FINAL REPORT

A review of the impact of food processing on antimicrobial-resistant bacteria in secondary processed meats and meat products

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1. Summary

For meat and meat products, secondary processes are those that relate to the downstream of the primary chilling of carcasses. Secondary processes include maturation chilling, deboning, portioning, mincing and other operations such as thermal processing (cooking) that create fresh meat, meat preparations and ready-to-eat meat products. This review systematically identified and summarised information relating to antimicrobial resistance (AMR) during the manufacture of secondary processed meat and meat products (SPMMP).

Systematic searching of eight literature databases was undertaken and the resultant papers were appraised for relevance to AMR and SPMMP. Consideration was made that the appraisal scores, undertaken by different reviewers, were consistent. Appraisal reduced the 11,000 initially identified documents to 74, which indicated that literature relating to AMR and SPMMP was not plentiful. A wide range of laboratory methods and breakpoint values (i.e. the concentration of antimicrobial used to assess sensitivity, tolerance or resistance) were used for the isolation of AMR bacteria.

The identified papers provided evidence that AMR bacteria could be routinely isolated from SPMMP. There was no evidence that either confirmed or refuted that genetic materials capable of increasing AMR in non-AMR bacteria were present unprotected (i.e. outside of a cell or a capsid) in SPMMP. Statistical analyses were not straightforward because different authors used different laboratory methodologies. However, analyses using antibiotic organised into broadly-related groups indicated that *Enterobacteriaceae* resistant to third generation cephalosporins might be an area of upcoming concern in SPMMP. The effective treatment of patients infected with *Enterobacteriaceae* resistant to cephalosporins are a known clinical issue. No AMR associations with geography were observed and most of the publications identified tended to be from Europe and the far east.

AMR *Listeria monocytogenes* and lactic acid bacteria could be tolerant to cleaning and disinfection in secondary processing environments. The basis of the tolerance could be genetic (e.g. efflux pumps) or environmental (e.g. biofilm growth). Persistent, plant resident, AMR *L. monocytogenes* were shown by one study to be the source of final product contamination.

AMR genes can be present in bacterial cultures used for the manufacture of fermented SPMMP. Furthermore, there was broad evidence that AMR loci could be transferred during meat fermentation, with refrigeration temperatures curtailing transfer rates. Given the potential for AMR transfer, it may be prudent to advise food business operators (FBOs) to use fermentation starter cultures that are AMR-free or not contained within easily mobilisable genetic elements.

Thermal processing was seen to be the only secondary processing stage that served as a critical control point for numbers of AMR bacteria.

There were significant linkages between some AMR genes in *Salmonella*. Quaternary ammonium compound (QAC) resistance genes were associated with copper, tetracycline and sulphonamide resistance by virtue of co-location on the same plasmid. No evidence was found that either supported or refuted that there was any association between AMR genes and genes that encoded an altered stress response or enhanced the survival of AMR bacteria exposed to harmful environmental conditions.

2. Executive summary

Antimicrobial resistance (AMR) has an impact on the effective clinical treatment of infections. The issue is of global concern and current estimates are that around 700,000 die prematurely as a consequence of AMR infections (O'Neill 2014). Future projections estimate there will be 10 million AMR deaths annually by 2050 (O'Neill 2014). Relatively little is known regarding the role that food plays in the problem of AMR. Consequently; this review was concerned with the impact of secondary processing of red meat such as meat derived from cattle, pig and sheep, white meat such as meat derived from poultry and the associated secondary processed meat products (SPMMP) derived from these animals on populations of AMR bacteria, genes conferring resistance and the mechanisms of transfer of resistance loci. Secondary processes for the purposes of this review were any processes that were undertaken after the primary chilling of red and white meat carcasses and included maturation chilling, the cutting and deboning of carcasses and activities such as comminution (e.g. mincing) or thermal processing, which resulted in red meat or poultry meat preparations and ready-to-eat meat products.

The publications for review were identified from searches of eight literature databases. An iterative refinement process reduced an initial total of over eleven thousand identified documents to 143. Search effectiveness was assessed by the random selection of papers and then studying their bibliographies to see if they contained papers overlooked by the searches. Searches were repeated twice during the project to detect new papers in 2019, published after the initial searches. In total, 169 papers were systematically appraised using a standardised questionnaire by a minimum of two reviewers. A statistical test, Cohen’s kappa, was used to assess the degree of agreement between appraiser scores and ensure consistent assessment. Including for arbitration purposes, around 400 reviews were undertaken, leading to 74 papers being assessed as suitable for inclusion. The majority of papers related to AMR surveillance in SPMMP and not significant numbers of papers were identified that dealt with other aspects of secondary processing and AMR populations.

