A survey of Salmonella, Escherichia coli (E. coli) and antimicrobial resistance in frozen, part-cooked, breaded or battered poultry products on retail sale in the United Kingdom

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Authors: C Willis, F Jorgensen, S.A. Cawthraw, H Aird, S Lai, M Chattaway, I Lock, E. Quill and G Raykova
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Authors

- C. Willis: Food Water and Environmental Microbiology Laboratory Porton, UK Health Security Agency, Porton Down, Salisbury.
- F. Jorgensen: Food Water and Environmental Microbiology Laboratory Porton, UK Health Security Agency, Porton Down, Salisbury.
- S.A. Cawthraw: Animal and Plant Health Agency, Field Epidemiology and Surveillance, Department of Bacteriology, Addlestone.
- H. Aird: Food Water and Environmental Microbiology Laboratory York, UK Health Security Agency, Sand Hutton, York.
- S. Lai: Food Water and Environmental Microbiology Laboratory London, UK Health Security Agency, London.
- M. Chattaway: Gastrointestinal Bacteria Reference Unit, UK Health Security Agency, London.
- I. Lock: Fera Science Ltd, Sand Hutton, York.
- E. Quill: Fera Science Ltd, Sand Hutton, York.
- G. Raykova: Fera Science Ltd, Sand Hutton, York.

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Abbreviations

Acronym	Definition of term
AFBI	Agri-Food and Biosciences Institute
AMR	Antimicrobial resistance
AST	Antimicrobial susceptibility testing
APHA	Animal and Plant Health Authority
BPW	Buffered Peptone Water
BSAC	British Society for Antimicrobial Chemotherapy
C	Degrees Celsius
Cfu	Colony forming units
CI	Confidence Interval
CTX	Cefotaxime
ECDC	European Centre for Disease Prevention and Control
ECOFF	Epidemiological cut-off value
EFSA	European Food Safety Authority
EQA	External Quality Assurance
ESBL	Extended-spectrum beta-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FSA/FSS	Food Standards Agency/Food Standards Scotland
FW and E	Food Water and Environmental
g	gram
GBRU	Gastrointestinal Bacteria Reference Unit
h	Hour(s)
ISO	International Organisation for Standardisation
l	Litre
LIMS	Laboratory Information Management System
mg	Milligram
ml	Millilitre
MALDI-TOF	Matrix-assisted laser desorption/ionization - time of flight
MIC	Minimum-inhibitory concentration
MDR	Multi-drug resistant
MPN	Most probable number
n	number
PCR	Polymerase chain reaction
PCU	Population correction unit
PHE	Public Health England

Acronym	Definition of term
SNP	Single nucleotide polymorphism
SOP	Standard Operating Procedure
spp.	Species (plural)
UKAS	United Kingdom Accreditation Service
UKHSA	United Kingdom Health Security Agency
WGS	Whole Genome Sequencing

1.0 Lay Summary

Frozen chicken products coated in breadcrumbs, including food such as chicken nuggets, poppets, pops and goujons, have caused outbreaks of salmonella illness in the UK and other countries in recent years. The Salmonella bacteria known to be causing such illness have been detected in such products. It is true that most food poisoning associated with those products probably could been avoided if the products had been cooked thoroughly enough (meaning cooking as indicated in the instructions on the packaging that would kill the bacteria of concern). It is also possible, however, that kitchen hygiene practices employed allowed the Salmonella bacteria to transfer from the frozen chicken products (as they were before they were cooked) onto other foods that were ready-to-serve (for example, salads) and if these were not cooked there could be an increased chance of infection.

In this study we estimated how frequently Salmonella spp. were present in frozen, breaded or battered chicken products, intended to be cooked before consumption, on retail sale in the UK between April and July 2021.

These products were also tested for the presence of Escherichia coli (E. coli), which are bacteria that can be used to indicate the general level of hygiene in foods. Products were also examined for specific types of E. coli that are resistant to antimicrobials of particular importance in the treatment of severe human illnesses.

Overall, 310 samples of chicken products were tested, and Salmonella spp. were detected in five samples (1.6%). When these samples were cooked according to the instructions on the packet, the Salmonella organisms were killed. An additional 20 similar products found to contain Salmonella spp. during a previous study in 2020/21 were also cooked according to the instructions on their packets, and no salmonellas were found in these products after cooking.

Escherichia coli was found in approximately a third of samples (36%), but only 15 samples (5%) harboured levels of E. coli that might be considered to indicate significant problems in the hygiene of the tested products. Some of the detected isolates of both Salmonella and E. coli were resistant to selected antimicrobials, but in general, the incidences of resistance were lower than seen in similar studies carried out in the UK in previous years, which suggests a gradual improvement in the way antimicrobials are being used during the production of poultry.

These results demonstrate that it is important to cook breaded and battered chicken products properly in accordance with the instructions on the packaging. Adequate cooking, along with using good kitchen hygiene, e.g. hand-washing between handling uncooked and cooked foods, and cleaning preparation surfaces and utensils properly after using them for uncooked food items, significantly reduces the risks posed to consumers by Salmonella and E. coli.

2.0 Executive Summary

Frozen, breaded, ready-to-cook chicken products have been implicated in outbreaks of salmonellosis. Some of these outbreaks can be large. For example, one outbreak of Salmonella Enteritidis involved 193 people in nine countries between 2018 and 2020, of which 122 cases were in the UK. These ready-to-cook products have a browned, cooked external appearance, which may be perceived as ready-to-eat, leading to mishandling or undercooking by consumers. Continuing concerns about these products led FSA to initiate a short-term (four month), cross-sectional surveillance study undertaken in 2021 to determine the prevalence of Salmonella spp., Escherichia coli and antimicrobial resistance (AMR) in frozen, breaded or battered chicken products on retail sale in the UK.

This study sought to obtain data on AMR levels in Salmonella and E. coli in these products, in line with a number of other FSA instigated studies of the incidence and nature of AMR in the UK food chain, for example, the systematic review (2016).

Between the beginning of April and the end of July 2021, 310 samples of frozen, breaded or battered chicken products containing either raw or partly cooked chicken, were collected using representative sampling of retailers in England, Wales, Scotland and Northern Ireland based on market share data. Samples included domestically produced and imported chicken products and were tested for E. coli (including extended-spectrum beta-lactamase (ESBL)-producing, colistin-resistant and carbapenem-resistant E. coli) and Salmonella spp. One isolate of each bacterial type from each contaminated sample was randomly selected for additional AMR testing to determine the minimum inhibitory concentration (MIC) for a range of antimicrobials. More detailed analysis based on Whole Genome Sequencing (WGS) data was used to further characterise Salmonella spp. isolates and allow the identification of potential links with human isolates.

Salmonella spp. were detected in 5 (1.6%) of the 310 samples and identified as Salmonella Infantis (in three samples) and S. Java (in two samples). One of the S. Infantis isolates fell into the same genetic cluster as S. Infantis isolates from three recent human cases of infection; the second fell into another cluster containing two recent cases of infection. Countries of origin recorded on the packaging of the five Salmonella contaminated samples were Hungary (n=1), Ireland (n=2) and the UK (n=2). One S. Infantis isolate was multi-drug resistant (i.e. resistant to three different classes of antimicrobials), while the other Salmonella isolates were each resistant to at least one of the classes of antimicrobials tested. E. coli was detected in 113 samples (36.4%), with counts ranging from <3 to >1100 MPN (Most Probable Number)/g. Almost half of the E. coli isolates (44.5%) were susceptible to all antimicrobials tested. Multi-drug resistance was detected in 20.0% of E. coli isolates. E. coli isolates demonstrating the ESBL (but not AmpC) phenotype were detected in 15 of the 310 samples (4.8%) and the AmpC phenotype alone was detected in two of the 310 samples (0.6%) of chicken samples. Polymerase Chain Reaction (PCR) testing showed that five of the 15 (33.3%) ESBL-producing E. coli carried blaCTX-M genes (CTX-M-1, CTX-M-55 or CTX-M-15), which confer resistance to third generation cephalosporin antimicrobials. One E. coli isolate demonstrated resistance to colistin and was found to possess the mcr-1 gene.

The five Salmonella-positive samples recovered from this study, and 20 similar Salmonella-positive samples from a previous UKHSA (2020/2021) study (which had been stored frozen), were subjected to the cooking procedures described on the sample product packaging for fan assisted ovens. No Salmonella were detected in any of these 25 samples after cooking.

The current survey provides evidence of the presence of Salmonella in frozen, breaded and battered chicken products in the UK food chain, although at a considerably lower incidence than reported in an earlier (2020/2021) study carried out by PHE/UKHSA as part of an outbreak investigation where Salmonella prevalence was found to be 8.8%.

The current survey also provides data on the prevalence of specified AMR bacteria found in the tested chicken products on retail sale in the UK. It will contribute to monitoring trends in AMR prevalence over time within the UK, support comparisons with data from other countries, and provide a baseline against which to monitor the impact of future interventions. While AMR activity was observed in some of the E. coli and Salmonella spp. examined in this study, the risk of acquiring AMR bacteria from consumption of these processed chicken products is low if the products are cooked thoroughly and handled hygienically.

3.0 Introduction

Frozen, breaded chicken products such as chicken nuggets, goujons and dippers have been implicated in cases of human salmonellosis, in the UK and elsewhere. For example, an outbreak of S. Enteritidis sequence type (ST) 11 affected 193 people in 9 countries between May 2018 and December 2020, of which 122 cases were in the UK (ECDC and EFSA, 2021). The outbreak strain was detected in five batches of non-ready-to-eat, breaded chicken products manufactured in Poland, using chicken from multiple Polish farms. Similarly, a multistate outbreak of S. Enteritidis in the United States in 2021, involving at least 36 people, has also been traced to raw, frozen, breaded and stuffed chicken products (Centers for Disease Control and Prevention, 2021), and three outbreaks of S. Enteritidis infection have been reported to be associated with these products in Canada during 2014 and 2015 (Hobbs et al, 2017).

