidime & clavulanic acid	3	50	0.3
Chloramphenicol	2	33.3	0.2
Ciprofloxacin	0	0	0
Colistin	0	0	0
Ertapenem*	0	0	0
Gentamicin	0	0	0
Imipenem	0	0	0
Meropenem	0	0	0
Nalidixic acid	1	16.7	0.1
Sulfamethoxazole	4	66.7	0.4
Temocillin	0	0	0
Tetracycline	6	100	0.6
Tigecycline	0	0	0
Trimethoprim	2	33.3	0.2

* Interpreted using an EFSA-recommended ECOFF



Table S2.7.4: Susceptibility in *E. faecalis* interpreted using both EUCAST a) ECOFFs and b) CBPs from bulk milk samples from healthy dairy cattle in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

- Antibiotic 2023 (n=296) Ampicillin 0 Chloramphenicol 28 (9.5) Ciprofloxacin 0 1 (0.3) Daptomycin 41 (13.9) Erythromycin Gentamicin 0 Linezolid 0 0 Teicoplanin 226 (76.4) Tetracycline Tigecycline 0 Vancomycin 0
- a) ECOFF

Antibiotic	2023 (n=296)
Ampicillin	0
Chloramphenicol	-
Ciprofloxacin	0
Daptomycin	-
Erythromycin	-
Gentamicin	-
Linezolid	0
Teicoplanin	0
Tetracycline	-
Tigecycline	0
Vancomycin	0

Table S2.7.5: Susceptibility in *E. faecium* interpreted using both EUCAST a) ECOFFs and b) CBPs from bulk milk samples from healthy dairy cattle in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

Antibiotic	2023 (n=283)
Ampicillin	0
Chloramphenicol	0
Ciprofloxacin	3 (1.1)
Daptomycin	0
Erythromycin	25 (8.8)
Gentamicin	0
Linezolid	0
Quinupristin/dalfopristin	73 (25.8)
Teicoplanin	0
Tetracycline	51 (18.0)
Tigecycline	0
Vancomycin	0

a) ECOFF

Antibiotic	2023 (n=283)
Ampicillin	0
Chloramphenicol	-
Ciprofloxacin	13 (4.6)
Daptomycin	-
Erythromycin	-
Gentamicin	-
Linezolid	0
Quinupristin/dalfopristin	94 (33.2)
Teicoplanin	0
Tetracycline	-
Tigecycline	0
Vancomycin	0



Table S2.7.6: Susceptibility in *Klebsiella spp.* interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	<i>Klebsiella oxytoca</i> n=4	<i>Klebsiella pneumoniae</i> n=9
Amikacin	0	0
Ampicillin	-	-
Cefotaxime	0	0
Ceftazidime	0	0
Chloramphenicol	-	-
Ciprofloxacin	0	0
Colistin	0	2 (22.2)
Gentamicin	0	0
Meropenem	0	0
Tetracycline	1 (25.0)	5 (55.6)
Tigecycline	0	0
Trimethoprim*	-	0

* Interpreted using an EFSA-recommended ECOFF

Antibiotic	<i>Klebsiella oxytoca</i> n=4	<i>Klebsiella pneumoniae</i> n=9
Amikacin	0	0
Ampicillin	4 (100.0)	8 (88.9)
Cefotaxime	0	0
Ceftazidime	0	0
Chloramphenicol	0	0
Ciprofloxacin	0	0
Colistin	0	2 (22.2)
Gentamicin	0	0
Meropenem	0	0
Tetracycline	-	-
Tigecycline	-	-
Trimethoprim	0	0



Table S2.7.7: Susceptibility in *S. aureus* interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

Antibiotic	2023 (n=100)
Cefoxitin	1 (1.0)
Chloramphenicol	0
Ciprofloxacin	0
Clindamycin	3 (3.0)
Erythromycin	1 (1.0)
Fusidate	0
Gentamicin	0
Kanamycin**	0
Linezolid	0
Mupirocin	0
Penicillin	4 (4.0)
Rifampin	0
Streptomycin	0
Quinupristin/dalfopristin	0
Sulfamethoxazole*	4 (4.0)
Tetracycline	0
Tiamulin**	0
Trimethoprim	0
Vancomycin	0

a) ECOFFs

* Interpreted using an EFSA-recommended ECOFF

** Interpreted using a tentative EUCAST ECOFF

47

Antibiotic	2023 (n=100)
Cefoxitin	1 (1.0)
Chloramphenicol	-
Ciprofloxacin	0
Clindamycin	3 (3.0)
Erythromycin	1 (1.0)
Fusidate	0
Gentamicin	0
Kanamycin	-
Linezolid	0
Mupirocin	-
Penicillin	4 (4.0)
Rifampin	0
Quinupristin/dalfopristin	0
Streptomycin	-
Sulfamethoxazole	-
Tetracycline	0
Tiamulin	-
Trimethoprim	0
Vancomycin	0



Table S2.7.8: Susceptibility in methicillin-resistant *S. aureus* (MRSA) interpreted using EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

Antibiotic	2023 (n=14)
Cefoxitin	11 (78.6)
Chloramphenicol	0
Ciprofloxacin	2 (14.3)
Clindamycin	3 (21.4)
Erythromycin	1 (7.1)
Fusidate	3 (21.4)
Gentamicin	4 (28.6)
Kanamycin**	6 (42.9)
Linezolid	0
Mupirocin	1 (7.1)
Penicillin	12 (85.7)
Quinupristin/dalfopristin	3 (21.4)
Rifampin	2 (14.3)
Streptomycin	4 (28.6)
Sulfamethoxazole*	5 (35.7)
Tetracycline	9 (64.3)
Tiamulin**	5 (35.7)
Trimethoprim	3 (21.4)
Vancomycin	0

a) ECOFFs

* Interpreted using an EFSA-recommended ECOFF

** Interpreted using a tentative EUCAST ECOFF

49

Antibiotic	2023 (n=14)
Cefoxitin	11 (78.6)
Chloramphenicol	-
Ciprofloxacin	2 (14.3)
Clindamycin	3 (21.4)
Erythromycin	1 (7.1)
Fusidate	3 (21.4)
Gentamicin	4 (28.6)
Kanamycin	-
Linezolid	0
Mupirocin	-
Penicillin	12 (85.7)
Quinupristin/dalfopristin	3 (21.4)
Rifampin	-
Streptomycin	-
Sulfamethoxazole	3 (21.4)
Tetracycline	9 (64.3)
Tiamulin	-
Trimethoprim	3 (21.4)
Vancomycin	0

Table S2.7.9: Susceptibility in *Streptococcus dysgalactiae* interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	Streptococcus dysgalactiae (n=6)
Doxycycline*	3 (50.0)
Enrofloxacin	0
Erythromycin	1 (16.7)
Florfenicol	0
Lincomycin	0
Penicillin	-
Tetracycline	-
Trimethoprim/ sulfamethoxazole	0
Tylosin*	1 (16.7)

* Interpreted using a tentative EUCAST ECOFF

Antibiotic	Streptococcus dysgalactiae n=6
Doxycycline	3 (50.0)
Enrofloxacin	-
Erythromycin	1 (16.7)
Florfenicol	-
Lincomycin	-
Penicillin	0
Tetracycline	6 (100.0)
Trimethoprim/ sulfamethoxazole	0
Tylosin	-

