Superbugs on UK supermarket shelves

WORLD ANIMAL PROTECTION

Animal welfare standards influence level of antimicrobial resistance found on pork samples

Contents

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Superbugs on UK supermarket shelves: Animal welfare standards influence level of antimicrobial resistance found on pork samples

Cover image: A pig in a newly built industrial farm. Source: World Animal Protection.

1. Introduction

The health and wellbeing of animals, people and our planet are interdependent. Poor animal health and welfare in factory farming negatively affect food safety, our environment and climate. Ending factory farming will help curb the rise of antimicrobial resistance (AMR) from farm animals and stop superbugs in their tracks. It will bring better animal health and welfare, healthier diets for people and a climate-safe and sustainable food system.

The United Nations is calling for an end to unsustainable agricultural practices and acknowledges that intensive farming carries high risk of disease outbreaks and fallout for public and environmental health. It recognises that antibiotics are used to mask poor conditions for farm animals, and calls for investment in sustainable, agroecological food systems (1).

But we can only achieve a humane and sustainable food system if governments and industry take action to end factory farming. Ten years from now, to protect our animals, people and planet, the building of factory farms should have stopped. Existing factory farms should pay for the costs of their irresponsible practices, rather than communities paying the costs of poor public health and environmental pollution.

Pigs are one of the most intensively farmed animals in the world and in the UK receive the highest levels of antibiotics of all farmed land animals.

UK antibiotic use data shows that total antibiotic usage in pigs was 83.0 tonnes for 2020, which represents 105 mg per kg of live pig. This is a decrease of 5.5 mg/kg (5%) since 2019, and a reduction of 172.7 mg per kg (62%) since 2015 (2) (3).

The UK has until recently been a world leader in antibiotic reduction but is now falling behind the EU by not introducing crucial legislation that will reduce the risks of antimicrobial resistance and raise welfare for millions of farmed animals.

The UK uses more antibiotics in pig farming than other European countries. For example, antibiotic use in pigs in the UK is about five times higher than in Denmark (46mg/kg PCU[1]) and about 25 times higher than in Sweden (11mg/kg PCU)

[1]PCU refers to the 'Population Correction Unit' and takes into account the animal population as well as the estimated weight of each particular animal at the time of treatment with antibiotics

Image: These growing pigs have little space and have had their tails docked. Source: World Animal Protection.

2. UK Animal Welfare Standards

The UK claims to have some of the highest welfare standards in the world, however for the majority of pigs in the UK factory farming is the reality. An estimated 60% of mother pigs are caged in farrowing crates during the birth and rearing of their piglets (4). Many of the remaining piglets who are bred outdoors are moved to indoor pens for rearing. Despite routine tail docking being banned over 70% of pigs have their tails cut off at just a few days old due to a loophole (5). Some welfare improvements in UK law include the banning of pregnancy cages in 1999 and although it is not officially banned castration is not widely practised on UK pig farms.

There are several assured farming labels in the UK and this study looked at the prevalence of antimicrobial resistance depending on production method.

The pork samples from no assurance scheme (standard industry practice) are assumed to come from farms that meet UK minimum legislation but will not have the same level of monitoring that assured farms receive.

Red Tractor

The Red Tractor scheme, run by Assured Food Standards, certifies the food was produced in Britain and to certain quality standards for food safety, hygiene, and the environment, and reflects standard industry practice in the UK. Some requirements go beyond minimum legislation, such as prohibiting castration of meat pigs and the requirement for on-farm health and welfare monitoring.

RSPCA Assured

RPSCA Assured is the RSPCA's labelling and assurance scheme dedicated to improving welfare standards for farm animals. They have a number of welfare benefits above standard industry practice including more space, bedding, and enrichment. The scheme covers both indoor and outdoor rearing systems and on-farm health, and welfare monitoring is required.

Organic/Soil Association

Soil Association is one of the organic standards in the UK. They offer many welfare benefits exceeding standard industry practice, including prohibiting cage systems, providing bedding and environmental enrichment, providing free-range access with shade and shelter and monitoring welfare through outcome measures.

Image: Organic systems usually include outdoor access and have been shown to have reduced antibiotic resistance compared with raised without antibiotic factory farm systems. Source: World Animal Protection.