These products are often sold as partially cooked portions, with a brown, cooked appearance to the breadcrumb or batter coating, while the inside of such products remains raw. This may result in a misunderstanding by consumers regarding whether or not the products are ready-to-eat, or difficulty in judging when the items are fully cooked prior to consumption. Moreover, the process of producing the comminuted (ground) chicken meat that is used in many of these products is likely to distribute any bacterial contamination widely throughout one or more batches of product. Catford et al (2017) examined 18 breaded, frozen, comminuted chicken samples collected as part of an outbreak investigation in Canada in 2015-2016, and reported the detection of S. Enteritidis in 17 of the 18 samples. That study also detected S. Infantis in one sample and S. Kentucky in one sample (which was also contaminated with S. Enteritidis). Enumeration of the salmonellas in these samples indicated that the numbers of salmonella organisms ranged from 0.0018 to 3 MPN/g.

A study by Public Health England (PHE) in 2020 found Salmonella in 40 (8.8%) of 456 samples of frozen, reformed chicken products, with Salmonella Enteritidis isolates from 17 samples falling into genetic clusters associated with an outbreak involving four S. Enteritidis clusters (Jorgensen et al, 2022). Salmonella counts in this study ranged from <0.02 to 54 MPN/g, and the presence of Salmonella spp. was found to be associated with elevated levels of generic E. coli that are frequently used as a general hygiene indicator in food products.

A systematic review of antimicrobial resistance (AMR) in the food chain funded by the Food Standards Agency in 2016 recommended that gaps in evidence regarding AMR prevalence in UK food on retail sale should be addressed by developing research and surveillance to monitor AMR levels in foodborne pathogens and commensal bacteria in poultry and pork meat.

In the poultry industry, antimicrobials may be used to treat disease or may be administered to the entire flock to prevent disease (metaphylaxis). Whilst their use to promote animal growth occurs in some non-UK countries such as Brazil and China, antimicrobial growth promoters were banned in the EU in 2006 and in the US in 2017(Roth et al, 2019). The use of antimicrobials in the production of food animals may increase the risk of the development of antimicrobial resistance in pathogenic or commensal bacteria within the animal or the farm environment. Thus, meat may become contaminated with AMR pathogens from the animal during slaughter, or through cross-contamination from the environment. Moreover, contamination of food products with AMR

commensal organisms may result in the subsequent transfer of resistance genes to pathogens or normal commensal bacteria within the human gut (Salyers et al, 2004; Karami et al, 2007).

Surveillance of AMR in animals, humans and food has been carried out within the EU by the European Food Safety Authority (EFSA, 2016), according to the requirements set down in Commission Implementing Decision 2013/652/EU (European Commission, 2013). This surveillance includes the prevalence of resistance to key antimicrobials in Salmonella spp., Campylobacter spp., E. coli and Staphylococcus aureus. Data submitted from 28 EU Member States indicated that a relatively high proportion of isolates of Salmonella spp. and E. coli from broilers were resistant to fluoroquinolones (42.6% of salmonellas and 65.7% of E. coli were resistant to ciprofloxacin) and tetracyclines (40.4% of salmonellas and 50.1% of E. coli), whereas the proportion of Salmonella isolates from humans with resistance to fluoroquinolones remained generally low (8.8% resistant to ciprofloxacin on average across different EU countries). A study of chicken and pork on retail sale in the UK in 2017 demonstrated that 26% of E. coli isolates from 339 fresh chicken samples were resistant to ciprofloxacin and extended spectrum beta-lactamase (ESBL) producing E. coli were detected in 10% of 339 fresh chicken samples examined (Willis et al, 2018).

The EU harmonised surveillance of AMR in E. coli from retail meats showed that, of 315 fresh chicken samples collected from retail sale in the UK in 2020, 13% were positive for ESBL/AmpC-producing E. coli, while 0.95% were positive for the colistin resistance gene, mcr-1, but none were resistant to carbapenem antimicrobials.

This study aimed to investigate the prevalence of Salmonella spp. in frozen, breaded or battered chicken products on retail sale in the UK, as well as determining levels of E. coli as an indicator of the overall hygiene of the products. The Salmonella spp. isolates were further characterised using whole genome sequencing, and phenotypic AMR was determined in both Salmonella spp. and E. coli in line with the UK AMR National Action Plan, in order to make comparisons with previous studies in the UK and elsewhere, and inform future risk assessments.

4.0 Methods

The design of the sampling and testing procedures for the survey was agreed with the FSA before commencement of sample collection. The survey protocol is briefly described (enclosed as Appendix I).

4.1 Sample collection at retail and transportation to the testing laboratory

Between the beginning of April and end of July 2021, 310 samples of frozen breaded or battered chicken products, either raw or partly cooked, were collected by Hallmark Meat Hygiene Ltd. Sample collectors were instructed to sample from pre-determined retail outlets in Northern Ireland and within 10 geographic regions of Great Britain based on regional spend index and market share data. Within a retail outlet, sample collectors were allowed to select any suitable product of the appropriate type as if they were a typical shopper. The product types were well defined (as frozen, reformulated, breaded or battered chicken products) to ensure consistency between samplers. All samples were collected prior to their use by date.

After collection, samples were packed into cold boxes with sufficient ice packs to achieve and maintain a temperature of <3°C during transit. The samples were dispatched by overnight courier to one of four microbiology laboratories for examination (PHE Porton FW&E Laboratory, PHE London FW&E Laboratory, PHE York FW&E Laboratory or Agri-Food and Biosciences Institute (AFBI), Belfast)). Where samples were received at the laboratory above 3°C, an assessment was made of the potential impact on microbiology results. In practice, this affected two samples, and

as these included chicken pieces that were still frozen in the centre of the pack at the time of arrival at the laboratory, the conditions on receipt were considered to be acceptable and unlikely to have a significant effect on the results observed. Samples were either tested immediately or stored at -18°C until testing commenced. Once portions had been removed for testing, the remainder of each sample was stored at -18°C in its original packaging.

4.2 Examination of chicken samples for the presence of Salmonella and E. coli

A 10-1 homogenate of each breaded/battered chicken sample was prepared by diluting a 27 g aliquot of the chicken product in Buffered Peptone Water (BPW), according to ISO 6887-1:2017 (International Organisation for Standardisation 2017a). A portion of this homogenate (20 ml) was retained and used to enumerate generic E. coli using a Most Probable Number (MPN) method (Table 1).

The remaining 250 ml of homogenate was incubated at 37°C for 18 h and then sub-cultured for the detection of Salmonella spp., generic E. coli and presumptive ESBL-producing E. coli, colistin resistant E. coli and carbapenemase-producing E. coli (Table 1). For samples in which Salmonella spp. were detected, an MPN technique was subsequently used to enumerate Salmonella spp. in the chicken product.

Table 1 Summary of methods used for enumeration and detection of target organisms

Test Parameter	Method	Reference
Sample preparation	1 in 10 dilution in BPW, ISO 6887-1: 2017	ISO, 2017a
Escherichia coli (detection)	Pre-enrichment in BPW followed by sub-culture onto TBX agar and incubation at 44°C for 22 h.	In-house method
Escherichia coli (enumeration)	Most Probable Number (MPN) technique, using 3 tubes of Minerals Modified Glutamate Medium at each of three dilutions. Incubation of tubes at 37°C for 24 h followed by sub-culture of tubes demonstrating acid production onto TBX agar and incubation of agar plates at 44°C for 22 h; ISO 16649-3:2015.	ISO, 2015
Salmonella (detection)	Pre-enrichment in BPW and subsequent selective enrichment followed by sub-culture onto selective agar plates; ISO 6579:2017.	ISO, 2017b
Salmonella (enumeration)	MPN technique, based on ISO 6579:2017, using 5 tubes of BPW at each of three dilutions (10 g, 1 g and 0.1 g of sample). Incubation of tubes at 37°C for 18 h followed by detection of Salmonella using PCR assay and culture confirmation from one tube (containing with the lowest amount of sample).	In-house method
Presumptive ESBL-producing E. coli	Enrichment in BPW and subsequent sub-culture onto MacConkey Agar containing 1 mg/l cefotaxime (MacConkey CTX), and incubation of plates at 44°C for 20 h. Confirmation of identity by biochemical testing or MALDI-ToF.	In-house method
Presumptive colistin resistant E. coli	Enrichment in BPW and subsequent sub-culture onto MacConkey Agar containing 2 mg/l colistin (MacConkey COL), and incubation of plates at 44°C for 20 h. Confirmation of identity by biochemical testing or MALDI-ToF.	In-house method
Presumptive carbapenemase-producing E. coli	Enrichment in BPW and subsequent sub-culture onto CHROMID® Carba Smart agar plates (Biomerieux), and incubation of plates at 37°C for 22 h. Confirmation of identity by biochemical testing or MALDI-ToF.	In-house method

Where E. coli or Salmonella spp. were isolated, up to five isolates (or all isolates if less than five were available) from each isolation medium were selected at random and sub-cultured onto transport medium for overnight delivery to the Animal and Plant Health Authority laboratory at Weybridge for MIC determination and storage.

A Salmonella spp. isolate from each positive sample was also sent to the Gastrointestinal Bacteria Reference Unit (GBRU) at Colindale for Whole Genome Sequencing (WGS) (see Section 4.6).

4.3 Determination of minimum inhibitory concentrations for bacterial isolates

4.3.1 E. coli and Salmonella

For E. coli and Salmonella spp., one isolate from each isolation medium for each sample was selected to determine the minimum inhibitory concentrations (MIC) for a range of antimicrobials. A broth microdilution method was used to determine the MICs, using SensititreTM. Isolates were inoculated into Mueller Hinton broth at a suitable concentration and dispensed onto commercially prepared plates (Sensititre EUVSEC3, Thermofisher) containing two-fold dilution series of antimicrobials in accordance with European Decision 2013/652/EU. After incubation at 37°C for 18-24 h the plates were examined and growth end-points established for each antimicrobial to provide MICs. Microbiologically-resistant and susceptible interpretations for the MICs were obtained by comparison with epidemiological cut-off values (ECOFFs) published by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) if available (Table 2).