51

Table S2.7.10: Susceptibility in *Streptococcus uberis* interpreted using both EUCAST a) ECOFFs and b) CBPs unless otherwise indicated from bulk milk samples from healthy dairy cattle in GB. This table shows the number and percentage of isolates with higher resistance levels than expected background levels for that species for 2023.

a) ECOFF

Antibiotic	<i>Streptococcus uberis</i> (n=162)
Doxycycline	35 (21.6)
Enrofloxacin	2 (1.2)
Erythromycin	15 (9.3)
Florfenicol	0
Lincomycin	67 (41.4)
Penicillin*	68 (42.0)
Tetracycline	33 (20.4)
Trimethoprim/ sulfamethoxazole	0
Tylosin*	14 (8.6)

* Interpreted using a tentative EUCAST ECOFF

Antibiotic	<i>Streptococcus uberis</i> n=162
Doxycycline	33 (20.4)
Enrofloxacin	-
Erythromycin	15 (9.3)
Florfenicol	-
Lincomycin	-
Penicillin	15 (9.3)
Tetracycline	33 (20.4)
Trimethoprim/ sulfamethoxazole	0
Tylosin	-



S3.1: Methodology susceptibility testing

S3.1.1 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 (<u>BSAC</u>).

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 S3.1.1.1, 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. 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 S3.1.1.1** 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 or for all of the bacterial/antibiotic combinations which may require testing. In these cases, a uniform cutoff 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.

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.

Susceptibility was determined for certain antibiotics not authorised for use in any foodproducing 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 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 (<u>SONAAR</u>) report.



Please note, cefalexin, cefotaxime, ceftazidime, cefpodoxime, ceftiofur, ciprofloxacin, colistin and enrofloxacin are included in the Antimicrobial Advice *ad hoc* Expert Group (<u>AMEG</u>) category B and are referred to as high priority critically important antibiotics (HP-CIAs) throughout the report. 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 denote that no isolates were tested, or that no data is available.

Table S3.1.1.1: Disc diffusion breakpoints, corresponding MIC breakpoints and breakpoints under review for the main bacteria covered in the core data of this report in a) England and Wales, b) Northern Ireland and c) Scotland.

a) England and Wales

Please note that for erythromycin the R \leq 21 mm breakpoint is for beta-haemolytic streptococci and R \leq 19 mm for other streptococci, for penicillin the R \leq 19 mm breakpoint is for beta-haemolytic streptococci and R \leq 16 mm for other streptococci and the tetracycline R \leq 19 mm breakpoint is for beta-haemolytic streptococci and R \leq 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 (µg)	<i>Escherichia coli,</i> Enterobacteriaceae	Salmonella	Staphylococci	Streptococci	Pasteurella, Mannheimia
Amikacin (AK)	30	R ≤18 mm* R ≥16 mg/L*	R ≤18 mm* R ≥16 mg/L*	N/A	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	R ≤13 mm***
Amoxicillin/clavulanate	2/1	N/A	N/A	R ≤17 mm* R >1 mg/L*	R ≤13 mm***	N/A
Ampicillin (AMP)	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**	N/A	N/A	R ≤13 mm***

Antibiotic	Disc charge (µg)	<i>Escherichia coli,</i> Enterobacteriaceae	Salmonella	Staphylococci	Streptococci	Pasteurella, Mannheimia
Cefalexin	30	R ≤15 mm* R >16 mg/L*	N/A	R ≤13 mm***	R ≤24 mm* R >2 mg/L*	R ≤13 mm***
Cefotaxime (CTX)	30	R ≤29 mm* R ≥2 mg/L*	R ≤29 mm* R ≥2 mg/L*	N/A	N/A	N/A
Cefpodoxime	10	R ≤ 19 mm* R >1 mg/L*	N/A	N/A	N/A	R ≤13 mm***
Ceftazidime (CAZ)	30	R ≤ 26 mm* R ≥2 mg/L*	R ≤26 mm* R ≥2 mg/L*	N/A	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	N/A
Ciprofloxacin (CIP)	1	N/A	R ≤16 mm* R ≥1 mg/L*	N/A	N/A	N/A
Doxycycline	30	R ≤13 mm***	N/A	R ≤30 mm* R ≥2 mg/L*	N/A	R ≤13 mm***
Enrofloxacin	5	R ≤13 mm** R ≥4 mg/L**	N/A	R ≤13 mm***	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*	R ≤13 mm***
Florfenicol	30	R ≤13 mm** R >32 mg/L**	N/A	N/A	R ≤13 mm***	R ≤13 mm***
Furazolidone (FR)	15	N/A	R ≤13 mm***	N/A	N/A	N/A
Gentamicin (CN)	10	N/A	R ≤19 mm* R ≥4 mg/L*	N/A	N/A	N/A
Lincomycin	10	N/A	N/A	R ≤13 mm***	R ≤13 mm***	R ≤13 mm***

Antibiotic	Disc charge (µg)	<i>Escherichia coli,</i> Enterobacteriaceae	Salmonella	Staphylococci	Streptococci	Pasteurella, Mannheimia
Nalidixic acid (NA)	30	N/A	≤13 mm	N/A	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	N/A
Neomycin	30	N/A	N/A	R ≤13 mm***	R ≤13 mm***	N/A
Novobiocin	30	N/A	N/A	R ≤13 mm***	R ≤13 mm***	N/A
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*	N/A
Spectinomycin	25	R ≤13 mm***	N/A	N/A	N/A	R ≤13 mm***
Streptomycin (S)	10	R ≤12 mm* R >8 mg/L*	R ≤13 mm R > ~8 mg/L	N/A	N/A	R ≤13 mm***
Sulfonamide compounds (S)	3/300	N/A	≤13 mm	N/A	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*	R ≤25 mm* R >1 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*	R ≤13 mm***
Tylosin	30	N/A	N/A	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

▲ 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*.

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

b) Northern Ireland

Sulfonamides (S)	3/300	≤12	13–16	≥17
Tetracycline (TE)	30	≤11	12–14	≥15
Trimethoprim (W)	5	≤10	11–15	≥16

c) Scotland

		Escherichia coli, Er	nterobacteriaceae	Salmonella	
Antibiotic	Disc charge (µg)	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
	25	R ≤14	-	R ≤14	-
Spectinomycin (SH)	100	-	R ≤17	-	R ≤17

		Escherichia coli, Er	nterobacteriaceae	Salmonella	
Antibiotic	Disc charge (µg)	Cattle and sheep (mm)	Pigs and poultry (mm)	Cattle and sheep (mm)	Pigs and poultry (mm)
			I ≤20		I ≤20
Streptomycin (S)	10	R ≤11 I ≤14	-	N/A	-
	25	-	R ≤10 I ≤14	-	R ≤10 I ≤14
	10	R ≤19	-	R ≤19	-
Tetracycline (TE)	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

S3.1.2 Private Laboratory Initiative

The methods used to determine antimicrobial susceptibility, are based on those in CLSI Vet01 July 2013¹. 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 S3.1.3.1 below.

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	R <14	R <14	R <14
Cloxacillin	I <18 N/A	I <18 R <18	l <18 R <18	I <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

Table 3.1.3.1: Disc diffusion breakpoints applied by Vale Veterinary Laboratories for the interpretation of resistance of bovine mastitis pathogens in millimetres.



