Surveillance Study of Antimicrobial Resistance (AMR) in Campylobacter in Chicken and Salmonella in Pork Sampled at Retail

PROTOCOL

September 2017

OUTLINE

Background

The Agency has identified antimicrobial resistance (AMR) in *Campylobacter* on chickens and AMR in *Salmonella* species (spp.) on pork as a surveillance priority following the AMR systematic review.[1] This surveillance study has been commissioned to help fill evidence gaps and provide a baseline for further ongoing surveillance in this area.

Data on AMR in retail chicken and pork is required to inform AMR risk assessment in the food chain, to monitor trends in emerging AMR issues, to track progress with interventions aimed at tackling AMR and to contribute to the wider international effort on AMR surveillance.

An invitation to tender was issued on 15th February 2017 to commission work on the design and execution of a UK-wide surveillance study on AMR in *Campylobacter* spp. on fresh/frozen chicken sold at retail and AMR in *Salmonella* spp. on fresh/ frozen minced pork sold at retail. In addition, selected commensal organisms (e.g. *Escherichia coli, Enterococcus* spp. and *Klebsiella* spp.) will also be included in this study as AMR is not an issue restricted to pathogens. The study will be representative of all four countries using UK market share data.

The Specification required the study to be tendered in two lots

1. Study design, sample collection at retail and transportation to the testing laboratory;
2. Microbiological testing, data analysis and reporting.

Following the tender exercise successful contractors were assigned to undertake each aspect of the survey, Hallmark for Lot 1 and Public Health England for Lot 2.

Objectives

A. To design a survey that will form a baseline for future ongoing surveillance on AMR in retail meats
B. To determine the prevalence and levels of *Campylobacter* in fresh/frozen, whole/portioned chicken sold in UK retail outlets by sampling over 2 month period during September - October 2017.
C. To determine the prevalence and levels of *Salmonella* in fresh minced pork sold in UK retail outlets by sampling over a 2 month period during September - October 2017.
D. To determine the prevalence and levels of commensal organisms (*E. coli, enterococci and Klebsiella*) in both chicken and pork samples during survey period.
E. To determine the prevalence and/or levels of susceptibility of positive isolates to a defined range of antimicrobial agents using minimum inhibitory concentration (MIC) testing.
F. To determine the presence of specific antibiotic resistance genes using Polymerase Chain Reaction (PCR)

[1] https://www.food.gov.uk/science/research/foodborneillness/b14programme/b14projlist/fs102127/a-systematic-review-of-amr-in-pork-and-poultry-dairy-products-seafood-and-fresh-produce

Requirement for Sampling and Testing for AMR in Retail Chicken and Pork

The requirement for the following combination of bacteria/antimicrobial testing for each food matrix was developed in consultation with the Advisory Committee on the Microbiological Safety in Food. In addition to this, screening for EC panel of antimicrobial resistance[2] and testing for the presence of specific resistance genes /resistance mechanisms will be carried out.

Table 1 – Bacteria/Antimicrobial Combinations

Parameter Pathogen/Commensal	***Testing***	***Antimicrobial***
Chicken		
*Campylobacter spp**	*Detection Enumeration*	• *Fluoroquinolones (Ciprofloxacin)** • *Macrolides (Erythromycin)*
Pork		
Salmonella spp	*Detection Enumeration*	• *ESBLs (eg. cefotaxime)* • *Ciprofloxacin/Nalidixic acid* • *Carbapenems*
Commensals for both chicken and pork samples		
*Escherichia coli** *Klebsiella spp*	*Detection Enumeration*	• *Tetracycline** • *ESBLs (eg. cefotaxime)* • *Polymyxins (Colistin, C resistance genes mrc1, mrc2)* • *Ciprofloxacin* • *Piperacillin-tazobactam* • *Carbapenems*
Enterococcus spp.	*Detection Enumeration*	• *Glycopeptides (vancomycoin)*

* Survey design must provide a reasonable level of statistical power for these bacteria/antimicrobial combinations:

- ***Campylobacter* spp. and Ciprofloxacin (chicken)**
- ***Escherichia coli* and Tetracycline (chicken and pork)**

Timetable

The survey will consist of a 2 month sampling period to enable better spread of data from 04 September 2017 – 27 October 2017. Microbiological testing will begin in September and depending on the number of positive isolates, AMR susceptibility testing will continue till November. Data analysis and validation will be required prior to the availability of complete results.

Publication of results

Information from all samples collected and analysed will be published on the Agency website as part of the final report, in line with the FSA's policy on open data Retailers/brand owners will be notified of their individual results and be given the opportunity to comment prior to publication of the final report which will be available in Spring 2018.