The identified papers provided evidence that SPMMP can be contaminated by AMR bacteria. However, no evidence was discovered to confirm or deny the possibility that naked genes, capable of creating AMR bacteria, were present in SPMMP. Much of

the surveillance literature was collected at retail, which made it difficult to differentiate between indigenous, natural contamination in the meat sampled, cross contamination during processing and contamination that occurred downstream of processing (e.g. during retail, in-store, slicing of cooked meats).

Papers relating to AMR *Listeria monocytogenes* that were tolerant to sanitisers dominated studies undertaken in the environment of secondary processing areas. Persistent, environmental, AMR *L. monocytogenes* were shown by one study to act as a source of final product contamination.

Starter cultures containing AMR can be deliberately added to meat to ensure effective fermentation. There were reports in the identified literature that AMR genes were present in fermented products and could be transferred to non-AMR bacteria during fermentation. Hence, the general precautionary approach and recommendation from the World Health Organisation (WHO 2015) that food business operators (FBOs) should be advised to use fermentation starter cultures that are AMR-free is also recommended by this review. As an interim measure, it might be reasonable to allow a time-restricted caveat to allow mobilizable AMR for historic strains to be cured before stricter implementation that allowed only intrinsic resistance. Three UK-based, fermented meat manufactures were informally questioned about AMR in their lactic acid bacteria (LAB) starter cultures. None had tested strains for AMR. FBOs might be advised to consider testing their starter cultures for AMR, and/or to build this requirement into their purchase specifications. There are no current, recommended standard protocols and guidance. If these were developed, FBOs could be provided with validated methodologies designed to cure AMR plasmids from their starter strains.

The study encountered difficulties caused by a range of microbiological methods being used to isolate AMR bacteria. AMR characterisation of isolates was also subject to diverse methodologies. AMR determination was commonly undertaken to comply with the Clinical and Laboratory Standards Institute (CLSI) protocols. The CLSI standard has evolved over time. Laboratories have a choice of AMR determination methods (e.g. disk diffusion or microtitre plate protocols) to define the minimum inhibitory concentration (MIC) for a given antibiotic, or report an organism as susceptible, intermediate, or resistant to an AM. Further, breakpoints are reactive

and change as typical susceptibilities for an organism change. A choice of laboratory methods with breakpoints that changed over time meant that it was not straightforward to combine information from different studies and use metanalysis to increase the statistical value of the data and draw conclusions for this study. Accordingly, a relatively simple statistical analysis was undertaken to analyse the summarised information identified. The analysis, which should be interpreted cautiously, indicated that *Enterobacteriaceae* that were resistant to cephalosporins; which is a known clinical issue, might be an upcoming concern in SPMMP.

Analyses to determine if AMR in SPMMP was influenced by geography did not detect differences between regions. The main issue was a disproportionately large number of papers relating to studies undertaken in Asia and Europe, which confounded balanced analysis. The comparatively fewer studies from the Americas and Oceania may underlie the observed outcome of no significant regional differences.

Plasmid-mediated transfer of AMR to and from SPMMP isolates has been observed. Identified publications were mainly *in vitro* mimics of industrial processes, although natural transformation was reported by two studies. Transconjugative transfer of AMR occurred during a four-week sausage fermentation using starter cultures of 10^8 cfu/g, using a model system that was considered a good mimic of a commercial fermentation.

For the *in vitro* transfer of AMR genes during SMP, lower temperatures were a significant barrier to transfer. By law in the UK, meat and meat products should be refrigerated to ≤4°C or ≤7°C (depending on species and product) to limit the growth of pathogens and spoilage organisms. However, effective chilling also had the benefit of reducing or preventing the spread of AMR genes by plasmid exchange. The effects of other parameters such as pH, [salt] and packaging atmosphere composition were strain specific. For example, Jayaratne (1987b) reported one *E. coli* donor strain was observed to have an increased transformation frequency under anaerobic conditions, another *E. coli* strain showed a decreased transformation efficiency and a third strain showed no significant difference between aerobic and anaerobic atmospheres. Consequently, because the responses were different in the small numbers of strains assessed, no general conclusions could be drawn from the evidence identified.

L. monocytogenes of undetermined AMR can have an ability to colonise processing environments, potentially contaminating product for extended periods. Consequently, there were studies reporting the impact of sanitising agents on *L. monocytogenes*. Persistence was associated with quaternary ammonium (QAC) resistance in some, not all, *L. monocytogenes*. Genes conferring resistance to QAC could be plasmid borne and transferred from resistant *L. monocytogenes* to other species. In addition, there were *L. monocytogenes* niches in that did not get full exposure to QAC treatments and some sub lethally exposed strains increased their intrinsic QAC resistance. Full exposure to QAC at manufacturer-stipulated concentrations was lethal, even for QAC tolerant strains. Biofilms could provide a physical barrier to sanitising compounds, reducing effective QAC exposures. Plasmid born loci were shown by a single paper to control the expression of chromosomally located genes regulating biofilm formation in *L. monocytogenes*. It was unclear if artificial biofilms, created in a laboratory, were an effective mimic for natural biofilms. There is likely benefit for undertaking work that creates standard strains and conditions that accurately model natural biofilms so that the relative roles of AMR and biofilms can be quantified in terms of strain, persistence and effective sanitation.