¹ The Vale Veterinary Laboratory, personal communications, 2021

S3.1.3 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. In this year's report, presented for the first time, are MIC results from a set of core respiratory pathogens, *Pasteurella multocida, Mannhaemia haemolytica, Actinobacillus pleuropneumoniae, Bibersteinia trehalosi* alongside *Streptococcus suis* and *Brachyspira hyodysenteriae,* which have been reported previously.

The samples came from diagnostic submissions to the Animal and Plant Health Agency (APHA) and its partner laboratories in 2023. 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 <u>CLSI</u> in the first instance, or <u>CA-SFM</u> when these are not available; if veterinary breakpoints were not available, <u>human</u> <u>CBPs</u> were used (see Table S3.1.2.1). For some veterinary antibiotic and organism combinations, there are no published breakpoints available and in these cases, resistance cannot be interpreted from MIC distributions.



Table S3.1.3.1: 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)		<i>a</i> (mg/L)	<i>Mannheimia haemolytica</i> (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 <u>></u> 8*	R <u>></u> 8*	R <u>></u> 8*	R <u>></u> 8*	R <u>></u> 8*	R <u>≥</u> 8*	R <u>></u> 8*
Doxycycline	R >8**	R >8**	R > 8**	R >8**	R > 8**	R > 8**	R > 8**
Enrofloxacin	R <u>></u> 2*	R <u>></u> 1*	R <u>></u> 2*	R <u>></u> 2*	R <u>></u> 2*	R <u>≥</u> 1*	R <u>></u> 2*
Florfenicol	R <u>></u> 8*	R <u>></u> 8*	R <u>></u> 8*	R <u>></u> 8*	R <u>></u> 8*	R <u>≥</u> 8*	R <u>></u> 8*
Gamithromycin	R <u>></u> 16*	R <u>></u> 16*	R <u>></u> 16*	R <u>></u> 16*	R <u>></u> 16*	N/A	R <u>></u> 16*
Spectinomycin	R <u>></u> 128*	R <u>></u> 128*	R <u>></u> 128*	R <u>></u> 128*	R <u>></u> 128*	N/A	R <u>></u> 128*
Tetracycline	R <u>></u> 8*	R <u>></u> 2*	R <u>></u> 8*	R <u>></u> 8*	R <u>></u> 8*	R <u>≥</u> 2*	R <u>></u> 8*
Tiamulin	N/A	N/A	N/A	N/A	N/A	R <u>≥</u> 32*	N/A

Antibiotic	Pasteu	rella multocida	a (mg/L)	Manni haemolyt	heimia ica (mg/L)	Actinobacillus pleuropneumoniae (mg/L)	Bibersteinia trehalosi (mg/L)
Tildipirosin	R <u>></u> 32*	S <u><</u> 4*	R <u>></u> 32*	R <u>></u> 16*	R <u>></u> 16*	S <u><</u> 16*	R <u>></u> 16*
Tilmicosin	R <u>></u> 32*	R <u>≥</u> 32*	R <u>></u> 32*				
Trimethoprim/ sulfonamide	R > 8**	R > 8**	R > 8**				
Tulathromycin	R <u>></u> 64*	S <u>≤</u> 64*	R <u>></u> 64*				

Key:

* CLSI veterinary clinical breakpoint

** CASFM veterinary clinical breakpoint

*** EUCAST human breakpoint



b) Other pathogens

Antibiotic	Streptococcus suis (mg/L)	Brachyspira hyodysenteriae (mg/L)
	Pigs	Pigs
Ceftiofur	R > 8*	N/A
Centolul	S <u>≤</u> 2*	N/A
Doxyoveline	R > 1***	R > 2****
Doxycycline	S <u>≤</u> 0.25***	N/A
Enrofloxacin	R > 2*	N/A
Entonoxacin	S <u>≤</u> 0.5*	N/A
Enthromyoin	R > 1*	N/A
Erythromycin	S <u><</u> 0.25*	N/A
Florfenicol	R > 8*	N/A
FIOHENICOI	S <u>≤</u> 2*	N/A
Lincomyoin	R > 8**	R > 8****
Lincomycin	S <u>≤</u> 2**	N/A
Penicillin	R > 1*	N/A
Penicillin	S <u><</u> 0.25*	N/A
Tetresveline	R > 2*	N/A
Tetracycline	S <u>≤</u> 0.5*	N/A
Tiamulin	N/A	R > 2****
	R > 2***	N/A
Trimethoprim/sulfonamide	S <u><</u> 1***	N/A
Tylosin	N/A	R > 8****
Tylvalosin	N/A	R > 8****

Key:

* CLSI veterinary clinical breakpoint

** CASFM veterinary clinical breakpoint

*** EUCAST human breakpoint

*** Suggested <u>broth microdilution clinical breakpoints</u> are considered to be one dilution lower than <u>clinical breakpoints for agar dilution</u>



S4.1: Clinical surveillance data for isolates of zoonotic pathogens from all species

Table S4.1.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *E. coli* from pigs, chickens, turkeys, cattle and sheep (combined) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amikacin	0/167 (0)	-	-
Amoxicillin/clavulanate	72/624 (11.5)	289/588 (49.1)	12/65 (18.5)
Ampicillin	492/1141 (43.1)	462/588 (78.6)	25/65 (38.5)
Apramycin	86/1090 (7.9)	79/466 (17.0)	3/65 (4.6)
Cefotaxime	10/168 (6.0)	-	0/29 (0)
Cefpodoxime	3/573 (0.5)	330/579 (57.0)	2/36 (5.6)
Ceftazidime	5/168 (3.0)	-	-
Chloramphenicol	34/167 (20.4)	-	-
Doxycycline	37/123 (30.1)	-	-
Enrofloxacin	12/1141 (1.1)	174/587 (29.6)	0/65 (0)
Florfenicol	42/217 (19.4)	215/401 (53.6)	6/65 (9.2)
Neomycin	128/1027 (12.5)	588/588 (100.0)	7/65 (10.8)
Spectinomycin	228/1089 (20.9)	6/115 (5.2)	17/65 (26.2)
Streptomycin	64/168 (38.1)	114/116 (98.3)	20/29 (69.0)
Tetracycline	524/1141 (45.9)	391/588 (66.5)	31/65 (47.7)
Trimethoprim/sulfonamide	289/1140 (25.4)	283/586 (48.3)	17/65 (26.2)



Table S4.1.2: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *Salmonella* from cattle, pigs, sheep, chickens and turkeys (combined) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amikacin	0/2886 (0)	-	-
Amoxicillin/clavulanate	2/2886 (0.1)	8/130 (6.2)	1/151 (0.7)
Ampicillin	334/2886 (11.6)	30/130 (23.1)	16/151 (10.6)
Apramycin	77/2886 (2.7)	9/130 (6.9)	3/151 (2.0)
Cefotaxime	0/2886 (0)	0/25 (0)	0/17 (0)
Cefpodoxime	-	5/105 (4.8)	0/134 (0)
Ceftazidime	0/2886 (0)	1/130 (0.8)	-
Chloramphenicol	219/2886 (7.6)	1/25 (4.0)	-
Ciprofloxacin	7/2886 (0.2)	1/25 (4.0)	-
Enrofloxacin	-	2/105 (1.9)	0/151 (0)
Florfenicol	-	4/105 (3.8)	7/151 (4.6)
Furazolidone	10/2886 (0.3)	0/25 (0)	-
Gentamicin	80/2886 (2.8)	3/25 (12.0)	-
Nalidixic acid	35/2886 (1.2)	1/25 (4.0)	0/134 (0)
Neomycin	130/2886 (4.5)	102/105 (97.1)	1/151 (0.7)
Spectinomycin	-	4/25 (16.0)	11/151 (7.3)
Streptomycin	364/2886 (12.6)	14/25 (56.0)	0/17 (0)
Sulfonamide compounds	580/2886 (20.1)	5/25 (20.0)	-
Tetracycline	375/2886 (13.0)	29/130 (22.3)	13/151 (8.6)
Trimethoprim/sulfonamide	441/2886 (15.3)	11/105 (10.5)	8/151 (5.3)

Table S4.1.3: Findings of LA-MRSA by government laboratories for England and Wales in 2023.