Table 2. Antimicrobials included in the testing, interpretative thresholds for resistance in Salmonella spp. and generic E. coli (first panel – EUVSEC3 plate) as provided for in Decision 2013/652/EU.

Antimicrobial	Interpretative threshold of resistance (ECOFF) for Salmonella (mg/l)	Interpretative threshold of resistance (ECOFF) for E. coli (mg/l)
Ampicillin	> 8	> 8
Cefotaxime	> 0.5	> 0.25
Ceftazidime	> 2	> 0.5
Meropenem	> 0.125	> 0.125
Nalidixic acid	> 16	> 16
Ciprofloxacin	> 0.064	> 0.064
Tetracycline	> 8	> 8
Colistin	> 2	> 2
Gentamicin	> 2	> 2
Trimethoprim	> 2	> 2
Sulfamethoxazole	NA; > 256b	> 64
Chloramphenicol	> 16	> 16
Azithromycin	NA; > 16b	NA; > 16b
Tigecycline	> 1	> 1
Ertapenem	See Table 3	See Table 3
Amikacin	NT	NT
Cefepime	See Table 3	See Table 3
Cefoxitin	See Table 3	See Table 3

NA: Not available; NT: Not tested; a) EUCAST epidemiological cut-off (ECOFF) values unless otherwise specified; b) ECOFF value not currently established – complementary threshold missing from Decision 2013/652/EU used; c) values used for analysis according to EUCAST clinical breakpoints or screening cut-offs; d) EUCAST ECOFF value not currently established; piperacillin threshold in the presence of a fixed tazobactam level of 4 mg/l according to levels originally published by BSAC.

The presence of carbapenemase, ESBL or AmpC enzyme-producers was determined initially by assessing isolate MICs against the microbiological breakpoints for meropenem, cefotaxime and ceftazidime. Isolates recovered from the MacConkey CTX plates, and any recovered from non-antibiotic selective media that were resistant to meropenem, cefotaxime or ceftazidime (on EUVSEC3 plates) were also tested on EUVSEC2 plates, containing extended ranges of meropenem, cefotaxime and ceftazidime and additional antimicrobials: imipenem, ertapenem, temocillin, cefoxitin, cefepime, cefotaxime with clavulanate and ceftazidime with clavulanate (Table 3).

Table 3. Panel of antimicrobials and interpretative thresholds for resistance used for testing only Salmonella spp. and generic E. coli isolates resistant to cefotaxime, ceftazidime or meropenem (second panel - EUVSEC2 plate).

Antimicrobial	Interpretative threshold of resistance (ECOFF) for Salmonella (mg/l)	Interpretative threshold of resistance (ECOFF) for E. coli (mg/l)
Cefoxitin	> 8	> 8
Cefepime	NAb, >0.125	> 0.125
Cefotaxime and clavulanic acid	Not applicable	Not applicable
Ceftazidime and clavulanic acid	Not applicable	Not applicable
Meropenem	> 0.125	> 0.125
Temocillin	NAb, >32	NAb, >32
Imipenem	> 1	> 0.5
Ertapenem	> 0.06	> 0.06
Cefotaxime	> 0.5	> 0.25
Ceftazidime	> 2	> 0.5

NA: Not available; a) EUCAST epidemiological cut-off (ECOFF) values unless otherwise specified; b) ECOFF value not currently established – complementary threshold used; c) interpretation (yes or no) based on synergy or no synergy (between the two antimicrobials).

The classification of isolates as having an ESBL phenotype was as follows: isolates that were susceptible to meropenem and cefoxitin, but resistant to one or both of cefotaxime and ceftazidime and also showed a reduction (synergy) in MIC of ? 8-fold against combined cefotaxime / clavulanate and/or ceftazidime / clavulanate when compared with the cephalosporin alone were classed as ESBL producers.

Isolates that were susceptible to meropenem, but that showed resistance to one or both of cefotaxime and ceftazidime, and also had an MIC of greater than 8 mg/l against cefoxitin and showed no reduction to MICs or a reduction of less than three dilution steps for cefotaxime or ceftazidime in the presence of clavulanate were considered to have an AmpC phenotype.

Isolates that were susceptible to meropenem but resistant to one or both of cefotaxime and ceftazidime and resistant to cefoxitin and also showed a reduction (synergy) in MIC of ? 8-fold against combined cefotaxime / clavulanate and/or ceftazidime / clavulanate when compared with the cephalosporin alone were considered to have an ESBL + AmpC phenotype.

Antimicrobial susceptibility profiles were determined using the ECOFF values, which separate the naive, susceptible bacterial populations from isolates that have developed reduced susceptibility to a given antimicrobial agent (Table 4) as recommended in the ECDC EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella spp. and Campylobacter spp. isolates (ECDC, 2016). The ECOFFs differ from breakpoints used for clinical purposes, which are defined against a background of clinically relevant data.

Table 4. Antimicrobial classes and examples of antimicrobial compounds within them

Antimicrobial group	Antimicrobial(s) included
Aminoglycosides	Gentamicin, streptomycin, amikacin, tobramycin
Penocillin beta-lactam antibiotics	Ampicillin, temocillin - β − lactamase resistant
β −€lactam/inhibitor combinations	Piperacillin/tazobactam, cefotaxime/clavulanic acid, ceftazidime/clavulanic acid
Macrolides	Erythromycin, azithromycin
Quinolones	Ciprofloxacin, nalidixic acid
Cephalosporins	Cefoxitin (2nd generation cephamycin), Ceftazidime and cefotaxime (3rd generation cephalosporins), Cefepime (4th generation cephalosporin)
Carbapenems	Meropenem, imipenem, ertapenem
Sulphonamides	Sulfamethoxazole
Chloramphenicol	Chloramphenicol
Tetracyclines	Tetracyclines
Polymyxins	Colistin
Glycylcyclines	Tigecycline
Trimethoprim	Trimethoprim

4.3.2 Multi-drug resistance

Multi-drug resistance was defined as reduced susceptibility to at least three unrelated antimicrobial classes as specified by the ECDC definition (Table 4; ECDC, 2016).

4.4 Detection of resistance genes using real time Polymerase Chain Reaction (PCR) and sequencing of amplicons

A multiplex PCR technique was used to establish the presence of blaCTX-M, blaOXA-1, blaSHV, or blaTEM beta-lactamase genes in E. coli isolates that exhibited an AmpC and/or ESBL phenotype, as previously described (Fang et al, 2008). PCR was performed using DNA preparations from pure cultures and resulting blaCTX-M amplicons were sequenced to determine the CTX-M variant (Randall et al, 2011).

4.5 Real time PCR for plasmid mediated mcr-1, mcr-2 and mcr-3 genes

Any isolate found to be colistin resistant (confirmed by MIC) was tested for the presence of plasmid-mediated resistance genes mcr-1, mcr-2 and mcr-3 by real time PCR, using an APHA in-house method (BAC0415). To make detection more sensitive, a “sweep” of approximately 10 to 20 colonies was taken to prepare the crude DNA for PCR.

4.6 Characterisation of Salmonella spp. isolates using genome sequencing and identification of links to related human cases

Salmonella spp. isolates were sent to GBRU for whole genome sequencing which was performed by the UKHSA Genome Sequencing and Development Unit using Nextera library preparation and Illumina HiSeq 2500 in fast-run mode according to the manufacturer’s instructions (Illumina Inc., Albany, USA). Species and serovar confirmation were derived as described previously (Ashton et al, 2016; Byrne et al, 2014; Dallman et al, 2015). Single nucleotide polymorphism (SNP) analysis was performed to identify any matches between isolates from chicken and from human cases as present in the UKHSA database in 2020 and 2021 at the 5 SNP level as previously described (Croucher et al, 2015; Dallman et al, 2018).

4.7 Evaluation of efficacy of manufacturers' cooking instructions on chicken products

Samples that were found to be positive for Salmonella spp. were sent to Fera Science Ltd. in York, using a same-day courier and packed in conditions that retained the products in a frozen condition until receipt. A further 20 chicken samples from a previous PHE study of frozen reformulated chicken products (collected from October to December 2020 and retained at -18°C in their original packaging) where Salmonella had been detected during initial testing were also sent to Fera Science Ltd for cooking (Table 10).

Frozen samples were received by Fera and stored at -18°C for 10 days. These were all suitable to cook from frozen and this was done as per the cooking instructions provided on the packaging. No defrosting was required. Fan assisted household grade ovens were used during the cooking process. The cooking times and temperatures on the back of each sample package were applied following the instructions for fan assisted appliances. The ovens were preheated, and the temperature of each oven was checked using a calibrated temperature probe prior to cooking. The product was placed in the centre of a sterile baking tray and placed in the centre of the oven. In some cases, the packaging instructions required turning of the product halfway through cooking. The products were turned using sterile tongs. Following the completion of the cooking time, the products were removed from the oven, left to cool briefly and transferred into sterile stomacher bags using sterile tongs.

Following cooking, samples were tested again for the presence of Salmonella in 25 g, using the procedure outlined in Table 1.

4.8 Quality assurance

All the chicken testing laboratories that participated in this study are UKAS- accredited to ISO 17025 and participate in External Quality Assurance (EQA) schemes. Laboratories carrying out MIC determination and further identification of strains also work within quality management systems (ISO 9001) and participate in EQA schemes. All analyses were performed by trained and competent staff.

5.0 Results

5.1 Sample numbers submitted for microbiological examination

A total of 310 samples of frozen chicken products were examined between April and July 2021. These included samples originating from 38 different approved establishments (i.e. establishments approved by the FSA, FSS or equivalent competent authority in EU Member States (MS) to undertake certain processes for which hygiene conditions are laid down in Regulation (EC) No 853/2004 for example, slaughtering of animals). The full list of approved processing plant premises numbers, including the details of each license, can be found on the FSA website. The establishments included 11 with UK approval codes, seven in Thailand, five in Poland, five in Germany, three in Hungary and one each in the Netherlands, Romania, Spain, Ireland, Brazil and China. One sample had an EC approval code (country not specified). Of the 310 samples tested, the largest proportion of samples were from a UK approval code (114 samples; 36.8%), followed by Poland (96 samples; 31.0%), Thailand (34 samples; 11.0%) and Germany (26 samples; 8.4%). The remaining samples were from other EU MS (32 samples; 10.3%) or other non-EU countries (8 samples; 2.6%). Based on UK market share data, the majority of samples were purchased from large national retail chains (n = 279), whilst the remaining 31 samples were collected from other retailers.

