Antimicrobial Resistance in *Salmonella enterica*: Trends and Risk Factor Analysis using Scanning Surveillance Data

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Background

The World Health Organisation (WHO) has classified antimicrobial resistance (AMR) as "one of the top 10 global public health threats facing humanity" (WHO, 2020). The use of antibiotics in human medicine and livestock production is contributing to the acquisition of resistance (WHO, 2015) and humans can be exposed to resistant bacteria through the consumption of animal derived food products and agricultural waste (WHO, 2015).

Salmonella is an important foodborne pathogen, with over 80,000 laboratory reports of human cases from 2010 to 2019 in England (UK Health Security Agency, 2021). Human infections are most commonly associated with *S*. Typhimurium and *S*. Enteritidis (UK Health Security Agency, 2021), while *S*. Typhimurium is the most common serovar isolated from pigs (AHDB, 2022). One way in which humans can become infected is through the consumption of undercooked or raw, contaminated pork products. AMR in *Salmonella* from pigs therefore represents a threat to both human and animal health. More evidence-based research is needed to identify factors associated with AMR to allow control efforts to be targeted areas of highest risk.

The objectives of this study were to use veterinary scanning surveillance data to describe trends in AMR in *Salmonella* isolates originating from pigs in England and Wales from 2010 to 2020, and to carry out risk factor analysis to identify factors associated with AMR.

Materials and Methods

The dataset was obtained via the veterinary diagnostic scanning surveillance programme operated in England and Wales by the APHA. This provided a convenient sample population that allowed AMR to be investigated over a long period of time. The dataset comprised of 1,590 pig *Salmonella* isolates derived from clinical or post-mortem samples submitted by veterinarians for diagnostic investigation of field cases of disease in pigs from 2010 to 2020. These isolates had been tested for susceptibility to a panel of up to 16 antibiotics using disc diffusion.

Where multiple isolates were collected from the same group of animals within a farm, one isolate was randomly selected. After this step, the final dataset comprised of 929 isolates. Only antibiotics with established or legacy disc diffusion breakpoints were included in the analysis. The classification scheme as per the UK-VARSS 2020 report was used to identify antibiotic classes (Table 1).

Trend analysis was carried out for individual antibiotics, antibiotic classes and multi-drug resistance (MDR), defined as resistance to three or more antibiotic classes. Cross tabulations were produced against year and Cuzick's tests for linear trend were carried out. The risk factor variables investigated were: year category; organic production; age category; farm type; outdoor access; serovar (*S*. Typhimurium vs 'other/unknown'), geographical region and season.

Risk factor analysis was carried out for individual antibiotics and MDR. Univariable and multivariable analyses were carried out using mixed-effects logistic regression. Farm ID was included as a random effect to account for clustering at the farm level. The year and season variables were included in the models *a priori*.

Table 1: Antibiotic classification scheme as per the UK-VARSS 2020 report

Antibiotic Class	Antibiotic	Disc diffusion breakpoint[b]
Cephalosporins (3rd Gen)[a]	Cefotaxime[a]	BSAC
	Ceftazidime[a]	BSAC
Quinolones[a]	Ciprofloxacin[a]	BSAC
Aminoglycosides	Amikacin	BSAC
	Apramycin	APHA
	Gentamicin	BSAC
	Neomycin	APHA
	Streptomycin	BSAC
Aminopenicillins/ Amoxicillin/ Clavulanate	Ampicillin	BSAC
	Amoxicillin Clavulanate	BSAC
Tetracyclines	Tetracycline	APHA
Amphenicols	Chloramphenicol	BSAC
Sulphonamides	Trimethoprim/Sulfamethoxazole (TMS)	BSAC

[a] Highest Priority Critically Important Antibiotic
[b] BSAC = BSAC legacy human clinical breakpoint; APHA = APHA derived veterinary disc diffusion zone size breakpoint:
  UK-VARSS 2020 Supplementary material (publishing.service.gov.uk)

Trend analysis results
The key results from the trend analysis are summarised below.

Extremely high levels of resistance were observed for streptomycin (80.7%), tetracycline (81.9%) and ampicillin (80.0%). There was no evidence of a linear trend for streptomycin, but at the aminoglycosides class level there was a significant downward trend from 2010 to 2020 (p<0.001). For tetracycline there was evidence of a downward linear trend (p=0.016), but no evidence of a linear trend for ampicillin.