Clonal complex	Species	Source of the sample	Resistance genes
CC398 (t4838)	Pig	Clinical	cfr

S4.2: Clinical surveillance data for isolates from pigs

Table S4.2.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *E. coli* from pigs (all ages) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistance in brackets.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amoxicillin/clavulanate	-	21/37 (56.8)	2/26 (7.7)
Ampicillin	221/453 (48.8)	36/37 (97.3)	5/26 (19.2)
Apramycin	80/453 (17.7)	13/37 (35.1)	2/26 (7.7)
Cefotaxime	-	-	0/26 (0)
Cefpodoxime	3/450 (0.7)	18/36 (50.0)	-
Enrofloxacin	5/453 (1.1)	9/37 (24.3)	0/26 (0)
Florfenicol	-	6/37 (16.2)	0/26 (0)
Neomycin	61/453 (13.5)	37/37 (100)	0/26 (0)
Spectinomycin	138/453 (30.5)	-	6/26 (23.1)
Streptomycin	-	-	18/26 (69.2)
Tetracycline	247/453 (54.5)	34/37 (91.9)	9/26 (34.6)
Trimethoprim/sulfonamide	171/453 (37.7)	25/37 (67.6)	7/26 (26.9)

Table S4.2.2: Resistance (and interpreted using clinical breakpoints) in *E. coli* from pigs in a) England and Wales, b) Northern Ireland and c) Scotland for 2023, split by age category. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Please note that no post-weaning or adult data is available for Northern Ireland and no neonatal or post-weaning data is available for Scotland.

a) England and Wales

Antibiotic	Neonatal	Post-weaning	Adult
Ampicillin	31/58 (53.4)	155/287 (54)	11/37 (29.7)
Apramycin	4/58 (6.9)	71/287 (24.7)	0/37 (0)
Cefpodoxime	0/58 (0)	3/284 (1.1)	0/37 (0)
Enrofloxacin	2/58 (3.4)	2/287 (0.7)	1/37 (2.7)
Neomycin	1/58 (1.7)	54/287 (18.8)	2/37 (5.4)
Spectinomycin	16/58 (27.6)	104/287 (36.2)	5/37 (13.5)
Tetracycline	31/58 (53.4)	170/287 (59.2)	13/37 (35.1)
Trimethoprim/sulfonamide	25/58 (43.1)	127/287 (44.3)	4/37 (10.8)



b) Northern Ireland

Antibiotic	Neonatal
Amoxicillin/clavulanate	1/4 (25.0)
Ampicillin	3/4 (75.0)
Apramycin	2/4 (50.0)
Cefpodoxime	1/4 (25.0)
Enrofloxacin	0/4 (0)
Florfenicol	0/4 (0)
Neomycin	4/4 (100.0)
Tetracycline	3/4 (75.0)
Trimethoprim/sulfonamide	1/4 (25.0)

c) Scotland

Antibiotic	Adult
Amoxicillin/clavulanate	2/26 (7.7)
Ampicillin	5/26 (19.2)
Apramycin	2/26 (7.7)
Cefotaxime	0/26 (0)
Cefpodoxime	-
Enrofloxacin	0/26 (0)
Florfenicol	0/26 (0)
Neomycin	0/26 (0)
Spectinomycin	6/26 (23.1)
Streptomycin	18/26 (69.2)
Tetracycline	9/26 (34.6)
Trimethoprim/sulfonamide	7/26 (26.9)



Table S4.2.3: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *Salmonella* from pigs (all ages) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amikacin	0/328 (0)	-	-
Amoxicillin/clavulanate	0/328 (0)	7/17 (41.2)	0/15 (0)
Ampicillin	247/328 (75.3)	17/17 (100)	10/15 (66.7)
Apramycin	71/328 (21.6)	7/17 (41.2)	1/15 (6.7)
Cefotaxime	0/328 (0)	0/3 (0)	0/15 (0)
Cefpodoxime	-	1/14 (7.1)	-
Ceftazidime	0/328 (0)	0/17 (0)	-
Chloramphenicol	198/328 (60.4)	0/3 (0)	-
Ciprofloxacin	1/328 (0.3)	0/3 (0)	-
Enrofloxacin	-	1/14 (7.1)	0/15 (0)
Florfenicol	-	1/14 (7.1)	3/15 (20.0)
Furazolidone	0/328 (0)	0/3 (0)	-
Gentamicin	71/328 (21.6)	3/3 (100)	-
Nalidixic acid	2/328 (0.6)	0/3 (0)	-
Neomycin	106/328 (32.3)	14/14 (100)	0/15 (0)
Spectinomycin	-	3/3 (100)	7/15 (46.7)
Streptomycin	190/328 (57.9)	3/3 (100)	15/15 (100)
Sulfonamide compounds	246/328 (75.0)	3/3 (100)	-
Tetracycline	189/328 (57.6)	16/17 (94.1)	9/15 (60.0)
Trimethoprim/sulfonamide	210/328 (64.0)	7/14 (50.0)	5/15 (33.3)

Table S4.2.4: Resistance (tested by broth microdilution and MIC values interpreted using clinical breakpoints) of *Brachyspira hyodysenteriae* from infections of pigs in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Brachyspira hyodysenteriae
Doxycycline	0/31 (0)
Lincomycin	9/31 (29.0)
Tiamulin	6/31 (19.4)
Tylosin	18/31 (58.1)
Tylvalosin	8/31 (25.8)



Table S4.2.5: Resistance (tested by broth microdilution and MIC values interpreted using clinical breakpoints) of *Actinobacillus pleuropneumoniae* and *Pasteurella multocida* from respiratory infections of pigs in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Actinobacillus pleuropneumoniae	Pasteurella multocida	
Ampicillin	2/6 (33.3)	1/30 (3.3)	
Amoxicillin/clavulanate	-	0/30 (0)	
Ceftiofur	0/6 (0)	0/30 (0)	
Doxycycline	0/6 (0)	0/30 (0)	
Enrofloxacin	0/6 (0)	0/30 (0)	
Gamithromycin	-	0/30 (0)	
Florfenicol	0/6 (0)	0/30 (0)	
Spectinomycin	-	0/30 (0)	
Tetracycline	1/6 (16.7)	4/30 (13.3)	
Tiamulin	0/6 (0)	-	
Tildipirosin	0/6 (0)	0/30 (0)	
Tilmicosin	0/6 (0)	0/30 (0)	
Trimethoprim/sulfonamide	1/6 (16.7)	5/30 (16.7)	
Tulathromycin	0/6 (0)	0/30 (0)	