3. Methodology

World Animal Protection commissioned Fera Science Ltd to deliver a survey examining the prevalence of antimicrobial resistant Enterococci in pork meat from different food assurance schemes as well as some which had no assurance scheme.

To achieve this, a total of 103 samples from supermarkets located in or around York and online retailers were collected. The meat was then analysed in the laboratory for the presence of Enterococci bacteria. When these bacteria were found, they were then tested for susceptibility to different antibiotics, in other words whether antibiotics were effective in killing them or slowing their growth. Samples collected were split as follows:

- 27 samples of fresh diced / chops of pork / loin of pork with no assurance certification,
- 22 samples of fresh diced / chops of pork / loin of pork with Red Tractor assurance certification,
- 27 samples of fresh diced / chops of pork / loin of pork with RSPCA assurance certification,
- 27 samples of fresh diced / chops of pork / loin of pork or pork mince with Soil Association or equivalent organic assurance certification.

It was anticipated that pork may have become difficult to find during this study due to labour shortages and other supply issues. These included the nationwide CO_2 shortage which resulted in large numbers of animals being culled.

As far as practicable samples were collected from different retailers and at different times, in an effort to collect samples from a number of different suppliers. Due to lack of samples within the narrow geographic sampling area, the organic / Soil Association samples were all bought from online retailers (with World Animal Protection approval). All other samples were purchased directly from supermarkets at different locations and / or times.

Samples were purchased and either delivered to Fera on the same day or kept at +4°C overnight before delivery to Fera. Once received on site, samples were logged in and allocated a unique sample number using the Laboratory Information Management System (LIMS). The information recorded included the date of purchase, supermarket, use by date, label details and assurance scheme specified (if any). Samples were stored at -20°C once logged in and labelled to allow sample collection to be completed and bulk testing to be delivered. This reduced any variables associated with the initial sample testing.

Once thawed, samples were processed using sterile equipment. The processing involves making a solution using a small sample of meat. This meat solution is then placed on special plates used to promote the growth of Enterococci bacteria that may be present in the meat samples. Once this meat solution had been spread over the whole surface of the plates, they were incubated at a specific temperature to encourage the growth of bacterial colonies.

Antibiotic Susceptibility Testing

Pure bacterial colonies were used to make a special solution using a sterile implement and a special broth. Once the solution was ready, a sterile cotton swab was used to take a sample of the solution and transfer it to a new growth plate. The bacterial solution was added to the plate surface in a specific way which was suitable for testing multiple antibiotics on the same plate. This way, the effect of different antibiotics on the bacterial colonies growing on the plate could be visually compared. In addition to the test plates, control plates were prepared using strains of bacteria already known to be resistant and susceptible to specific antibiotics, in order to ensure that the testing method was working. In this test, all control strains reacted as expected.

Susceptibility Testing

A selection of antibiotics were chosen by World Animal Protection to test the Enterococci against. For these tests, a well-established method was used where the specially prepared plates (described in the paragraph above) were used to see which antibiotics were able to stop bacterial growth. Antibiotic discs were bought with a specific concentration of antibiotic added to them. These were put onto the surface of the specially prepared agar plates. Plates were then incubated and then a visual check was carried out. Where the antibiotic was able to stop the bacteria from growing, a clear ring or 'zone' was observed around the antibiotic disc. This clear ring is known as a zone of inhibition. If the antibiotic was not able to stop bacterial growth, there was no clear zone visible as the bacteria were able to grow right up to the edge of the disc without being inhibited.

Detailed methodology can be found in the Appendix

4. Findings

A total of 101 Enterococci isolates (the bacterial cultures recovered from the samples) were taken through for antimicrobial resistance (AMR) susceptibility testing. These came from twenty-seven different samples. The range of resistance and sensitivity to the panel of antibiotics screened against varied greatly. Some isolates demonstrated very little resistance to any antibiotics whereas others appeared to be resistant to many of those tested against. Red Tractor provided the highest number of positives for Enterococci.