There was also evidence that some plant environmental LABs could be resistant to routine commercial disinfectants. *In vitro*-determined resistance in some LABs varied between 30µg/ml and >200µg/ml QAC. QAC may not be able to effectively sanitise *E. coli* and *Salmonella* biofilms, at low (4^{o}C) temperatures, with evidence that higher temperatures provided better disinfection. An isolate of *P. mirabilis* was determined to be resistant to QAC. The resistance was conferred by a plasmid-borne qacH gene. The plasmid transferred to *E. coli* by transconjugation, but there was insufficient literature to identify if this was a common or a rare event. Further studies that clarified if persistently colonised processing environments were a cross-contamination source for AMR bacteria and facilitated the transfer of AMR genetic materials isolated from final SPMMP would be helpful to address this gap identified in the literature

Cooking was the only secondary process that served as a critical control point for AMR bacteria. However, effective refrigeration could reduce the numbers of certain bacterial genera, and their ability to transfer AMR. No evidence was found that low temperatures had an impact on the maintenance of AMR plasmids or were beneficial

for plasmid curing. After repeated rounds of freeze/thaw, AMR plasmids were still detected.

Evidence identified a role for cross resistance between QAC and cadmium in *L. monocytogenes* with broad-spectrum *bcrABC* efflux pumps being able to export both compounds. The *qacH* efflux pump did not confer increased tolerance to additional AMs at clinically relevant concentrations in *L. monocytogenes*. There were significant linkages between some AMR genes in *Salmonella*. QAC resistance genes were associated with copper, tetracycline and sulphonamide resistance by virtue of co-location on the same plasmid. For the literature identified for SPMMP for this review, no evidence was found to support or refute any association of AMR genes and altered stress responses or enhanced survival of AMR bacteria.

In conclusion, the topic of AMR as it related to SPMMP has been a limited focus for study using a variety of bacterial species. AMR bacteria can be routinely isolated from SPMMP. Some SPMMP matrices supported the transfer of plasmids between identical and diverse species under in vitro conditions. It is important to note that some of the transfer conditions were excellent models for some commercial fermentations and nearly indistinguishable to industrial conditions. Thus, it is quite likely that some commercial fermentations might facilitate AMR transfer. However, the diversity of methodologies applied in the determination of resistance breakpoints, a lack of basic knowledge in biofilm formation and composition and the largely unknown AMR status of many deliberate process bacterial additions such as fermentation starter cultures, confounded the drawing of definitive conclusions for many aspects of SPMMP and populations of antimicrobial resistant (AMR) bacteria. Some of the knowledge gaps could be addressed by further work as described in Section 9.

3. Glossary

AM	Antimicrobial.
AMR	Antimicrobial resistance.
Aminoglycoside	Aminoglycosides; a class of antibiotic that is characterised by a sugar backbone that has been modified, typically by substituting a hydroxyl (OH^-) group with an amine (NH_2). Aminoglycosides commonly have antimicrobial activities for Gram negative bacteria.
BC	Benzalkonium chloride.
β-lactams	See penicillin
CCP	Critical control point.
CFU	Colony forming units.
CSM	Cooked, sliced meats.
Carbapenems	Carbapenems are a derivative of the β-lactam class of antibiotics. Their mechanism of action is to bind to penicillin-binding proteins, which inhibits cell wall synthesis.
Cephalosporin	Cephalosporins are a derivative of the β-lactam class of antibiotics. Their mechanism of action is the same as most □-lactams, whereby they interfere with peptidoglycan synthesis and assembly of cell walls. Cephalosporins are commonly active against Gram positive bacteria.
Co-resistance	The presence of two or more resistance determinants in a single microorganism.
Co-selection	The combined selective pressures exerted by two or more types of antibiotics on a single microorganism. For survival, any co-selected organism must acquire resistance to all of the