5.2 Detection of target organisations in chicken samples

The number of samples giving positive results for each target organism is shown in Table 5. Salmonella spp. were detected in 5 out of 310 (1.6%) samples. The Salmonella MPN/g for these five samples ranged between 0.020 and 0.230 MPN/g. Using WGS for serovar prediction, three of these were identified as S. Infantis and two as S. Java. Two of the samples from which S. Infantis was isolated were different batches of the same chicken goujon product, originating from a single Irish Approval Number, and were purchased in two different branches of the same supermarket chain. The third S. Infantis isolate was from an unrelated product with a Hungarian Approval Number. The S. Infantis isolates were genetically distant from each other (> 25 SNPs). The two samples containing S. Java originated from a single UK Approval Number but were different product types (one spicy chicken breast and one chicken nuggets) purchased in different retail outlets. The two S. Java isolates were not closely related (> 25 SNPs).

Table 5. Number of frozen reformulated chicken samples with positive results for Salmonella spp., generic E. coli and specific phenotypically resistant E. coli.

Test	Number of positive samples	% positive samples	95% CI
Salmonella	5	1.6	0.6 to 3.8
Generic E. coli	113	36.4	31.3 to 42.0
ESBL/AmpC E. coli	17	5.5	3.2 to 8.6
Colistin resistance E. coli	1	0.3	0.0 to 1.8
Carbapenem resistant E. coli	0	0	0.0 to 1.2

a Confirmed ESBL or AmpC shown; from one additional sample a presumptive ESBL was detected but could not be recovered for confirmation testing.

Escherichia coli was detected in 113 samples (36.4%). Of these, 30 samples had E. coli levels of <3 MPN/g, with the remainder ranging from 3 to >1100 MPN/g (Table 6). E. coli levels in the five samples in which Salmonella spp. were detected were <3, <3, 4, 9 and 240 MPN/g.

Table 6. Generic Escherichia coli levels detected in chicken samples

Generic E. coli level	Number of samples as a percentage	95% CI for percentage
Not detected in 25g	197 (63.5)	58.1 to 68.7
Detected; < 3 MPN/g	30 (9.7)	6.8 to 13.5
3 to <10 MPN/g	24 (7.7)	5.2 to 11.3
10 to <100 MPN/g	44 (14.2)	10.7 to 18.5
100 to <1000MPN/g	12 (3.9)	2.2 to 6.7
? 1000 MPN/g	3 (1.0)	0.2 to 2.9

5.3 Genetic association between Salmonella isolates from chicken and from human cases

One of the S. Infantis isolates was genetically very similar (ie it fell into the same 5-SNP cluster) to three isolates from human cases of infection detected in the UKHSA database (with patient specimen sample dates between March and April 2021). A second S. Infantis isolate fell into the same 5-SNP cluster as isolates from two human cases, with specimen sample dates between February and March 2021. No contemporaneous S. Java isolates from cases of human infection were detected in the UKHSA database but isolates from some historic cases were genetically very similar; one isolate from a human case (specimen data from 2018) fell into the same 5-SNP cluster as the S. Java isolated from spicy chicken breast and isolates from three human cases

(with specimen dates in 2014 and 2015) fell into the same 5-SNP cluster as the S. Java isolate from a chicken nugget product.

5.4 Determination of minimum inhibitory concentration (MIC) for bacterial isolates

5.4.1 Salmonella spp

The three S. Infantis isolates were all resistant to nalidixic acid, ciprofloxacin and tetracycline, with one also showing resistance to ampicillin (and thus being classified as multi-drug resistant). These isolates also had reduced susceptibility to sulfamethoxazole but no ECOFF value is available for this drug for Salmonella spp., and therefore it was not possible to interpret these as resistant in relation to an ECOFF value. The three isolates were susceptible to all other antimicrobials tested (for example, amikacin, azithromycin, cefotaxime, ceftazidime, chloramphenicol, colistin, gentamicin, meropenem, tigecycline and trimethoprim).

The two S. Java isolates were resistant to trimethoprim only, but also had reduced susceptibility to sulfamethoxazole.

5.4.2 E. coli

From the 113 samples giving positive generic E. coli results (detected by means of the enumeration procedure or enrichment method using media with no added antimicrobials), a total of 110 E. coli isolates were subjected to MIC determination (Table 7). The highest percentages of resistance to individual antimicrobials by isolates were: ampicillin (31.8%), sulfamethoxazole (28.2%), tetracycline (23.6%), ciprofloxacin (18.2%) and trimethoprim (15.5%). No isolates were resistant to amikacin, cefotaxime, ceftazidime, chloramphenicol, colistin, meropenem or tigecycline.

Table 7. Generic E. coli isolates from frozen chicken showing resistance to various antimicrobials (n = 110)

Antimicrobial	Number of isolates	% of isolates resistant to antimicrobial	95% CI
Amikacin	0	0.0	0 to 0.04
Ampicillin	35	31.8	23.8 to 41.0
Azithromycin	4	3.6	1.1 to 9.3
Cefotaxime	0	0.0	0 to 0.04
Ceftazidime	0	0.0	0 to 0.04
Chloramphenicol	0	0.0	0 to 0.04
Ciprofloxacin	20	18.2	12.0 to 26.5
Colistin	0	0.0	0 to 0.04
Gentamicin	4	3.6	1.1 to 9.3
Meropenem	0	0.0	0 to 0.04
Nalidixic acid	19	17.3	11.3 to 25.5
Sulfamethoxazole	31	28.2	20.6 to 37.3
Tetracycline	26	23.6	16.6 to 32.4
Tigecycline	0	0.0	0 to 0.04
Trimethoprim	17	15.5	9.8 to 23.5
Fully susceptible	49	44.5	35.6 to 53.9
Resistant to ? 3 antimicrobial groups	22	20.0	13.0 to 28.7

Resistance to three or more groups of antimicrobials (multi-drug resistance) was seen in 20.0% (22/110) of isolates. Of these, one isolate was resistant to six different antimicrobial groups, three isolates were resistant to five groups, eight were resistant to four groups and 10 were resistant to three groups (Table 8).

Table 8: Antimicrobial resistance profiles of multi-drug resistant generic E. coli

Resistance profile	Number of isolates	ESBL/AmpC isolated from same sample
Ampicillin, azithromycin, nalidixic acid / ciprofloxacin, sulfamethoxazole, tetracycline, trimethoprim	1	Yes
Ampicillin, nalidixic acid / ciprofloxacin, sulfamethoxazole, tetracycline, trimethoprim	1	Yes
Ampicillin, azithromycin, nalidixic acid / ciprofloxacin, sulfamethoxazole, trimethoprim	1	No
Ampicillin, gentamicin, sulfamethoxazole, tetracycline, trimethoprim	1	No
Ampicillin, sulfamethoxazole, tetracycline, trimethoprim	4	Yes (1) No (3)
Ampicillin, nalidixic acid / ciprofloxacin, sulfamethoxazole, tetracycline	2	Yes (1) No (1)
Ampicillin, gentamicin, sulfamethoxazole, tetracycline	1	No
Ampicillin, nalidixic acid / ciprofloxacin, sulfamethoxazole, trimethoprim	1	No
Ampicillin, sulfamethoxazole, trimethoprim	5	Yes (2) No (3)
Nalidixic acid / ciprofloxacin, sulfamethoxazole, tetracycline	2	Yes (1) No (1)
Nalidixic acid / ciprofloxacin, sulfamethoxazole, trimethoprim	1	No
Ampicillin, nalidixic acid / ciprofloxacin, trimethoprim	1	Yes
Sulfamethoxazole, tetracycline, trimethoprim	1	No

Escherichia coli with a presumptive AmpC or ESBL (or both) phenotype were detected in 18 of 310 samples (5.8%) using an enrichment procedure and subsequent sub-culture onto agar plates containing cefotaxime. These were subjected to extended MIC testing to confirm their AmpC and/or ESBL phenotype except for one isolate (from a sample from a plant in Germany) which could not be recovered after storage (Table 9).

Table 9. Number (and percentage) of presumptive ESBL-producing E. coli isolates from frozen chicken showing resistance to antimicrobials and typical resistance phenotypes (ESBL (n=15) or AmpC (n=2))

Antimicrobial	Number of isolates	% of isolates resistant to antimicrobial	95% CI
Ampicillin	17	100	80.5 to 100
Azithromycin	0	0	0.0 to 19.5
Cefotaxime	17	100	80.5 to 100
Ceftazidime	17	100	80.5 to 100
Chloramphenicol	6	35.3	14.2 to 61.7
Cirpofloxacin	8	47.2	23.0 to72.2
Colistin	0	0	0.0 to 19.5

Antimicrobial	Number of isolates	% of isolates resistant to antimicrobial	95% CI
Gentamicin	1	5.6	0.2 to 28.7
Meropenem	0	0	0.0 to 19.5
Nalidixic acid	6	35.3	14.2 to 61.7
Sulfamethoxazole	10	58.8	32.9 to 81.6
Tetracycline	8	47.2	23.0 to 72.2
Tigecycline	0	0	0.0 to 19.5
Trimethoprim	5	29.4	10.3 to 56.0
Cefepime	0	0	0.0 to 19.5
Cefoxitin	2	11.8	1.5 to 36.4
Ertapenem	0	0	0.0 to 19.5
Imipenem	0	0	0.0 to 19.5
Temocillin	0	0	0.0 to 19.5
Cefotaxime + clavulanic acid synergy	2	11.8	1.5 to 36.4
Cefotaxime+clavulanic acid synergy	2	11.8	1.5 to 36.4
ESBL	15	88.2	63.9 to 98.5
AmpC	2	11.8	1.5 to 36.4

a One additional presumptive ESBL E. coli isolate was not subjected to MIC testing and could therefore not be confirmed as ESBL or AmpC phenotype.