No Enterococci isolate was resistant to all of the antibiotics screened against. The highest resistance profile was for sample S22-010032, Red Tractor Assured pork loin steaks from a major UK supermarket. Two of the five isolates from this sample were resistant to Vancomycin, Ciprofloxacin, Ofloxacin and Ampicillin. One of these was also resistant to Clindamycin. Several

isolates showed resistance to three or more of the eight antibiotics. Macrolide resistance was present in many of the isolates from the Red Tractor and non-assured samples and greater attention should be given to this in future studies.

Table 1: shows summary of incidence of resistance for all isolates to all antibiotics screened against.

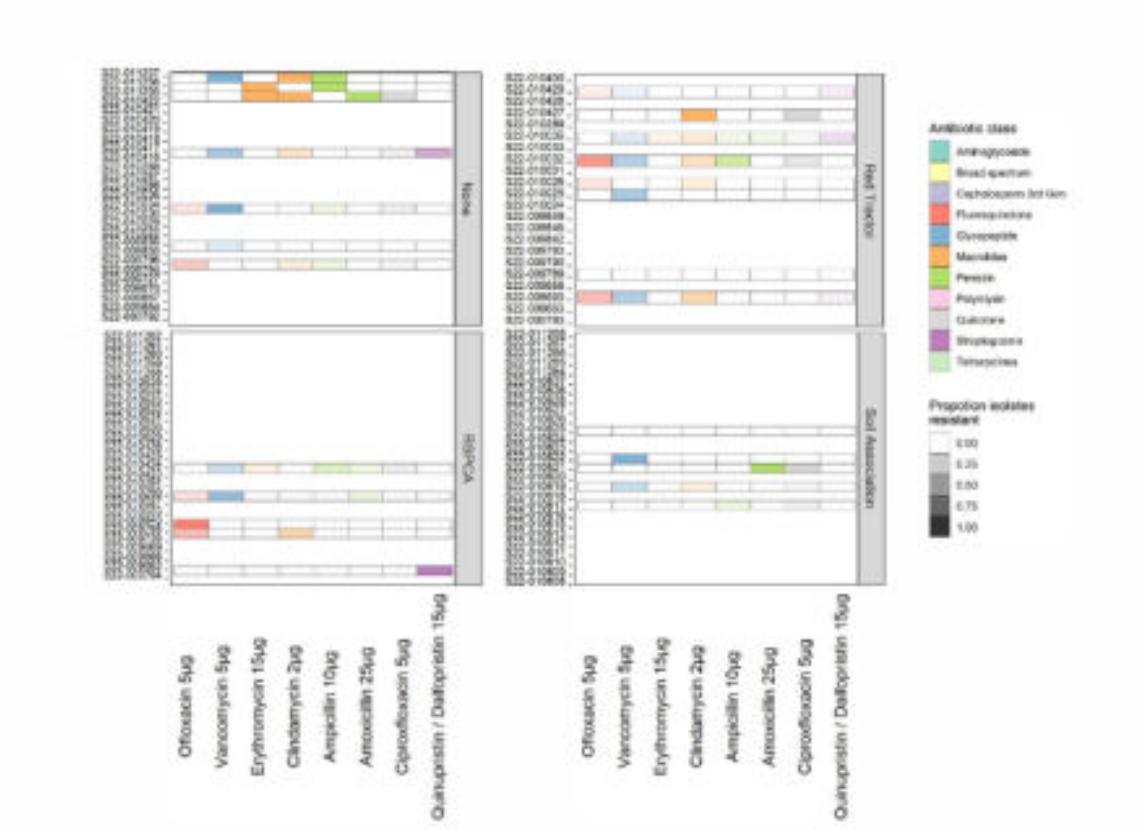

Table 2: Number of samples of which at least five isolates were tested, divided by production method

Assurance Type	Enterococci detected	Resistance to antibiotics	Resistance to one antibiotic	Resistance to two antibiotics	Multi-drug resistance	Macrolide resistance	Glycopeptide resistance
Non-Assured	7	7	1	1	5	5	4
Red Tractor Assured	8	7	1	2	4	5	5
RSPCA Assured	5	5	2	1	2	2	2
Organic/Soil Association	5	4	1	3	0	1	2

5. Recommendations

This study is a snapshot of the UK market, and we strongly recommend that all data collected by the Veterinary Medicines Directorate for the annual UK Veterinary Antibiotic Resistance and Sales Surveillance Report and by The Food Standards Agency for the AMR surveillance programme should be divided by production method as this study indicates a potential trend with more intensive production methods and higher AMR burden. While the UK pig industry uses the majority of antibiotics on farms other species should also be included in the division of data collection by production method. This will enable greater surveillance and identification of where industry can and must improve. Methods of production can be easily identified by assurance scheme as with this study.