Table S4.2.6 Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Erysipelothrix rhusiopathiae, Staphylococcus hyicus* and *Staphylococcus xylosus* from infections of pigs in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Erysipelothrix rhusiopathiae	Staphylococcus hyicus	Staphylococcus xylosus
Ampicillin	0/4 (0)	3/5 (60.0)	0/1 (0)
Enrofloxacin	0/4 (0)	0/5 (0)	0/1 (0)
Lincomycin	0/4 (0)	3/5 (60.0)	0/1 (0)
Penicillin	0/4 (0)	3/5 (60.0)	0/1 (0)
Tetracycline	0/4 (0)	3/5 (60.0)	0/1 (0)
Trimethoprim/sulfonamide	3/4 (0)	0/5 (0)	0/1 (0)
Tylosin	0/4 (0)	1/5 (20.0)	0/1 (0)

71

Table S4.2.7: Resistance (tested by broth microdilution and MIC values interpreted using clinical breakpoints) of *Streptococcus suis* from infections of pigs in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Streptococcus suis	
Ceftiofur	0/56 (0)	
Doxycycline	39/56 (69.6)	
Enrofloxacin	0/56 (0)	
Erythromycin	19/56 (33.9)	
Florfenicol	0/56 (0)	
Lincomycin	18/56 (32.1)	
Penicillin	0/56 (0)	
Tetracycline	47/56 (83.9)	
Trimethoprim/sulfonamide	2/56 (3.6)	

S4.3: Clinical surveillance data for isolates from poultry

Table S4.3.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *E. coli* from chickens (all ages) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amoxicillin/clavulanate	5/59 (8.5)	29/60 (48.3)	0/2 (0)
Ampicillin	46/121 (38.0)	47/60 (78.3)	0/2 (0)
Apramycin	1/121 (0.8)	16/60 (26.7)	1/2 (50.0)
Cefotaxime	-	-	0/2 (0)
Cefpodoxime	0/121 (0)	46/60 (76.7)	-
Doxycycline	36/121 (29.8)	-	-
Enrofloxacin	2/121 (1.7)	7/59 (11.9)	0/2 (0)
Florfenicol	-	-	0/2 (0)
Neomycin	1/59 (1.7)	60/60 (100)	0/2 (0)
Spectinomycin	16/120 (13.3)	-	0/2 (0)
Streptomycin	-	-	1/2 (50.0)
Tetracycline	37/121 (30.6)	41/60 (68.3)	0/2 (0)
Trimethoprim/sulfonamide	16/121 (13.2)	11/60 (18.3)	0/2 (0)



Table S4.3.2: Resistance (tested by disc diffusion and interpreted using breakpoints) in all *E. coli* from turkeys (all ages) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amoxicillin/clavulanate	-	0/1 (0)	0/1 (0)
Ampicillin	2/2 (100)	0/1 (0)	0/1 (0)
Apramycin	0/2 (0)	0/1 (0)	0/1 (0)
Cefotaxime	-	-	0/1 (0)
Cefpodoxime	0/2 (0)	0/1 (0)	-
Doxycycline	1/2 (50.0)	-	-
Enrofloxacin	0/2 (0)	0/1 (0)	0/1 (0)
Florfenicol	-	-	0/1 (0)
Neomycin	-	1/1 (100)	0/1 (0)
Spectinomycin	0/2 (0)	-	1/1 (100)
Streptomycin	-	-	1/1 (100)
Tetracycline	1/2 (50.0)	0/1 (0)	1/1 (100)
Trimethoprim/sulfonamide	0/2 (0)	0/1 (0)	0/1 (0)



Table S4.3.3: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *Salmonella* from chickens and turkeys (all ages) in England and Wales and Scotland in 2023. In Northern Ireland only chickens were tested in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

	England and Wales		Northern Ireland	Scotla	and
Antibiotic	Chickens	Turkeys	Chickens	Chickens	Turkeys
Amikacin	0/1945 (0)	0/76 (0)	-	-	-
Amoxicillin/ clavulanate	2/1945 (0.1)	0/76 (0)	1/20 (5.0)	0/1 (0)	0/1 (0)
Ampicillin	49/1945 (2.5)	16/76 (21.1)	1/20 (5.0)	0/1 (0)	0/1 (0)
Apramycin	4/1945 (0.2)	0/76 (0)	0/20 (0)	0/1 (0)	0/1 (0)
Cefotaxime	0/1945 (0)	0/76 (0)	0/20 (0)	0/1 (0)	0/1 (0)
Ceftazidime	0/1945 (0)	0/76 (0)	0/20 (0)	-	-
Chloramphenicol	12/1945 (0.6)	0/76 (0)	1/20 (5.0)	-	-
Ciprofloxacin	6/1945 (0.3)	0/76 (0)	1/20 (5.0)	-	-
Enrofloxacin	-	-	-	0/1 (0)	0/1 (0)
Florfenicol	-	-	-	0/1 (0)	0/1 (0)
Furazolidone	10/1945 (0.5)	0/76 (0)	0/20 (0)	-	-
Gentamicin	8/1945 (0.4)	0/76 (0)	0/20 (0)	-	-
Nalidixic acid	21/1945 (1.1)	6/76 (7.9)	0/20 (0)	-	-
Neomycin	23/1945 (1.2)	1/76 (1.3)	-	0/1 (0)	0/1 (0)
Spectinomycin	-	-	1/20 (5.0)	0/1 (0)	0/1 (0)
Streptomycin	109/1945 (5.6)	17/76 (22.4)	11/20 (55.0)	1/1 (100)	0/1 (0)
Sulfonamide compounds	264/1945 (13.6)	30/76 (39.5)	2/20 (10.0)	-	-
Tetracycline	117/1945 (6.0)	34/76 (44.7)	0/20 (0)	0/1 (0)	0/1 (0)
Trimethoprim/ sulfonamide	216/1945 (11.1)	12/76 (15.8)	-	0/1 (0)	0/1 (0)

Table S4.3.4: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Klebsiella pneumoniae* from respiratory infections of chickens in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Klebsiella pneumoniae
Amoxicillin/clavulanate	0/1 (0)
Ampicillin	1/1 (100)
Apramycin	0/1 (0)
Cefpodoxime	0/1 (0)
Doxycycline	0/1 (0)
Enrofloxacin	0/1 (0)
Neomycin	0/1 (0)
Spectinomycin	0/1 (0)
Tetracycline	0/1 (0)
Trimethoprim/sulfonamide	0/1 (0)

Table S4.3.5: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Erysipelothrix rhusiopathiae*, *Staphylococcus aureus* and *Staphylococcus xylosus* from infections of chickens in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates.