Of 17 isolates tested, 15 (88.2%) were confirmed as having the ESBL only phenotype, whilst two (11.8%) demonstrated an AmpC only phenotype; none had an ESBL+AmpC phenotype (Table 9). E. coli isolates demonstrating the ESBL phenotype were detected in 15 (4.8%) of the 310 chicken samples tested. E. coli demonstrating the AmpC phenotype was detected in two (0.6%) of the 310 chicken samples tested.

One E. coli isolate recovered from selective agar containing colistin showed resistance to colistin and possessed the mcr-1 gene. This isolate was from a sample of chicken fingers with a UK approval number. None of the E. coli isolates were resistant to carbapenem, either recovered from the generic E. coli test or where the specific selective method was used.

5.4.3 Characterisation of resistance genes in E. coli isolates

The 17 E. coli isolates displaying the AmpC or ESBL phenotypes during MIC analysis were tested for blaCTX-M, blaOXA-1, blaSHV and blaTEM genes. Resulting blaCTX-M amplicons were sequenced to determine the CTX-M sequence type. In total five isolates were positive for blaCTX-M type genes, of which four were also positive for blaTEM and one also for blaOXA-1 (Table 10). It is likely that the two isolates that were negative for any of the gene types tested for (those being blaCTX-M, blaOXA-1, blaSHV and blaTEM) might harbour an AmpC gene such as blaCIT.

All five isolates that were positive for blaCTX-M genes were of the Group 1 CTX-M type (Table 10). Of the five isolates, two were of the CTX-M-1 type and these isolates were from chicken products from production plants registered in UK. Another two of these E. coli isolates were of the CTX-M-55 type and these were from chicken products made in UK registered plants. The final CTX-M positive E. coli isolate was of a CTX-M-15 type and isolated from a frozen butter basted chicken breast joint product labelled with a production plant in Hungary.

Table 10. Summary of the ESBL/AmpC-producing E. coli isolates (n=17) showing multiplex PCR results for blaCTX-M, blaOXA-1-like, blaSHV and blaTEM genes

Genes (and CTX-M type) detected	Number of isolates	Number of isolates with ESBL/AmpC only phenotype	Number of isolates with UK/non-UK origin
blaCTX-M (two CTX-M-55 and two CTX-M-1) and blaTEM	4	4/0	4/0
blaCTX-M (CTX-M-15) and blaOXA-1-like	1	1/0	0/1
blaTEM	2	2/0	1/1
blaSHV and blaTEM	5	5/0	4/1
blaSHV	3	3/0	1/2
Negative for all genes tested	2	0/2	2/0

a Country of origin based on country of production plant unless otherwise stated on pack.
b Hungarian plant code. All other non-UK samples had an approval code for one plant in Ireland

5.5 Evaluation of efficacy of manufacturers' cooking instructions

Following cooking according to the instructions provided on the product packaging, Salmonella spp. were not recovered from any of the 25 samples that had previously been found to be positive for Salmonella spp. (Table 11).

Table 11. Product descriptions and Salmonella enumeration results for samples subjected to recommended cooking time/temperature treatments

Product description	Salmonella serovar detected	Salmonella MPN/g	Salmonella result after cooking (in 25g sample)
Chicken breast joint butter basted (1)	S.Infantis	0.02	Not detected
Chicken goujons (1)	S.Infantis	0.23	Not detected
Chicken goujons (1)	S.Infantis	0.092	Not detected
Spicy chicken breast mini-fillets (1)	S. Java	0.045	Not detected
Chicken nuggets (1)	S. Java	0.02	Not detected
BBQ bites (2)	S.Infantis	24	Not detected
Chicken goujons (2)	S.Infantis	1.3	Not detected
Southern fried chicken steaks (2)	S.Infantis	54	Not detected
Southern friend chicken pops (2)	S.Infantis	4.9	Not detected
Chicken poppets (2)	S. Enteritidis	28	Not detected
Chicken strips (2)	S. Enteritidis	Not determined	Not detected
Chicken poppets (2)	S. Enteritidis	Not determined	Not detected
Chicken nuggets (2)	S. Enteritidis	0.13	Not detected
Chicken poppets (2)	S. Enteritidis	1.7	Not detected
Chicken nuggets (2)	S.Infantis	0.45	Not detected
Chicken nuggets (2)	S. Enteritidis	1.7	Not detected
Chicken strips (2)	S. Livingstone	0.21	Not detected
Chicken poppets (2)	S. Livingstone	0.21	Not detected
Chicken nuggets (2)	S. Java	0.05	Not detected
Chicken poppets (2)	S. Newport	0.02	Not detected
Chicken strips (2)	S. Newport	0.02	Not detected
Chicken dippers (2)	S.Infantis	0.04	Not detected
Chicken burgers (2)	S.Infantis	0.13	Not detected
Chicken nuggets (2)	S.Infantis	1.3	Not detected
Chicken bites (2)	S.Infantis	0.49	Not detected

1. Samples from current study

2. Samples from UKHSA study (2020-21)

Discussion

The presence of Salmonella spp in breaded and battered, frozen chicken products sold in the UK is a public health concern due to the association with cases of illness between 2018 and 2021. A study by Jørgensen et al (2022), which described a survey carried out in response to an outbreak, found that Salmonella spp. were detected in 8.8% of 456 samples of frozen, breaded chicken in 2020. In contrast, in the current study, Salmonella spp. were detected in 1.6% of 310 samples. While the Jørgensen et al study was not based on market share information and may have involved some bias towards certain product types, the data obtained in the current study suggest that there has been a reduction in Salmonella contamination rates in frozen, breaded and battered chicken products between 2020 and 2021. The FSA led the investigation into the food supply chain in relation to the contaminated products detected in 2020, resulting in affected supermarket chains changing to different suppliers. These changes explain at least some of the observed improvement in results, as Salmonella contamination appeared to be linked particularly to a small number of producers.

Two of the five Salmonella spp. isolates detected in this study matched recent isolates from human cases of illness at the 5-SNP level: one S. Infantis strain matched three human cases detected in March and April 2021, two in the North West of England and one in the South West; a second S. Infantis strain matched two human cases with sample dates in February and March 2021, one in the South East of England and one in Wales. The two S. Java isolates both showed matches to isolates from two historical human cases (with sample dates between 2014 and 2018). The detection of closely related isolates from both chicken samples and human cases indicates a clear link between the chicken products and human infection, and indeed suggests that such chicken products were the probable source of the infections in humans. Further controls are required to ensure that the risks posed to consumers are reduced.

Commission Regulation (EC) No. 2073/2005 (as amended) specifies that meat preparations made from poultry meat intended to be eaten cooked should not have any Salmonella detected in a 25 g sample, when placed on the market and examined during their shelf-life. Therefore, the five samples from which Salmonella spp. were detected in this study should be recognised as unsatisfactory. For this reason, FSA was made aware of these undesirable results as soon as they became apparent to ensure that timely, appropriate actions were taken.

Previous outbreaks of salmonellosis linked to these products in Canada led Hobbs et al (2017) to make several suggestions to reduce future infections and outbreaks associated with this product type. These included setting acceptability criteria for Salmonella in such products; selling only cooked or irradiated products; ensuring that labelling and cooking instructions are clear on both the inner and outer packaging; and displaying cooked and uncooked products separately in retail outlets.

Cross-contamination of Salmonella organisms from the uncooked chicken products to ready-to-eat foods may occur prior to cooking of these chicken products. An FSA survey of consumer practices identified that only 58% of respondents always washed their hands after handling coated frozen chicken products, and the majority of respondents stated that the uncooked products always, often or sometimes came into contact with other surfaces such as worktops or plates.

When the five Salmonella-positive samples identified in this study, and twenty further similar products known to be contaminated with Salmonella, were subjected to cooking according to the

instructions on the product packaging, no Salmonella organisms were subsequently detected in any of the 25 samples. This observation confirmed that the provided cooking instructions were effective if followed accurately. However, since numerous cases of illness have been associated with these types of chicken product in the UK and other countries, it appears that either consumers do not always apply effective cooking processes or cross-contamination plays a significant role in causing infections. The current study only evaluated one cooking method (fan assisted oven). However, it should be noted that other cooking technologies are available that are not included in the instructions provided by manufacturers. For example, in an FSA consumer survey of practices in relation to coated frozen chicken products, 8% of respondents stated that they typically cooked the products using an air fryer. Inadequate cooking may be either due to variations in the effectiveness of cooking appliances or failure of consumers to follow the instructions accurately, possibly exacerbated by misunderstandings regarding the status of the products as uncooked. Cases of infection associated with the S. Enteritidis outbreak in the UK were particularly associated with juveniles and young adults (ECDC and EFSA 2021) who may be less experienced in applying adequate food hygiene procedures and more likely to use rapid methods of cooking such as microwave ovens.

Higher concentrations of generic E. coli in foods are commonly recognised as an indicator of poor hygiene. EC 2073/2005 (as amended) specifies a maximum acceptable level of 5000 cfu/g of E. coli in meat preparations, with improvements in production hygiene being required where this criterion is exceeded. Moreover, if more than two samples from a batch (when five samples are selected for testing) exceed a level of 500 cfu/g, the batch is also considered to be unsatisfactory. Only three samples (1%) in this study exceeded the 500 cfu/g limit, with the five samples positive for Salmonella spp. showing acceptable E. coli levels according to the above criteria (<3, <3, 4, 9 and 240 cfu/g respectively).

Of 110 generic E. coli isolates, 22 (20.0%) were multi-drug resistant. This compares well with a study of fresh chicken on retail sale in the UK in 2017 which found that 56.5% of generic E. coli isolates were multi-drug resistant (Willis et al, 2018). While the processes involved in producing frozen, coated chicken products may result in lower overall contamination levels compared to fresh chicken, it is unlikely that these processes would significantly affect the proportion of resistant versus sensitive organisms within the overall E. coli population on the products. Therefore, comparison of the two datasets seems useful in reviewing trends in E. coli strains associated with chicken products over recent years. When a selective method was used to specifically detect ESBL-producing E. coli (by culturing 25 g of chicken sample in an enrichment broth and then sub-culturing onto agar plates containing cefotaxime), these bacteria were detected in 4.8% of frozen, coated chicken samples. These results indicate a decrease in the proportion of samples positive for ESBL-producing E. coli in comparison with earlier UK studies of fresh chicken, as shown in Table 12.