On 28 January 2022, the EU banned all forms of routine farm antibiotic use, including prophylactic group treatments[2]. Using antibiotics to compensate for inadequate husbandry or poor hygiene is illegal. This is a major step forward for more responsible and sustainable antibiotic use in European farming. If properly implemented, it should lead to a large reduction in farm antibiotic use, help tackle the serious crisis of antibiotic resistance, and protect human and animal health.

We recommend the UK government adopts this level of legislation as a minimum. This will not only ensure higher welfare practises continue to be brought in across UK production but will once again set the UK as a world leader in fighting antimicrobial resistance. This legislation would also allow for greater protection from imported animal products from countries with far higher antibiotic use on farms.

6. Appendix

Detailed methodology

World Animal Protection commissioned Fera Science Ltd to deliver a survey examining the prevalence of antimicrobial resistant Enterococci in pork meat from different food assurance schemes as well as some which had no assurance scheme.

To achieve this, a total of 103 samples from supermarkets located in or around York and online retailers were collected. The meat was then processed in the laboratory so that samples could be tested for presence of bacteria. When bacteria were found, they were then tested for susceptibility to different antibiotics, in other words whether antibiotics were effective in killing them or slowing their growth. Samples collected were split as follows:

[2] Antibiotics given to whole herds of healthy animals to prevent disease

· 27 samples of fresh diced / chops of pork / loin of pork with no assurance certification,

· 22 samples of fresh diced / chops of pork / loin of pork with Red Tractor assurance certification,

· 27 samples of fresh diced / chops of pork / loin of pork with RSPCA assurance certification,

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It was anticipated that pork may have become difficult to find during this study due to labour shortages and other supply issues. These included the nationwide CO2 shortage which resulted in large numbers of animals being culled.

As far as practicable samples were collected from different retailers and at different times, in an effort to collect samples from a number of different suppliers. Due to lack of samples within the narrow geographic sampling area, the organic / Soil Association samples were all bought from online retailers. All other samples were purchased directly from supermarkets at different locations and / or times.

Samples were purchased and either delivered to Fera on the same day or kept at +4°C overnight before delivery to Fera. Once received on site, samples were logged in and allocated a unique sample number using the Laboratory Information Management System (LIMS). The information recorded included the date of purchase, supermarket, use by date, label details and assurance scheme specified (if any). Samples were stored at -20°C once logged in and labelled to allow sample collection to be completed and bulk testing to be delivered. By analysing all samples over two days, there were fewer variables associated with the testing (e.g., media, incubators etc).

Antibiotic Preparation

Erythromycin (from Sigma) was prepared from the stock by adding 10mg to 10mL of distilled sterile water. This was filter sterilised using a 0.2µm pore size filter to remove any potential contaminants. Once filter sterilised, solutions were kept for 72hrs at +4°C. Erythromycin was added at a concentration of 1 mg/L to 3.5 L of S&B agar which equates to approximately 1 µg per ml. All media for the enumeration of target organisms was made in-house the day before testing.

Sample preparation

Samples were thawed at +4°C overnight prior to testing. Additional samples (where available) were analysed to increase the probability of recovering target organisms.

The method for the analysis of Enterococci was based on an in-house method used by Fera for over 15 years, The method is validated for testing of meat samples.

Once thawed samples were analysed immediately by aseptically opening the packaging and removing 10 g of pork meat ± 0.2 g. This was placed into a sterile filtered stomacher bag.

Laboratory sampling of pork.

Forceps and scissors used for sampling were sterilised by flaming in 70% ethanol prior to use. Packaging was surface decontaminated with 70% ethanol-soaked cotton and allowed to air dry prior to cutting open with the sterilised utensils. Balances used were checked with check-weights

on the day of use and these weights fell within the acceptable tolerance. 7 90 millilitres of Maximum recovery diluent (MRD) was added to the sterile stomacher bag containing the 10 g pork and homogenised in a laboratory stomacher, speed setting 2 for 2 minutes ± 10 seconds.