Antibiotic	Erysipelothrix rhusiopathiae	Staphylococcus aureus	Staphylococcus xylosus
Amoxicillin/clavulanate	-	0/3 (0)	0/1 (0)
Ampicillin	0/1 (0)	0/3 (0)	1/2 (50.0)
Doxycycline	0/1 (0)	0/3 (0)	0/2 (0)
Enrofloxacin	0/1 (0)	0/3 (0)	0/2 (0)
Erythromycin	-	0/3 (0)	-
Lincomycin	0/1 (0)	0/3 (0)	0/2 (0)
Penicillin	0/1 (0)	0/3 (0)	1/2 (50.0)
Tetracycline	0/1 (0)	0/3 (0)	0/2 (0)
Trimethoprim/sulfonamide	1/1 (100)	0/3 (0)	0/2 (0)
Tylosin	0/1 (0)	0/3 (0)	0/2 (0)

S4.4: Clinical surveillance data for isolates from cattle

Table S4.4.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in *E. coli* mastitis isolates from England and Wales for 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	2023
Amoxicillin/clavulanate	3/33 (9.1)
Ampicillin	15/33 (45.5)
Cefpodoxime	0/33 (0)
Enrofloxacin	0/33 (0)
Neomycin	0/33 (0)
Streptomycin	3/33 (9.1)
Tetracycline	2/33 (6.1)
Trimethoprim/sulfonamide	2/33 (6.1)

Table S4.4.2: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Staphylococci* and *Streptococci* from mastitis cases from England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Staphylococcus aureus	Streptococcus dysgalactiae	Streptococcus uberis
Amoxicillin/clavulanate	1/20 (5.0)	0/13 (0)	0/26 (0)
Ampicillin	6/20 (30.0)	0/13 (0)	0/26 (0)
Cefalexin	0/20 (0)	0/13 (0)	0/26 (0)
Neomycin	0/20 (0)	1/12 (8.3)	16/23 (69.6)
Novobiocin	0/20 (0)	0/12 (0)	0/23 (0)
Penicillin	6/20 (30.0)	0/13 (0)	0/26 (0)
Tetracycline	0/20 (0)	13/13 (100)	13/26 (50.0)
Tylosin	0/20 (0)	2/13 (15.4)	3/26 (11.5)

76

Table S4.4.3: Resistance (tested by disc diffusion and interpreted clinical using breakpoints) of *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Trueperella pyogenes* from mastitis cases from England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested in brackets.

Antibiotic	Klebsiella pneumoniae	Pseudomonas aeruginosa	Trueperella pyogenes
Amoxicillin/clavulanate	0/16 (0)	5/5 (100)	0/1 (0)
Ampicillin	15/16 (93.8)	5/5 (100)	0/1 (0)
Cefalexin	-	-	0/1 (0)
Cefotaxime	-	5/5 (100)	-
Cefpodoxime	0/12 (0)	-	-
Ceftazidime	-	1/5 (20.0)	-
Enrofloxacin	0/16 (0)	0/5 (0)	-
Neomycin	0/7 (0)	-	0/1 (0)
Novobiocin	-	-	0/1 (0)
Penicillin	-	-	0/1 (0)
Streptomycin	0/3 (0)	-	-
Tetracycline	0/16 (0)	5/5 (100)	0/1 (0)
Trimethoprim/sulfonamide	0/16 (0)	5/5 (100)	-
Tylosin	-	-	0/1 (0)

Table S4.4.4: Resistance (tested by disc diffusion and interpreted using clinical

breakpoints) in all *E. coli* from cattle (all ages) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amikacin	0/102 (0)	-	-
Amoxicillin/clavulanate	53/306 (17.3)	212/416 (51.0)	7/19 (36.8)
Ampicillin	151/306 (49.3)	333/416 (80.0)	15/19 (78.9)
Apramycin	3/283 (1.1)	46/306 (15.0)	0/19 (0)
Cefotaxime	9/103 (8.7)	-	-
Cefpodoxime	-	235/415 (56.6)	2/19 (10.5)
Ceftazidime	5/103 (4.9)	-	-
Chloramphenicol	30/102 (29.4)	-	-
Enrofloxacin	2/306 (0.7)	149/416 (35.8)	0/19 (0)
Florfenicol	37/125 (29.6)	191/304 (62.8)	5/19 (26.3)
Neomycin	56/283 (19.8)	416/416 (100.0)	7/19 (36.8)
Spectinomycin	41/283 (14.5)	6/109 (5.5)	4/19 (21.1)
Streptomycin	48/102 (47.1)	108/110 (98.2)	-
Tetracycline	149/306 (48.7)	281/416 (67.5)	11/19 (57.9)

77

Table S4.4.5: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in *E. coli* from cattle in a) England and Wales, b) Northern Ireland and c) Scotland for 2023, split by age category. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Please note that no pre-weaning or adult data is available for Northern Ireland.

- Antibiotic Neonatal **Pre-weaning** Adult Amikacin 0/84 (0) 0/18 (0) Amoxicillin/clavulanate 26/109 (23.9) 17/87 (19.5) 0/52 (0) Ampicillin 79/109 (72.5) 46/87 (52.9) 5/52 (9.6) Apramycin 2/108 (1.9) 1/79 (1.3) 0/50 (0) Cefotaxime 8/84 (9.5) 1/19 (5.3) Ceftazidime 4/84 (4.8) 1/19 (5.3) -21/84 (25.0) 9/18 (50.0) Chloramphenicol Enrofloxacin 1/109 (0.9) 1/87 (1.1) 0/52 (0) Florfenicol 19/85 (22.4) 13/26 (50.0) 30/108 (27.8) 0/50 (0) Neomycin 21/79 (26.6) Spectinomycin 21/108 (19.4) 12/79 (15.2) 2/50 (4.0) Streptomycin 38/84 (45.2) 10/18 (55.6) 59/109 (54.1) 8/52 (15.4) Tetracycline 55/87 (63.2) Trimethoprim/sulfonamide 34/109 (31.2) 34/87 (39.1) 4/52 (7.7)
- a) England and Wales

b) Northern Ireland

Antibiotic	Neonatal
Amoxicillin/clavulanate	30/52 (57.7)
Ampicillin	45/52 (86.5)
Apramycin	7/52 (13.5)
Cefpodoxime	26/52 (50.0)
Enrofloxacin	25/52 (48.1)
Florfenicol	36/52 (69.2)
Neomycin	52/52 (100.0)
Tetracycline	41/52 (78.8)
Trimethoprim/sulfonamide	38/52 (73.1)



c) Scotland

Antibiotic	Neonatal	Pre-weaning	Adult
Amoxicillin/clavulanate	2/7 (28.6)	4/6 (66.7)	1/6 (16.7)
Ampicillin	6/7 (85.7)	6/6 (100)	3/6 (50.0)
Apramycin	0/7 (0)	0/6 (0)	0/6 (0)
Cefpodoxime	0/7 (0)	2/6 (33.3)	0/6 (0)
Enrofloxacin	2/7 (28.6)	0/6 (0)	0/6 (0)
Florfenicol	0/7 (0)	4/6 (66.7)	1/6 (16.7)
Neomycin	1/7 (14.3)	4/6 (66.7)	2/6 (33.3)
Spectinomycin	1/7 (14.3)	1/6 (16.7)	2/6 (33.3)
Tetracycline	2/7 (28.6)	5/6 (83.3)	4/6 (66.7)
Trimethoprim/sulfonamide	4/7 (57.1)	4/6 (66.7)	2/6 (33.3)