Table 12. Proportion of chicken samples from which ESBL-producing E. coli were detected in previous UK studies and the current study

Year	Sample types	Percentages of samples positive for ESBL producing E. coli	Reference
2013 to 2014	Fresh chicken at retail sale in the UK	65.4	Randall et al, 2017
2016	Fresh chicken at retail sale in the UK	29.7	APHA, 2017
2017	Fresh and frozen chicken at retail sale in the UK	10.1	Willis et al, 2018
2018	Fresh chicken at retail sale in the UK	8.4	APHA, 2019
2021	Fresh coated chicken products at retail sale in the UK	4.8	Current study

In the current study, molecular testing (PCR) showed that 29% (5 of 17) of ESBL-producing E. coli carried blaCTX-M genes which confer resistance to third generation cephalosporin antimicrobials. In a previous study (APHA 2019), blaCTX-M genes were detected more frequently in fresh chicken meat i.e. 87% (27 of 31 isolates) of ESBL-phenotype E. coli were confirmed to be blaCTX-M positive, with 26 being blaCTX-M-1 and 1 being blaCTX-M-55 (APHA, 2019). Samples in that study were fresh and largely of UK origin (apart from 2 of 309 samples that originated from Poland). The differences in product type and country of origin may explain some of the observed differences in the prevalence of resistance genes.

In the current study, the absence of any carbapenem-resistant E. coli isolates, and the detection of only one isolate with colistin resistance is encouraging, given that these antimicrobials are particularly important drugs of last resort when treating human infections (Mediavilla et al, 2016; Meletis, 2016).

The decrease in the percentage E. coli with AMR isolated from chicken is consistent with data from the Veterinary Medicines Directorate (VMD) showing a downward trend in the use of antimicrobials in meat poultry in the UK (Veterinary Medicines Directorate, 2021). VMD reported that antibiotic usage in the chicken sector was 16.3 mg/kg in 2020, which was a decrease from 18.5 mg/kg in 2019, and 32.5 mg/kg (67%) lower than in 2014. It should however be noted that at least 63% of samples in the study reported here were produced using chicken from approved establishments outside the UK, with 31% coming from Poland, 11% from Thailand and 8% from Germany. Figures for antibiotic usage in food animals in European countries in 2018 show considerably higher usage of antimicrobials in Poland (167.4 mg/PCU) and Germany (88.4 mg/PCU) compared to the UK (29.5 mg/PCU) (European Medicines Agency, 2020). While it is encouraging that antibiotic usage and AMR are reducing in the UK food chain, the global trade in foods including meat products means that it is important to continue to monitor AMR in foods imported to the UK from other countries, as well as antimicrobial usage in food animals in the originating countries.

Conclusion

This study has demonstrated the presence of Salmonella in a small proportion of raw or partially cooked, frozen, breaded and battered chicken products in 2021, with an apparent decrease in prevalence since a similar PHE study carried out 2020. Antimicrobial resistance was observed amongst both E. coli and Salmonella isolates, including the presence of ESBL-producing E. coli in almost 5% of chicken samples. However, the proportion of chicken samples from which ESBL-producing E. coli were detected was lower than in previous FSA and APHA studies of fresh retail chicken.

The correct application of manufacturers' cooking instructions reduced Salmonella numbers in raw chicken products to undetectable levels and is likely to significantly reduce the risks posed by any AMR bacteria in the cooked product.

Consumers education and advice should emphasise the importance of:

- correctly following manufacturer's cooking instructions
- understanding the importance of differentiating between raw and ready to eat chicken products
- general good hygiene practices in the kitchen and preparation of raw food to prevent foodborne illness associated with eating raw chicken products.

References

APHA (2017). RDFS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in E. coli from Retail Meats (Year 2 - Chicken). Accessed November 2021.

APHA (2019). RDFS102109 - EU Harmonised Surveillance of Antimicrobial Resistance (AMR) in E. coli from Retail Meats in UK (2018 - Year 4, chicken).

Ashton PM, Nair S, Peters TM, Bale JA, Powell DG, Painset A, Tewolde R, Schaefer U, Jenkins C, Dallman TJ, de Pinna EM, Grant KA and Salmonella Whole Genome Sequencing Implementation Group (2016). Identification of Salmonella for public health surveillance using whole genome sequencing. PeerJ 4:e1752. DOI: 10.1186/s13073-017-0480-7

Byrne L, Elson R, Dallman TJ, Perry N, Ashton P, Wain J, Adak GK, Grant KA and Jenkins C (2014). Evaluating the use of multilocus variable number tandem repeat analysis of Shiga toxin-producing Escherichia coli O157 as a routine public health tool in England. PLoS One 9:e85901. DOI: 10.1371/journal.pone.0085901

Catford A, Ganz K and Tamber S (2017). Enumerative analysis of Salmonella in outbreak-associated breaded and frozen comminuted raw chicken products. J Food Prot 80: 814-818. DOI: 10.4315/0362-028X.JFP-16-496

Centers for Disease Control and Prevention (2021). Salmonella outbreak linked to raw frozen breaded stuffed chicken products. Accessed November 2021.

Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR (2015). Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res. 43:e15. doi:10.1093/nar/gku1196. DOI: 10.1093/nar/gku1196

Dallman TJ, Byrne L, Ashton PM, Cowley LA, Perry NT, Adak G, Petrovska L, Ellis RJ, Elson R, Underwood A, Green J, Hanage WP, Jenkins C, Grant K and Wain J (2015). Whole-genome sequencing for national surveillance of Shiga toxin-producing Escherichia coli O157. Clin Infect Dis 61:305-12. DOI: 10.1093/cid/civ318

Dallman T, Ashton P, Schafer U, Jironkin A, Painset A, Shaaban S, Hartman H, Myers R, Underwood A, Jenkins C, Grant K (2018). SnapperDB: A database solution for routine sequencing analysis of bacterial isolates. Bioinformatics. DOI: 10.1093/bioinformatics/bty212.

European Centre for Disease Prevention and Control (ECDC) and European Food Safety Authority (EFSA) (2021). Multi-country outbreak of Salmonella Enteritidis sequence type (ST) 11 infections linked to poultry products in the EU/EEA and the United Kingdom. Stockholm: ECDC/ EFSA. Accessed October 2021.

European Commission (2013). Commission implementing decision of 12 November 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria. Official J European Commun L303: 26-39.

European Centre for Disease Prevention and Control (ECDC) (2016). EU protocol for harmonised monitoring of antimicrobial resistance in human Salmonella and Campylobacter isolates – June 2016. Stockholm: ECDC.

EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control), (2016). The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2014. EFSA Journal 14:4380.

European Medicines Agency (2020). Sales of veterinary antimicrobial agents in 31 European countries in 2018. Accessed November 2021.

Fang H, Ataker F, Hedin G, Dornbusch K. (2008). Molecular epidemiology of extended-spectrum beta-lactamases among Escherichia coli isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. J Clin Microbiol. 46:707-12. DOI: 10.1128/JCM.01943-07

Hobbs JL, Warshawsky B, Maki A, Zitterman S, Murphy A, Majury A and Middleton D (2017). Nuggets of wisdom: Salmonella Enteritidis outbreaks and the case for new rules on uncooked frozen processed chicken. J Food Prot 80: 703-709. DOI: 10.4315/0362-028X.JFP-16-431

International Organisation for Standardization (ISO), (2015). ISO 16649-3:2015. Microbiology of the food chain – horizontal method for the enumeration of beta-glucuronidase-positive Escherichia coli – Part 3: Detection and most probable number technique using 5-bromo-4-chloro-3-indolyl-ß-D-glucuronide. ISO: Geneva.

International Organisation for Standardization (ISO), (2017a). ISO 6887–1:2017. Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination. Part 1: General rules for the preparation of the initial suspension and decimal dilutions. ISO: Geneva.

International Organisation for Standardisation (ISO) (2017b). ISO 6579:2017. Microbiology of food and animal feeding stuffs- horizontal method for the detection of Salmonella spp. ISO: Geneva.

Jørgensen F McLauchlin J, Verlander NQ, Aird H, Balasegaram S, Chattaway MA, Dallman T, Herdman MT, Hoban A, Lai S, Larkin L, McCormick J, Sadler-Reeves L and Willis C (2022). Levels of Salmonella and Escherichia coli in frozen ready-to-cook chicken and turkey products in England tested in 2020 as part of the response to an outbreak of Salmonella Enteritidis. Int J Food Microbiol 369. https://doi.org/10.1016/j.ijfoodmicro.2022.109609

Karami N, Martner A, Enne VI, Swerkersson S, Adlerberth I and Wold AE (2007). Transfer of an ampicillin resistance gene between two Escherichia coli strains in the bowel microbiota of an infant treated with antibiotics. J Antimicrob Chemother 60: 1142–1145. DOI: 10.1093/jac/dkm327

Mediavilla JR, Patrawalla A, Chen L, Chavda KD, Mathema B, Vinnard C, Dever LL and Kreiswirth BN (2016). Colistin- and carbapenem-resistant Escherichia coli harboring mcr-1 and blaNDM-5, causing a complicated urinary tract infection in a patient from the United States. mBio 30: e01191-16. DOI: 10.1128/mBio.01191-16

Meletis G (2016). Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis 3:15-21. DOI: 10.1177/2049936115621709

Randall LP, Clouting C, Horton RA, Coldham NG, Wu G, Clifton-Hadley FA, et al. (2011). Prevalence of Escherichia coli carrying extended-spectrum beta-lactamases (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. J Antimicrob Chemother 66:86-95. DOI: 10.1093/jac/dkq396

Randall LP, Lodge MP, Elviss NC, Lemma FL, Hopkins KL, Teale CJ and Woodford N (2017). Evaluation of meat, fruit and vegetables from retail stores in five United Kingdom regions as sources of extended-spectrum beta-lactamase (ESBL)-producing and carbapenem-resistant Escherichia coli. Int J Food Microbiol 241: 283-290. DOI: 10.1016/j.ijfoodmicro.2016.10.036

Roth N, Käsbohrer A, Mayrhofer S, Zitz U, Hofacre C and Domig KJ (2019) The application of antibiotics in broiler production and the resulting antibiotic resistance in Escherichia coli: a global overview. Poult Sci 98: 1791-1804. DOI: 10.3382/ps/pey539

Salyers AA, Gupta A and Wang Y (2004). Human intestinal bacteria as reservoirs for antibiotic resistance genes. Trends in Microbiol 12: 412-416. DOI: 10.1016/j.tim.2004.07.004

Veterinary Medicines Directorate (2021). UK veterinary antibiotic resistance and sales surveillance report: UK-VARSS 2020. Addlestone: Veterinary Medicines Directorate. Accessed November 2021.