A high level of MDR was observed (76.1%). However, there was a notable decrease from 2014 to 2018 and evidence of a downward linear trend from 2010 to 2020 (p<0.001). The only antibiotic for which there was evidence of an increasing linear trend was amoxicillin clavulanate (p=0.021), though the level of resistance to this antibiotic was low at 1.1%.

Risk factor analysis results
For the four antibiotic resistance outcomes with the highest overall levels of resistance, the final multivariable models are summarised in Table 2.

There was strong evidence (all p<0.001) that *S*. Typhimurium isolates were more likely to be resistant than isolates in the 'other/unknown' serovar category for all four outcomes: streptomycin, ampicillin, tetracycline and MDR.

Isolates from samples collected in summer were less likely to be resistant than isolates from samples collected in spring, as seen for all four outcomes.

Associations between year category and AMR were observed for the tetracycline and MDR outcomes, with isolates from '2017-2020' less likely to be resistant than isolates from '2010-2012'.

For ampicillin, isolates from rearer farms were more likely to be resistant than isolates from breeder farms.

Conclusion
This study has identified a high prevalence of resistance to certain antibiotics in *Salmonella* from pigs from 2010 to 2020 and highlighted trends in AMR over time. In addition, it has provided evidence of associations between risk factors and resistance to specific antibiotics. The findings suggest that AMR reduction strategies should be targeted at the Typhimurium serovar, particularly given the importance of this serovar for human health, and also at rearing farms. Antimicrobial usage in pigs has shown a substantial decline over recent years and it will be interesting to replay this analysis in the future to investigate impacts on trends of resistance.

There are some limitations of the study: As the analysis is based on clinical cases, the findings may not be generalisable to the healthy animal population. In addition, the panel of antibiotics against which isolates were tested varied between years and between individual isolates. Some strains of *Salmonella* typically possess genetic structures, such as genomic islands or plasmids, which contain linked resistance genes and this will have influenced the outputs obtained, since these structures tend to be associated with particular serovars.

Table 2: Final multivariable risk factor models for the four antibiotic resistance outcomes with the highest overall levels.

Variable	Streptomycin			Ampicillin			Tetracycline			MDR[a]		
	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value	OR	95% CI	p-value
Year												
2010-2012	1	-	-	1	-	-	1	-	-	1	-	-
2013-2016	1.08	0.59 – 2.00	0.794	1.26	0.63 – 2.50	0.51	0.95	0.46 – 1.98	0.897	0.93	0.50 – 1.76	0.835
2017-2020	0.64	0.33 – 1.28	0.208	1.29	0.53 – 3.13	0.581	0.35	0.16 – 0.74	0.006	0.24	0.12 – 0.47	<0.001
Serovar												
Other/ Unknown	1	-	-	1	-	-	1	-	-	1	-	-
Typhimurium	86.57	30.74 – 243.83	<0.001	200.69	46.87 – 859.37	<0.001	54.46	20.18 – 147.01	<0.001	135.56	40.16 – 457.62	<0.001
Season												
Spring (Mar-May)	1	-	-	1	-	-	1	-	-	1	-	-
Summer (Jun-Aug)	0.45	0.21 – 0.96	0.04	0.38	0.16 – 0.91	0.029	0.34	0.14 – 0.82	0.017	0.41	0.19 – 0.88	0.021
Autumn (Sep-Nov)	0.59	0.27 – 1.27	0.18	0.56	0.25 – 1.28	0.172	0.58	0.25 – 1.37	0.216	0.71	0.34 – 1.47	0.357
Winter (Dec-Feb)	0.75	0.35 – 1.64	0.48	0.82	0.35 – 1.97	0.664	0.7	0.29 – 1.66	0.419	0.67	0.32 – 1.42	0.299
Farm type												
Breeder				1	-	-						
Rearer				2.18	1.02 – 4.65	0.044						
Finisher				1.26	0.53 – 3.01	0.603						
Other				0.7	0.22 – 2.19	0.534						

[a]MDR = multi-drug resistance, defined as resistance to three or more antibiotic classes.