Table S4.4.6: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *Salmonella* from cattle (all ages) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amikacin	0/471 (0)	-	-
Amoxicillin/clavulanate	0/471 (0)	0/69 (0)	1/96 (1.0)
Ampicillin	21/471 (4.5)	6/69 (8.7)	3/96 (3.1)
Apramycin	1/471 (0.2)	2/69 (2.9)	2/96 (2.1)
Cefotaxime	0/471 (0)	0/2 (0)	-
Cefpodoxime	-	3/67 (4.5)	0/96 (0)
Ceftazidime	0/471 (0)	0/69 (0)	-
Chloramphenicol	8/471 (1.7)	0/2 (0)	-
Ciprofloxacin	0/471 (0)	0/2 (0)	-
Enrofloxacin	-	1/67 (1.5)	1/96 (1.0)
Florfenicol	-	2/67 (3.0)	3/96 (3.1)
Furazolidone	0/471 (0)	0/2 (0)	-
Gentamicin	0/471 (0)	0/2 (0)	-
Nalidixic acid	6/471 (1.3)	1/2 (50.0)	0/96 (0)
Neomycin	0/471 (0)	65/67 (97.0)	0/96 (0)
Spectinomycin	-	0/2 (0)	2/96 (2.1)
Streptomycin	46/471 (9.8)	0/2 (0)	-
Sulfonamide compounds	39/471 (8.3)	0/2 (0)	-
Tetracycline	34/471 (7.2)	8/69 (11.6)	3/96 (3.1)
Trimethoprim/sulfonamide	2/471 (0.4)	3/67 (4.5)	2/96 (2.1)



Table S4.4.7 Resistance (tested by broth microdilution and MIC values interpreted using clinical breakpoints) of *Mannheimia haemolytica* and *Pasteurella multocida* from respiratory infections of cattle in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Mannheimia haemolytica	Pasteurella multocida
Amoxicillin/clavulanate	0/70 (0)	1/128 (0.8)
Ampicillin	1/70 (1.4)	2/128 (1.6)
Ceftiofur	0/70 (0)	0/128 (0)
Doxycycline	0/70 (0)	0/128 (0)
Enrofloxacin	1/70 (1.4)	0/128 (0)
Erythromycin	-	-
Florfenicol	2/70 (2.9)	5/128 (3.9)
Gamithromycin	0/70 (0)	21/128 (16.4)
Lincomycin	-	-
Neomycin	-	-
Penicillin	-	-
Spectinomycin	0/70 (0)	43/128 (33.6)
Tetracycline	3/70 (4.3)	70/128 (54.7)
Tildipirosin	0/70 (0)	20/128 (15.6)
Tilmicosin	1/70 (1.4)	24/128 (18.8)
Trimethoprim/sulfonamide	0/70 (0)	0/128 (0)
Tulathromycin	0/70 (0)	21/128 (16.4)

Table S4.4.8: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Histophilus somni* from respiratory infections of cattle in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Histophilus somni
Amoxicillin/clavulanate	0/27 (0)
Ampicillin	0/27 (0)
Cefpodoxime	0/27 (0)
Enrofloxacin	0/27 (0)
Florfenicol	0/27 (0)
Tetracycline	0/27 (0)
Trimethoprim/sulfonamide	0/27 (0)



Table S4.4.9: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Listeria monocytogenes* and *Staphylococcus xylosus* from infections of cattle in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested.

Antibiotic	Listeria monocytogenes	Staphylococcus xylosus
Amoxicillin/clavulanate	0/4 (0)	0/2 (0)
Ampicillin	0/4 (0)	1/2 (50.0)
Cefalexin	0/4 (0)	0/2 (0)
Neomycin	-	0/1 (0)
Novobiocin	-	0/1 (0)
Penicillin	0/4 (0)	1/2 (50.0)
Tetracycline	0/4 (0)	0/2 (0)
Trimethoprim/sulfonamide	0/4 (0)	-
Tylosin	0/4 (0)	0/2 (0)

S4.5: Clinical surveillance data for isolates from sheep

Table S4.5.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *E. coli* from sheep (all ages) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amikacin	0/65 (0)	-	-
Amoxicillin/clavulanate	14/259 (5.4)	20/52 (38.5)	3/17 (17.6)
Ampicillin	72/259 (27.8)	34/52 (65.4)	5/17 (29.4)
Apramycin	2/231 (0.9)	3/51 (5.9)	0/17 (0)
Cefotaxime	1/65 (1.5)	-	-
Cefpodoxime	-	25/50 (50.0)	0/17 (0)
Ceftazidime	0/65 (0)	-	-
Chloramphenicol	4/65 (6.2)	-	-
Enrofloxacin	3/259 (1.2)	7/52 (13.5)	0/17 (0)
Florfenicol	5/92 (5.4)	15/52 (28.8)	1/17 (5.9)
Neomycin	10/232 (4.3)	52/52 (100)	0/17 (0)
Spectinomycin	33/231 (14.3)	-	6/17 (35.3)
Streptomycin	16/66 (24.2)	-	-
Tetracycline	90/259 (34.7)	30/52 (57.7)	10/17 (58.8)
Trimethoprim/sulfonamide	17/258 (6.6)	13/51 (25.5)	0/17 (0)



Table S4.5.2: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in *E. coli* from sheep in a) England and Wales, b) Northern Ireland and c) Scotland from 2023, split by age category. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Please note that no pre-weaning or adult data is available for Northern Ireland.

- Antibiotic Neonatal **Pre-weaning** Adult Amikacin 0/53 (0) Amoxicillin/clavulanate 6/89 (6.7) 3/45 (6.7) 4/63 (6.3) Ampicillin 30/89 (33.7) 20/45 (44.4) 10/63 (15.9) Apramycin 0/87 (0) 1/43 (2.3) 1/54 (1.9) Cefotaxime 0/53 (0) 1/6 (16.7) 0/53 (0) Ceftazidime 0/6 (0) -2/53 (3.8) 1/6 (16.7) Chloramphenicol Enrofloxacin 2/89 (2.2) 1/45 (2.2) 0/63 (0) 2/12 (16.7) Florfenicol 2/55 (3.6) 1/8 (12.5) Neomycin 3/87 (3.4) 5/43 (11.6) 1/55 (1.8) Spectinomycin 19/87 (21.8) 8/43 (18.6) 5/54 (9.3) Streptomycin 10/53 (18.9) 6/6 (100) 25/45 (55.6) 16/63 (25.4) Tetracycline 37/89 (41.6) Trimethoprim/sulfonamide 8/89 (9.0) 7/45 (15.6) 2/63 (3/2)
- a) England and Wales

b) Northern Ireland

Antibiotic	Neonatal
Amoxicillin/clavulanate	13/28 (46.4)
Ampicillin	24/28 (85.7)
Apramycin	2/28 (7.1)
Cefpodoxime	16/27 (59.3)
Enrofloxacin	5/28 (17.9)
Florfenicol	10/28 (35.7)
Neomycin	28/28 (100)
Tetracycline	18/28 (64.3)
Trimethoprim/sulfonamide	9/27 (33.3)



c) Scotland

Antibiotic	Neonatal	Pre-weaning	Adult
Amoxicillin/clavulanate	3/13 (23.1)	-	0/4 (0)
Ampicillin	5/13 (38.5)	-	0/4 (0)
Apramycin	0/13 (0)	-	0/4 (0)
Cefpodoxime	0/13 (0)	-	0/4 (0)
Enrofloxacin	0/13 (0)	-	0/4 (0)
Florfenicol	1/13 (7.7)	-	0/4 (0)
Neomycin	0/13 (0)	-	0/4 (0)
Spectinomycin	5/13 (38.5)	-	2/4 (50.0)
Tetracycline	9/13 (69.2)	-	2/4 (50.0)
Trimethoprim/sulfonamide	0/13 (0)	-	0/4 (0)