Willis C, Jorgensen F, Elviss N, Cawthraw S, Randall L, Ellington MJ, Hopkins KL, Swift C and Woodford N (2018). Surveillance study of antimicrobial resistance in bacteria isolated from chicken and pork sampled on retail sale in the United Kingdom. London: Food Standards Agency. Accessed October 2021.

Appendix 1: Procedure for testing chicken samples

Examination of frozen poultry products for salmonella and antimicrobial resistant E. coli

Introduction

Scope

This method describes the test procedures required for the examination of frozen chicken products for an FSA-funded study of Salmonella prevalence and antimicrobial resistance.

Background

The Food Standards Agency requires data on the prevalence of Salmonella and the rates of antimicrobial resistance (AMR) amongst Salmonella and E. coli in frozen poultry products on retail sale within the UK, in response to recent recalls and withdrawals of frozen, partially cooked breaded chicken products due to contamination with Salmonella.

Principle

Frozen poultry samples will be collected from retailers in England, Wales, Northern Ireland and Scotland and tested for the presence of Salmonella and enumeration of E. coli using standard methods. Any target organisms isolated will be forwarded to a specialist laboratory for AMR testing.

The enumeration of E. coli resistant to antimicrobial agents involves the following stages:

- pre-enrichment in a non-selective liquid medium (BPW) with adjustments as necessary
- incubation at 37°C for 16-20h
- subculture of sample to three selective agars including MacConkey with cefotaxime, MacConkey with colistin and CHROMID CARBA SMART Agar
- examination of selective agar for the presence of typical colonies
- confirmation these typical colonies as E. coli using biochemical testing or Maldi Tof
- referral of typical colonies

Definitions

Antimicrobial resistance: Growth of bacteria in Minimum Inhibitory Concentration (MIC) tests in the presence of agreed antibiotics at levels above the cut-off points shown on the EUCAST website.

Salmonella species: Micro-organisms that form typical or less typical colonies on solid selective agar media and which display the biochemical and serological characteristics described in ISO 6579:2017.

E. coli: Micro-organisms which, under the test conditions specified, grow in the presence of bile salts at 44°C and show a positive ?-glucuronidase reaction.

Extended Spectrum Beta-Lactamase Producing E. coli (ESBLs): Micro-organisms which grow with typical morphology on the selective agar medium described, and which are confirmed by biochemical array and/or MALDI-TOF as being E. coli.

Safety considerations

General Safety Considerations

Normal microbiology laboratory precautions apply.

All laboratory activities associated with this SOP must be risk assessed to identify hazards. Appropriate controls must be in place to reduce the risk to staff or other groups. Staff must be trained to perform the activities described and must be provided with any personal protective equipment (PPE) specified in this method. Review of this method must also include a review of the associated risk assessment to ensure that controls are still appropriate and effective. Risk assessments are site specific and are managed within safety organiser.

Information Note: Throughout this method safety critical tasks are highlighted in yellow and identified using the exclamation mark symbol. Safety Critical tasks (or processes) are defined as "…one that if carried out incorrectly or not at all could lead to death, significant injury, ill health, loss of containment or serious plant/equipment damage".

Hazards are identified using red text. Where a means of controlling a hazard has been identified this is shown in green text.

Specific Safety Considerations

Isolation and identification must be performed by trained laboratory personnel (green text) in a properly equipped laboratory (green text) and under the supervision (green text) of a qualified microbiologist. Care must be taken in the disposal and sterilisation of all test materials (green text). Procedures involving sub-culturing from pre-enrichment broths and handling of cultures (red text) during identification procedures must be performed in a designated area of the laboratory (green text).

For specific safety information relevant to this method including method specific risk assessments refer to FNEW303 Safety Matrix for London, FNEW304 Safety Matrix for Porton and FNEW305 Safety Matrix for York.

Laboratory Containment

This method can be carried out routinely in the CL2 laboratory.

Equipment

Usual laboratory equipment and in addition:

- top pan balance capable of weighing to 0. g
- gravimetric diluter (optional)
- stomacher
- vortex mixer

- incubators: 37±1°C, 41.5±1°C and 44±1°C
- colony Counter (optional)
- stomacher bags (sterile)
- automatic pipettors and associated sterile pipette tips capable of delivering up to 10 mL and 1 mL volumes (optional)
- pipettes (sterile total delivery) 10 mL and 1 mL graduated in 0.1 mL volumes (optional)
- sterile spreaders

Culture media and reagents

Equivalent commercial dehydrated media may be used; follow the manufacturer's instructions.
Media for the detection of Salmonella are as described in FNES16 (F13; based on ISO 6579:2017).
Media for the enumeration of E. coli are as described in FNES28 (F22; based on ISO 16649-3:2015)

Buffered peptone water (ISO formulation) BPW

Element	Amount in grams
Peptone	10.0g
Sodium chloride	5.0g
Anyhdrous disodium hydrogen phosphate	3.5g
Potassium dihydrogen phosphate	1.5g
Water	1 litre
pH 7.0 $\pm$ 0.2 at 25°C	-

Information note: BPW must be pre-warmed to room temperature before use.

Tryptone Bile Glucuronide agar (TBX)

Element	Amount in grams
Enzymatic digest of casein	20.0g
Bile salts No.3	1.5g
5-bromo-4-chloro-3-indoyl-?-D-glucuronic acid (BCIG) cyclohexylammonium salt	75mg
Dimethyl sulfoxide (DMSO)	3ml
Agar	15.0g
Water	1 litre
pH 7.0 $\pm$ 0.2 at 25°C	-

MacConkey with cefotaxime (1.0mg/l) (McCon+CTX)

Element	Amount in grams
Pancreatic digest of gelatin	17.0g
Peptones (meat and casein)	3.0g
Lactose	10.0g
Bile salts No.3	1.5g
Sodium chloride	5.0g
Neutral red	0.03g

Element	Amount in grams
Crystal violet	0.001g
Agar	13.5g
Water	1 litre
pH 7.1 ± 0.2 at 25°C	-

Selective Supplement: Cefotaxime sodium salt stock solution prepared in bi-distilled water. Aliquots of aqueous cefotaxime stock solution (concentration 1 mg/mL) can be stored at -20 ? C.

Information Note: It is important to take the activity of the drug into account to ensure that 1 mg/mL active compound is used. E.g. if the manufacturer has given an activity of 50%, 2 mg/mL should be prepared to give an active concentration of 1 mg/mL.

MacConkey with colistin (2.0mg/l) (McCon+COL)

As for MacConkey with cefotaxime, but with the addition of 2.0 mg/l colistin.

CHROMID CARBA SMART Agar

MacConkey Agar (MAC)

Element	Amount in grams
Bile salts	5.0g
Enzymatic digest of gelatine	17.0g
Enzymatic digest of casein	1.5g
Enzymatic digest of animal tissues	1.5g
Lactose	10.0g
Sodium chloride	5.0g
Neutral red	0.05g
Agar	12.0g
Water	1 litre
pH 7.4 ± 0.2 at 25°C	-

Nutrient agar slopes

Dorset egg slopes

Sample processing

This method is capable of isolation of very low numbers of bacteria and will typically be carried out on samples that are not ready to eat. Procedures must be in place to avoid cross contamination of samples and guidance on how to prevent cross contamination is available in Standard Method FNES18 (Q4), Public Health Response: Involvement of PHE FW&E Microbiology Laboratory Staff in the Investigation of Outbreaks of Food and Waterborne Disease.

Sample receipt

Samples will be delivered either in person or by courier from the sampling contractor, Hallmark Meat Hygiene Ltd. The samples will be accompanied by a Hallmark sample submission letter. On receipt, the following information must be recorded:

1. Date and time of receipt
2. Temperature of samples (if above 3°C, determine whether the deviation is sufficiently significant to affect results)
3. Unique ID number (if possible, use a barcode reader to avoid transcription errors)
4. Initial post code letters of retailer
5. Whether any crumbs or other debris are visibly coming loose from the packaging
6. If relevant, the batch code of any inner packaging that cannot be seen prior to opening the outer box / bag.

Full sample details will be recorded by Hallmark on an electronic spreadsheet that will be available for the laboratory to check and refer to. However, in order to allow the lab's own records to be accurately cross-checked against Hallmark's records, retailer postcode will be recorded on the LIMS system along with the unique ID allocated by Hallmark.

Test the samples promptly on arrival or store in a -18°C freezer. Do not allow to fully defrost.
Note: If possible, return the insulated sample container to Hallmark for re-use.

Sample preparation and dilutions

Prepare the sample using the procedure described in National Standard Method FNES26 (F2) - Preparation of samples and dilutions, plating and sub-culture. Using sterile instruments and aseptic technique, weigh a representative 27 g sample of each food into a sterile stomacher bag with wire closures. Add nine times that weight or volume of buffered peptone water (BPW) pre-warmed to room temperature. Avoid touching the inside of the bag with the hands.

Promptly place the remaining sample in a sealed bag in the freezer.

If the amount of food available is less than 27 g, maintain the sample: diluent volume at 1:9 (1 in 10).

Homogenise for between 30 seconds and 3 minutes in a stomacher. The homogenisation time required will depend on the manufacturer instructions and the type of sample being examined.