[2] http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-Salmonella-Campylobacter-harmonised-monitoring.pdf

SURVEY DESIGN

Sample numbers

A total of 680 samples will be taken as this should be sufficient to provide a reasonable level of statistical power when assessing changes in the prevalence of AMR for the selected matrices (the bacterium/antimicrobial combinations shown in Table 1). The study design will provide 80% power for detecting a 50% reduction in the prevalence of AMR in chicken, based on the campylobacter/ciprofloxacin combination. Calculations made by the FSA suggest 340 samples of chicken collected in all 4 UK countries would be sufficient for this purpose. For pork, the data available to inform such a calculation are less robust in terms of the prevalence of *Salmonella* but more robust in terms of prevalence of commensals in pork. Therefore based on *E.coli*/tetracycline combination, 340 samples of minced pork collected from all 4 UK countries will provide a reasonable level of statistical power.

Market share data

A provisional survey design for the study was established by the sampling contractor for Lot 1. This was subjected to expert peer review as part of the project evaluation process. Market share data purchased from Kantar WorldPanel in July 2017 was used to create a more detailed survey design reflecting the market share for raw fresh and frozen chicken (whole and portioned) and for raw fresh pork mince sold at retail in the UK. The final survey design was shared with and approved by the FSA before commencement of sample collection.

The contractor will ensure that the sampling is evenly distributed throughout the period of the survey and that the samples provide a geographic spread across the UK (see Annex 1). Chicken will be sampled per cut (whole, breast fillet, leg/thigh/mixed and miscellaneous). For pork only fresh minced pork will be sampled due to low availability of frozen pork mince. If any deviations are necessary, these will be noted in the final report.

Only unseasoned, fresh chilled/frozen chickens (whole and portioned) and fresh chilled mince pork should be sampled. Samples that are marinated, basted, seasoned, herbed, stuffed or processed will not be included in this survey. All samples should be sampled within their Use-by date. Chicken and pork samples will be taken randomly and it is assumed that a portion of the samples will be of non-UK origin.

The contractor will provide smaller independent retail outlets with a leaflet from the Agency informing them that samples have been taken from their premises in order to carry out a survey. For larger retail chains this is not necessary, as the relevant contact at head office will be sent a list of the premises from which samples have been obtained by the Agency.

SAMPLING

Sample Collection

It is essential that cross-contamination be avoided during the collection of chicken samples. Precautions will therefore be taken at all stages to ensure that the equipment used during sampling, transport and storage is not contaminated with the pathogens investigated in the Survey. A single sample from the selected retailer is to be collected and placed into one of the large grip seal bags, sealed and then placed into a second numbered large tamperproof sample bag and sealed. The samples are to be packed into the chilled Insulated Shipping Box and sent to the selected laboratory for testing.

Surveyor will consult the Sampling Request Form and organise collections for that week. On arriving at the retailer, Surveyors will identify location of samples and collect at random from the refrigerator cabinet – not necessarily from the front of the display. The surface temperature of the sample will be checked and recorded prior to purchase. This is achieved using the calibrated infrared thermometer supplied.

Samples will be kept chilled/frozen (as required) from the time of sampling until delivery to the laboratory by storing samples inside the Insulated shipping Box. Chilled and frozen samples will be segregated, packed and labelled in separate Shipping boxes. This will facilitate the laboratories identifying chilled and frozen samples which could be stored for later analysis (within the self-life). Correct packaging and temperature control is paramount; temperature and package integrity will be checked and recorded on arrival at the laboratory.

Samples must reach the laboratory within 24 hours of purchase. Samples will be dispatched to the Laboratories from Monday to Wednesday avoiding bank holidays or public holidays, so the sample does not arrive outside of laboratory hours. Samples will be purchased early in the day so they can be sent on the same day to the laboratory.

Data Recording

Surveyors are required to record a high level of detailed information about samples collected in accordance with the agreed protocols. Required information includes Sample Number; Date/time of purchase; Brand name (if any); Product description; Weight/volume of each sample purchase and number of units purchased to make sample size; Manufacturer name; Retailer name and address; Country of origin; Durability ; Batch code/lot; Approval Number; Form of packaging; Whether it was chilled or frozen; Date/time of delivery of sample to laboratory; Sample price etc.

High quality digital photographs of packaging, product labels, receipts etc. will be taken, labelled with appropriate sample number and stored within the contractors live database.

TESTING

Microbiological testing for this survey will be carried out and managed by contractor for Lot 2, Public Health England (FW&E London and Porton laboratories), who will undertake the examination and testing together with expert sub-contracted laboratories (APHA and AFBI).