Table S4.5.3: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *Salmonella* from sheep (all ages) in England and Wales, Northern Ireland and Scotland in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	England and Wales	Northern Ireland	Scotland
Amikacin	0/64 (0)	-	-
Amoxicillin/clavulanate	0/64 (0)	0/24 (0)	0/38 (0)
Ampicillin	1/64 (1.5)	6/24 (25.0)	3/38 (7.9)
Apramycin	1/64 (1.5)	0/24 (0)	0/38 (0)
Cefotaxime	0/64 (0)	-	-
Cefpodoxime	-	1/24 (4.2)	0/38 (0)
Ceftazidime	0/64 (0)	1/24 (4.2)	-
Chloramphenicol	1/64 (1.5)	-	-
Ciprofloxacin	0/64 (0)	-	-
Enrofloxacin	-	0/24 (0)	0/38 (0)
Florfenicol	-	1/24 (4.2)	1/38 (2.6)
Furazolidone	0/64 (0)	-	-
Gentamicin	1/64 (1.5)	-	-
Nalidixic acid	0/64 (0)	-	0/38 (0)
Neomycin	0/64 (0)	23/24 (95.8)	1/38 (2.6)
Spectinomycin	-	-	2/38 (5.3)
Streptomycin	2/64 (3.0)	-	-
Sulfonamide compounds	1/64 (1.5)	-	-
Tetracycline	1/64 (1.5)	5/24 (20.8)	1/38 (2.6)
Trimethoprim/sulfonamide	1/64 (1.5)	1/24 (4.2)	1/38 (2.6)

83

Table S4.5.4: Resistance (tested by broth microdilution and MIC values interpreted using clinical breakpoints) of *Bibersteinia trehalosi*, *Mannheimia haemolytica* and *Pasteurella multocida* from respiratory infections of sheep in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates.

Antibiotic	Bibersteinia trehalosi	Mannheimia haemolytica	Pasteurella multocida
Amoxicillin/clavulanate	1/47 (2.1)	0/96 (0)	0/23 (0)
Ampicillin	2/47 (4.3)	0/96 (0)	0/23 (0)
Ceftiofur	0/47 (0)	0/96 (0)	0/23 (0)
Doxycycline	0/47 (0)	0/96 (0)	0/23 (0)
Enrofloxacin	0/47 (0)	0/96 (0)	0/23 (0)
Florfenicol	0/47 (0)	0/96 (0)	0/23 (0)
Gamithromycin	0/47 (0)	0/96 (0)	1/23 (4.3)
Spectinomycin	0/47 (0)	0/96 (0)	1/23 (4.3)
Tetracycline	0/47 (0)	0/96 (0)	0/23 (0)
Tildipirosin	1/47 (2.1)	0/96 (0)	1/23 (4.3)
Tilmicosin	1/47 (2.1)	0/96 (0)	1/23 (4.3)
Trimethoprim/sulfonamide	0/47 (0)	0/96 (0)	0/23 (0)
Tulathromycin	0/47 (0)	0/96 (0)	1/23 (4.3)

Table S4.5.5: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Trueperella pyogenes* from respiratory infections of sheep in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Trueperella pyogenes
Amoxicillin/ clavulanate	0/1 (0)
Ampicillin	0/1 (0)
Cefalexin	0/1 (0)
Penicillin	0/1 (0)
Tetracycline	0/1 (0)
Tylosin	0/1 (0)

84

Table S4.5.6: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Staphylococcus aureus* and *Streptococcus dysgalactiae* from infections of sheep in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Staphylococcus aureus	Streptococcus dysgalactiae
Amoxicillin/ clavulanate	0/18 (0)	0/20 (0)
Ampicillin	0/18 (0)	0/20 (0)
Cefalexin	0/18 (0)	0/20 (0)
Neomycin	0/12 (0)	-
Penicillin	0/18 (0)	0/20 (0)
Tetracycline	4/18 (22.2)	20/20 (100)
Trimethoprim/ sulfonamide	0/6 (0)	0/5 (0)
Tylosin	0/18 (0)	1/20 (5.0)

Table S4.5.7: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) of *Erysipelothrix rhusiopathiae, Listeria ivanovii* and *Listeria* monocytogenes from infections of sheep in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Erysipelothrix rhusiopathiae	Listeria ivanovii	Listeria monocytogenes
Amoxicillin/ clavulanate	0/1 (0)	0/4 (0)	0/3 (0)
Ampicillin	0/1 (0)	0/4 (0)	0/3 (0)
Cefalexin	0/1 (0)	0/4 (0)	0/3 (0)
Florfenicol	-	0/4 (0)	-
Penicillin	0/1 (0)	0/4 (0)	0/3 (0)
Tetracycline	0/1 (0)	0/4 (0)	0/3 (0)
Trimethoprim/ sulfonamide	-	0/4 (0)	0/3 (0)
Tylosin	0/1 (0)	0/4 (0)	0/3 (0)

S4.6: Clinical surveillance data for isolates from dogs

Table S4.6.1: Resistance (tested by disc diffusion and interpreted using clinical breakpoints) in all *Salmonella* from dogs in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	England and Wales
Amikacin	0/757 (0)
Amoxicillin/clavulanate	4/757 (0.5)
Ampicillin	74/757 (9.8)
Apramycin	6/757 (0.8)
Cefotaxime	7/757 (0.9)
Ceftazidime	7/757 (0.9)
Chloramphenicol	23/757 (3.0)
Ciprofloxacin	8/757 (1.1)
Furazolidone	8/757 (1.1)
Gentamicin	10/757 (1.3)
Nalidixic acid	26/757 (3.4)
Neomycin	18/757 (2.4)
Streptomycin	74/757 (9.8)
Sulfonamide compounds	75/757 (9.9)
Tetracycline	82/757 (10.8)
Trimethoprim/sulfonamide	34/757 (4.5)



S4.7: Clinical surveillance data for isolates from trout

Table S4.7.1: Resistance (tested by broth microdilution and MIC values interpreted using a combination of CLSI and internally generated epidemiological cut-off values of *Aeromonas salmonicida* and *Yersinia ruckeri* from infections of trout in England and Wales in 2023. The table shows the number of resistant isolates out of the total number tested and the percentage of resistant isolates in brackets.

Antibiotic	Aeromonas salmonicida	Yersinia ruckeri
Ampicillin	-	1/11 (9.1)
Ceftazidime	0/2 (0)	1/11 (9.1)
Enrofloxacin	0/2 (0)	6/11 (54.5)
Florfenicol	0/2 (0)	0/11 (0)
Gentamicin	0/2 (0)	0/11 (0)
Meropenem	1/2 (50)	1/11 (9.1)
Oxolinic acid	1/2 (50)	6/11 (54.5)
Oxytetracycline	0/2 (0)	0/11 (0)
Sulfamethoxazole	1/2 (50)	10/11 (90.9)
Trimethoprim/ sulfamethoxazole	0/2 (0)	0/11 (0)