Decant 20 ml of homogenate into a sterile container for inoculation of enumeration tests.

Retain the remaining 250 ml to be incubated as an enrichment broth.

Inoculation and incubation

Inoculate MMGM tubes for E. coli enumeration as described in FNES28 (F22). Incubate the remaining 250 ml BPW homogenate at 37 ± 1°C for 18 ± 2 hours.

Sub-culture to selective media
Transfer 1 ml of broth to a screw-capped tube to prepare a boilate for detection of Salmonella by PCR, as described in FNES88 (M11) and FNES43 (M2).
Also sub-culture the BPW pre-enrichment broth (using a 10 ?L loop) to:

- TBX – incubate at 44 ± 1°C for 22 ± 2 h.
- MacConkey+CTX – incubate at 44 ± 1°C for 20 ± 2 hrs.
- MacConkey+COL - incubate at 44 ± 1°C for 20 ± 2 hrs.
- CHROMID CARBA SMART agar – incubate at 37 ± 1°C for 22 ± 2 hrs (Note that there are different types of agar in each half of the plate, so a single sample broth should be inoculated onto both sides of the plate).

For samples that are Salmonella-positive by PCR:

- continue with selective enrichment for Salmonella as described in FNES16 (F13).
- perform an enumeration of Salmonella by MPN (see Appendix 2).

Identification and confirmation of colonies

E. coli

Detection: For the TBX plate inoculated from the BPW enrichment, record E. coli growth as detected or not detected. Sub-culture a single colony to a MacConkey agar plate. Incubate at 37°C for 18-22 hrs to check purity, then inoculate a nutrient agar slope and store locally. (At intervals throughout the project, a review will be undertaken of the number of E. coli isolates obtained from enumeration and detection procedures, and a decision will be made about whether any of these isolates from detection need to be sent to the APHA for MIC testing).

Enumeration: Record the MPN according to FNES28. Sub-culture up to 5 single colonies to a MacConkey agar plate (each taken from a separate segment of the TBX plate). Incubate at 37°C for 18-22 hrs to check purity, then inoculate each isolate onto a Dorset egg slope to send to APHA.

ESBL-producing E. coli

ESBL-producing E. coli form purple / red, non-mucoid colonies on MacConkey+CTX agar.
Sub-culture five suspect colonies (or all if less than five present) by re-streaking onto MacConkey+CTX agar. Incubate at 37 °C for 18-22 hrs.
Confirm E. coli identity using a validated method (e.g. 20E API, MALDI-TOF or oxidase & indole tests). Inoculate a single colony of each isolate onto a Dorset egg slope to send to APHA.

Colistin resistant E. coli

Colistin-resistant E. coli form purple / red, non-mucoid colonies on MacConkey+COL agar.

Sub-culture five suspect colonies (or all if less than five) from each plate by re-streaking onto MacConkey+COL agar. Incubate at 37 °C for 18-22 hrs.
Confirm E. coli identity using a validated method for example, 20E API, MALDI-TOF or oxidase and indole tests).
Sub-culture a single colony of each isolate onto a Dorset egg slope to send to APHA.

Carbapenemase-producing E.coli

Carbapenemase-producing E. coli appear as burgundy colonies on CHROMID CARBA SMART agar (see Figure 1).

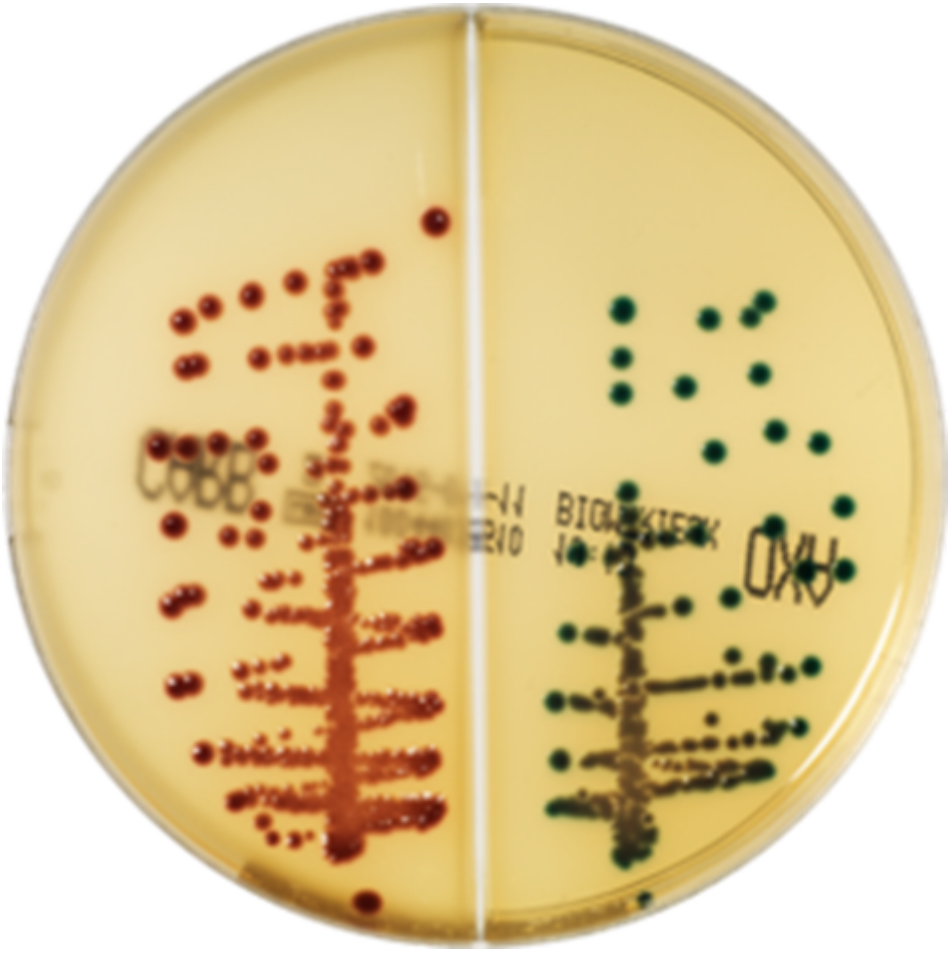

Figure 1: E. coli (burgundy) on CHROMID CARBA SMART agar.

Sub-culture five suspect colonies (or all if less than five) from each plate by re-streaking onto the same agar again. Incubate at 37 °C for 18 to 22 hours.

Confirm E. coli identity using a validated method (for example, 20E API, MALDI-TOF or oxidase and indole tests).

Sub-culture a single colony of each isolate onto a Dorset egg slope to send to APHA.

Salmonella
Pick 5 (or all if less than five present) colonies and confirm as described in FNES16 (F13).

Check purity and sub-culture a single colony of each isolate onto a Dorset egg slope to send to APHA 9 label as picks 1 to 5).

The first isolate above must also be inoculated onto a further Dorset egg slope to send to GBRU for typing.

If positive tubes are subsequently obtained from the Salmonella enumeration by MPN, retain an isolate from the most dilute tube and send this to GBRU in addition to the original isolate.

Quality control
Media for Salmonella detection and E. coli enumeration will be checked using the quality control procedures described in FNES82.
Agar plates for ESBL, colistin resistant and carbapenemase-producing E. coli procedures will be prepared by APHA, and QC checks performed prior to distribution to participating laboratories.

Reporting of results
All results are reported using the StarLims system as described in Standard Method FNES17 (Q13) Technical Validation and release of result in StarLims. The test report must specify the method used, all details necessary for complete identification of the sample and details of any incidents that may have influenced the result.

The actual weight or volume of sample examined must be reported, for example, 25g.

Report the E. coli result as detected or not detected in 25 g, as well as recording the most probable number (MPN) per gram, as described in FNES28 (F22).

Report the Salmonella as detected or not detected in 25 g. If detected, report the MPN per gram. Report the ESBL, colistin-resistant and carbapenemase-producing E. coli as detected or not detected in 25 g.

Results of public health significance

The presence of Salmonella in poultry products is unsatisfactory according to EC 2073/2005 (as amended). This must therefore be reported to the Project Lead, caroline.willis@phe.gov.uk, as soon as possible after the result has been confirmed (and no more than two weeks after the commencement of testing the sample).

Anomalous results

Where anomalous results are obtained (for example, an unusual number of samples being positive for a particular organism on the same day of testing), appropriate checks must be made on results before they are reported. These checks should include:

1. Review of sample type and other test results for the same sample, to assess general plausibility of results
2. Review of sterility controls for the day of testing
3. Review of results for other samples tested at the same time, to ensure that there is not an unusual proportion of failures overall
4. Review of result entry on the LIMS system, to ensure that there are no transcription errors, that confirmatory tests have been performed as appropriate and that any calculations have been performed correctly.
5. Review of confirmatory controls for the day that confirmatory tests were set up
6. Consideration of any relevant IQC, EQA or media QC tests that may have been performed on the same days as the samples in question (for example, to rule out cross-contamination with control strains).
7. Review of recent environmental monitoring results for the relevant laboratory areas.
8. Review of sample details entered onto the LIMS system, to ensure any results are reported in relation to the correct sample and location.

Once appropriate checks have been made, report such results to the Project Lead, caroline.willis@phe.gov.uk, as soon as possible.

Referral of cultures

A single isolate of Salmonella should be sent as soon as possible to GBRU for WGS, as detailed below. Isolates should be sent on a nutrient agar slope.

All other organisms should be sent on Dorset Egg agar slopes to APHA and may be collected together for submission once per week. Isolates should be sent in accordance with National method FNES65 Referral of CL2 and CL3 isolates.

Organism	Laboratory	Address	Contact Name

E. coli including: ESBL, colistin resistant, carbapenama se-producing E. coli	APHA	Field Epidemiology and Surveillance (Bld 17), Department of Bacteriology, Woodham Lane, Addlestone, Surrey, KT15 3NB	Shaun Cawthraw
Salmonella	PHE GBRU and APHA	61 Colindale Avenue, London, NW9 5EQ	Marie Chattaway