Receipt of samples

On receipt of the samples, laboratories will check the information recorded by the Surveyor using live data system allowing real-time tracking of the sampling process. The integrity of the packaging will be noted and the temperature of the samples will also be recorded as any sample over 8^{o}C will not be tested. Any samples that are rejected will be notified to the sampling contractor who will make appropriate arrangements with the Surveyor to take further samples**.**

Methodology

On receipt, samples will be stored at a temperature of 2 – 5 ˚C and tested within the specified shelf-life of the product but preferably with 48 hours of the sampling time. For detailed methodology refer to Annex 2.

Isolates will be retained on a nutrient agar slope (*Salmonella, E. coli, enterococci* and *Klebsiella)* or charcoal swab (*Campylobacter*) prior to Minimum Inhibitory Concentration Testing (MIC). This will be performed on isolates ensuring harmonized data entries and any necessary repeat of MIC testing will be undertaken.

PCR will be performed to detect colistin resistance and/or carbapenemase genes in isolates showing phenotypic resistance to colistin and/or the carbapenems. The approach of performing PCR based on the specific mcr genes has been validated and is considered the most appropriate option.

Retention of Isolates: It is envisaged that 10% of isolates will be archived. Five isolates (or less, depending on availability) from each positive sample will be taken and archived for two years following analysis.

Data Handling and reporting

At the end of monthly testing periods progress reports will be submitted to the Agency that provides details of the number of samples giving positive results to date for *Salmonella*, *Campylobacter*, *E. coli*, *Enterococci* and *Klebsiella*, including counts where applicable. Those selected for MIC will also be indicated.

Data will be validated by contracted laboratory on the bacterial testing and for antimicrobial resistance profiles for isolated strains. All results will be collated into a single spreadsheet for transfer to FSA together with final report.

The contractor will notify the FSA immediately of any unusual/non-compliant results or of any deviations which may affect the specifications and timing of the work programme

or the functions of the project. All records and electronic files will be maintained for a period of 3 years from the end of the contract.

Quality Assurance

The contractor is a designated official control laboratory and consequently accredited to the ISO 17025 standard. Microbiological testing performed in PHE laboratories is audited by United Kingdom Accreditation Service (UKAS) annually to ensure compliance with the International Standard – General Requirements for the Competence of testing and Calibration laboratories (ISO/IEC 17025:2005). AFBI and APHA laboratories are also UKAS accredited against ISO 17025:2005. Each laboratory holds accreditation for the isolation and identification of some the bacteria to be tested for this project. Prior to this study starting, PHE will distribute spiked samples for testing by all four laboratories, to ensure equivalent results are obtained in each laboratory.

ANNEX 1: SAMPLING PLAN

A sample size of 680 samples consisting of 340 samples of fresh/frozen chicken (whole/portioned) and 340 samples of fresh chilled minced pork will be required to achieve a reasonable level of statistical power. Sampling will be kept under review and can be revised to ensure that statistically meaningful analyses can be carried out and if further sampling is required. The sampling will aim to take place evenly over a 2-month period. The sampling plan is structured to reflect the market share data that was sourced from Kantar WorldPanel in July 2017.

Table 1: Number of samples vs. UK countries

	Total number of chicken	Total number of pork
Total UK	340	340
England	279	279
Wales	29	25
Northern Ireland	7	10
Scotland	25	26

Table 2: Number of samples vs. geographic region

Geographic region	No of chicken samples	No. of pork samples
London	77	80
Midlands	48	47
Lancashire	35	27
Yorkshire	35	34
South England	33	34
Wales	29	25
East England	27	37
Scotland	25	26
North East England	13	11
South West England	11	9
Northern Ireland	7	10

Table 3: Number of samples vs. retailer types

Retailer	No. of chicken samples	Number of pork samples
Top 10 retailers	293	316
Butchers	17	12
Miscellaneous	30	12

Table 4: Sample collection

Month	Week Number	Day	Date	Weekly totals
September	1	Monday	04-Sep	91
		Tuesday	05-Sep	
		Wednesday	06-Sep	
	2	Monday	11-Sep	82
		Tuesday	12-Sep	
		Wednesday	13-Sep	
	3	Monday	18-Sep	84
		Tuesday	19-Sep	
		Wednesday	20-Sep	
	4	Monday	25-Sep	75
		Tuesday	26-Sep	
		Wednesday	27-Sep	
October	5	Monday	02-Oct	90
		Tuesday	03-Oct	
		Wednesday	04-Oct	
	6	Monday	09-Oct	79
		Tuesday	10-Oct	
		Wednesday	11-Oct	
	7	Monday	16-Oct	90
		Tuesday	17-Oct	
		Wednesday	18-Oct	
	8	Monday	23-Oct	89
		Tuesday	24-Oct	
		Wednesday	25-Oct	