Plating out

The resulting homogenate was then surface plated onto the corresponding selective media plates (both supplemented and non-supplemented) as described below:

· 0.1mL of diluent was spread across non-supplemented TBX plates; 0.1mL of a further 1 in 10 dilution was spread across non-supplement TBX plate.

· 1mL of diluent was spread across three supplemented TBX plates; 333µL per plate. · TBX plates were incubated aerobically at 37°C ± 1.7 °C for 4 hours then moved to 43.5°C ± 0.7°C (where the temperature did not exceed 44°C) and incubated for 20h ± 2 h.

· 0.1mL of diluent was spread across non-supplemented S&B plates; 0.1mL of a further 1 in 10 dilution was spread across non-supplement S&B plate.

· 1mL of diluent was spread across three supplemented S&B plates: 333µL per plate.

· S&B plates were incubated aerobically at 37 °C ± 1.7 °C for 44h ± 4h.

Plate reading, isolate collection, and storage

After incubation, plates were checked and where growth was present, colonies present were counted and recorded. Information as to the type of media (e.g., supplemented, or non-supplemented) was written in addition to a brief morphological description of the colony appearance. Colony counts were calculated as colony forming units per gram of sample based on the totals presenting per millilitre of homogenate.

109 Colonies were streaked on to nutrient rich Columbia agar with 5% sheep blood to provide fresh 24h cultures for the sensitivity test element and for cryopreservation. These plates were incubated at 30°C ± 1.7°C for 24hrs to encourage recovery and ensure cultures were healthy for the sensitivity work. Protect beads were prepared for every isolate and labelled with the organism type, code S for supplemented and NS for non-supplemented, LIMS number and identifying factor (in morphological terms were there was more than one isolate for a sample).

Susceptibility testing was carried out as per the EUCAST disk diffusion method, Version 10.0, January 2022. Fresh 24h colonies on CBA were used to prepare colony suspensions to a 0.5 McFarland standard (by adding a suitable number of colonies with a sterile 10µl loop) into 10ml Mueller Hinton Broth (MHB) medium.

Immediately after preparing the 0.5 McFarland standard MHB, a sterile cotton tipped swab was immersed in this suspension and used to inoculate the surface of a Mueller Hinton Agar plate. This was done by inoculating a lawn of three-directional growth across the full agar surface (with the same cotton swab). This is the approved method for plate preparation for antibiotic susceptibility testing.

This lawn of three directional growth provides consistent and pure growth across the whole surface of the agar plate which means multiple

discs can be used on one plate, allowing direct comparison of sensitivities by visual assessment. Antibiotic disks (from MAST Diagnostics) were then applied using a multi-disk dispenser with a maximum of 5 disks per plate. No zones of inhibition were measured as agreed. Instead, resistance or sensitivity was recorded based on the presence or absence of a zone of inhibition (of any diameter). Where there was a zone of inhibition, the isolate was susceptible to the antibiotic at the specific concentration. Where there was no zone of inhibition zone, the isolate was resistant to the antibiotic at the specific concentration. Control plates were prepared for sensitive and resistant strains to ensure the method was working. E. faecalis ATCC 25912 sensitive E. faecium NCTC 13633 Vancomycin resistant (VanA positive)

All control strains gave expected sensitivity results.

The antibiotics tested can be found below. Concentrations of antibiotics used in testing were taken from the EUCAST (European Committee on Antibiotic Susceptibility Testing) guidelines as the cut-off values for resistance for each antibiotic.

- Erythromycin (1µg) (Macrolides)
- Vancomycin 5µg (Glycopeptides)
- Quinupristin / Dalfopristin 15µg (Streptogramins)
- Erythromycin 15µg (Macrolides)
- Clindamycin 2µg (Macrolides)
- Ciprofloxacin 5µg (Quinolones)
- Ofloxacin 5µg (Fluoroquinolones)
- Ampicillin 10µg (Penicillins)
- Amoxicillin 25µg (Penicillins)

7. References

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