ANNEX 2: LABORATORY METHODOLGY

Testing of chicken and pork samples will be managed by the Public Health England (FW&E laboratories at Porton and London) and part of the testing will be sub-contracted to AFBI laboratory in Northern Ireland and the APHA laboratory in Weybridge. It is proposed that generally only one single isolate of each bacterial type per sample will be selected for MIC testing in order to avoid biased results due to multiple counting of copy strains. Statistical analysis based on estimates of prevalence for *Campylobacter* in chicken and *E. coli* in pork indicates that using a single isolate of these organisms from each meat sample would give sufficient power (80 %) to demonstrate a 50 % reduction in ciprofloxacin resistance (in campylobacters) and tetracycline resistance (in *E. coli*).

Test Procedures for Detection and Enumeration of Bacteria types

The following procedures will be used to detect/enumerate the specified bacteria.

Chicken

- *Enumeration of Campylobacter – based on ISO 10272-2; 2006.* One isolate from every positive sample would be speciated and MIC tested.

Pork

- *Detection of Salmonella – based on ISO 6579; 2002.* One isolate from every positive sample would be MIC tested.

Commensals in chicken and pork

- *Enumeration of E.coli – based on ISO 16649-2; 2001.* One isolate from every positive sample would be MIC tested.
- *Detection of Klebsiella – In-house method.* One isolate from every positive sample would be MIC tested.
- *Enumeration of Enterococci – based on ISO 7899-2.* One isolate from every positive sample would be speciated and MIC tested.
- *Detection of Cefotaxime-Resistant E.coli – EU Method.*

Test Procedures for Determination of MICs for AMR

It is expected that at least 180 *Campylobacter* spp., 400 *E. coli* and 400 *enterococci* isolates will be subjected to MIC testing in addition to approximately 15 *Salmonella* spp. and 60 *Klebsiella* spp. isolates. The following procedures will be used:

1. *Campylobacter* - Minimum Inhibitory concentration (MIC) testing will be performed using dilution methods according to the methods described by the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Isolates will be tested against 4 antibiotics using agar dilution against EFSA breakpoints. In addition MIC testing for streptomycin will be included.

http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-Salmonella-Campylobacter-harmonised-monitoring.pdf

2. *E. coli* and *Salmonella* - Minimum inhibitory concentrations will be determined using dilution methods according to the methods described by EUCAST and the Laboratory Standards Institute (CLSI), accepted as the international reference method (ISO standard 20776-1:2006), EN 14.11.2013 Official Journal of the European Union L 303/33. MICs will be determined in accordance with the guidance located at http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:303:0026:0039:EN:PDF ; Isolates will be tested against an initial panel of 14 antibiotics. Isolates showing microbiological resistance to third generation cephalosporins (cefotaxime or ceftazidime) or meropenem using the epidemiological cut-off values described by EFSA, will be tested against a further panel of 10 antibiotics. Results will be interpreted using the epidemiological cut-off values outlined in the document http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:303:0026:0039:EN:PDF

3. *Klebsiella* - Minimum inhibitory concentrations will be determined using dilution methods according to the standard methods used in PHE's national Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit. Isolates will be tested against a panel of >20 antibiotics, including third generation cephalosporins (cefotaxime and ceftazidime), carbapenems and colistin. Results will be interpreted using the epidemiological cut-off values shown on the EUCAST website: https://mic.eucast.org/Eucast2/

4. *Enterococci* - Minimum inhibitory concentrations will be determined using dilution methods according to the standard methods used in PHE's AMRHAI reference unit. MICs for vancomycin will thus be determined according to this standard and results will be interpreted using the epidemiological cut-off values shown on the EUCAST website: https://mic.eucast.org/Eucast2/

PCR methods for characterisation of antibiotic resistance determinants

E. coli, Klebsiella and *Salmonella* isolates with phenotypic resistance to colistin or the carbapenems (MICs above the colistin ECOFF or EUCAST screening cut-offs for the carbapenems) will be screened for mcr-1 and mcr-2, and acquired carbapenemase genes, respectively. mcr genes will be sought according to the EURL-AR protocol outlined in the document http://www.crl-ar.eu/data/images/protocols/mcr-multiplex_pcr_protocol_v2_oct16.pdf . Carbapenemase genes will be sought by in-house PCRs (Ellington et al. 2016; Ellington et al. 2007 and Meunier et al., submitted).

Characterisation by Whole Genome Sequencing (WGS) (optional)

Selected isolates may be tested by WGS in order to confirm the species identity as well as comparing the in silico results of antibiotic resistance with those obtained by in vitro testing.