	antimicrobials to which it is exposed. Co-selection can be accomplished either through co-resistance or cross-resistance.
Cross-resistance	The presence of a single resistance determinant that provides protection against two or more antimicrobial compounds.
DNA	Deoxyribonucleic acid.
DRT	Decimal reduction time.
EHO	Environmental health officer.
EFSA	European Food Safety Authority.
EU	European Union.
EUCAST	The EU Committee on Antimicrobial Susceptibility Testing.
FAO	Food and Agriculture Organisation (of the United Nations).
FBO	Food business operators.
Fluoroquinolone	Fluoroquinolone; broad-spectrum antimicrobials that are commonly active against both Gram-positive and Gram-negative bacteria. Fluoroquinolones are characterised by a bicyclic core structure where two carbon atoms are shared between two adjacent six carbon rings.
FSA	UK-Food Standards Agency.
Glycopeptide	Glycopeptide; glycosylated cyclic or polycyclic peptides that are non-ribosomally synthesised. The non-ribosomal construction of peptides allows the concatenation of unusual amino acids including D-forms which may help confound the emergence of resistance.
GRAS	Acronym used in the United States for a chemical used in food processing because it is ‘generally regarded as safe’.

HC	High care.
HGT	Horizontal gene transfer.
HR	High risk.
Integron	Integrons commonly exist on plasmids although there have been reports of chromosomal integration. Integrons have a standard organisational structure. Commonly there is the presence of genes and insertion sequences that control integration into larger DNA structure and at least one promotor that sits in the 5' direction in front of the cassette of genes. Commonly, inside the cassette there are resistance genes. Integrons are commonly identified by the presence of an integrase gene that assists in the recombination of the integron into a larger DNA structure. Type II integrons are commonly associated with, and possibly derived from, the Tn7 family of transposons.
LA	Local Authority.
LAB	Lactic acid bacteria.
Macrolides	Macrolides are a class of antimicrobials characterised by a large macrocyclic lactone ring to which one or more deoxy sugars are attached. The lactone rings are can contain as many as 16 carbon or oxygen atoms in their circumference. Macrolides inhibit protein synthesis in a wide range of bacteria.
MAP	Modified atmosphere packaging.
MSM	Mechanically separated meat.
Nitrobenzine	Nitrobenzines are a class of antibiotics that are derived from a benzine ring that has been nitrated with NO_2. Nitrobenzines inhibit protein synthesis.

Nitrofuran	Nitrofuran antibiotics are composed of a four-carbon ring closed with an oxygen atom (i.e. a furan ring) that has been nitrated with NO_2. Nitrofurans are broad spectrum antimicrobials that have multiple activities including generation of destructive oxygen radicals.
Nosocomial infection	An infection that is acquired by a patient in a hospital (or other clinical treatment or care environment).
NPLM	Non-persistent L. monocytogenes.
ORF	Open reading frame.
Oxazolidinone	Oxazolidinones are five-member heterocyclic rings composed of three carbon atoms, an oxygen atom and a nitrogen. They are a novel class of antibiotics that inhibit protein synthesis by binding to 50S ribosomal subunits. Oxazolidinones active against a large spectrum of Gram-positive bacteria.
Penicillin	The penicillins are the original class of β-lactam antibiotics. Penicillins function by inhibiting the formation of peptidoglycan cross-links in bacterial cell walls.
PL	Potassium lactate.
PLM	Persistent L. monocytogenes.
PFGE	Pulsed field gel electrophoresis.
PPE	Personal protective equipment.
QAC	Quaternary ammonium compounds.
QMRA	Quantitative microbiological risk assessment.
RAPD	Randomly amplified polymorphic DNA.
RTE	Ready-to-eat.

SME	Small to medium-sized enterprises.
SPMMP	Secondary processed meat and meat products as defined by section 1.1.
SOP	Standard operating procedure.
Streptogramin	Streptogramin; a class of cyclic peptide antibiotics that inhibit the synthesis of bacterial proteins.
Sulphonamide	Sulphonamide; an antibiotic class comprising functional side chains bound to a sulphur molecule, two oxygen atoms and a nitrogen. Sulfonamide antibiotics are synthesised compounds active against both Gram-positive and Gram-negative bacteria.
Tetracycline	The tetracyclines typically have four adjacent heterocyclic rings each composed of six carbon atoms that are modified. Tetracyclines inhibit protein synthesis by blocking the binding of tRNA to ribosomes. Tetracyclines are active against both Gram-positive and Gram-negative bacteria.
Transposon	Transposable elements are DNA organisational structures that include sequences that allow the transposon to change its position within a genome. Transposons can create insertion mutations if they integrate into an open reading frame and commonly such mutations are reversed when the transposon shifts location.
UN	United Nations.
VBNC	Viable but non-culturable.
VP	Vacuum packed.
WGS	Whole genome sequencing.
WHO	World Health Organisation (of the United Nations).

w/w	A percentage concentration expressed as weight divided by weight.
w/v	A percentage concentration expressed as weight divided by volume.

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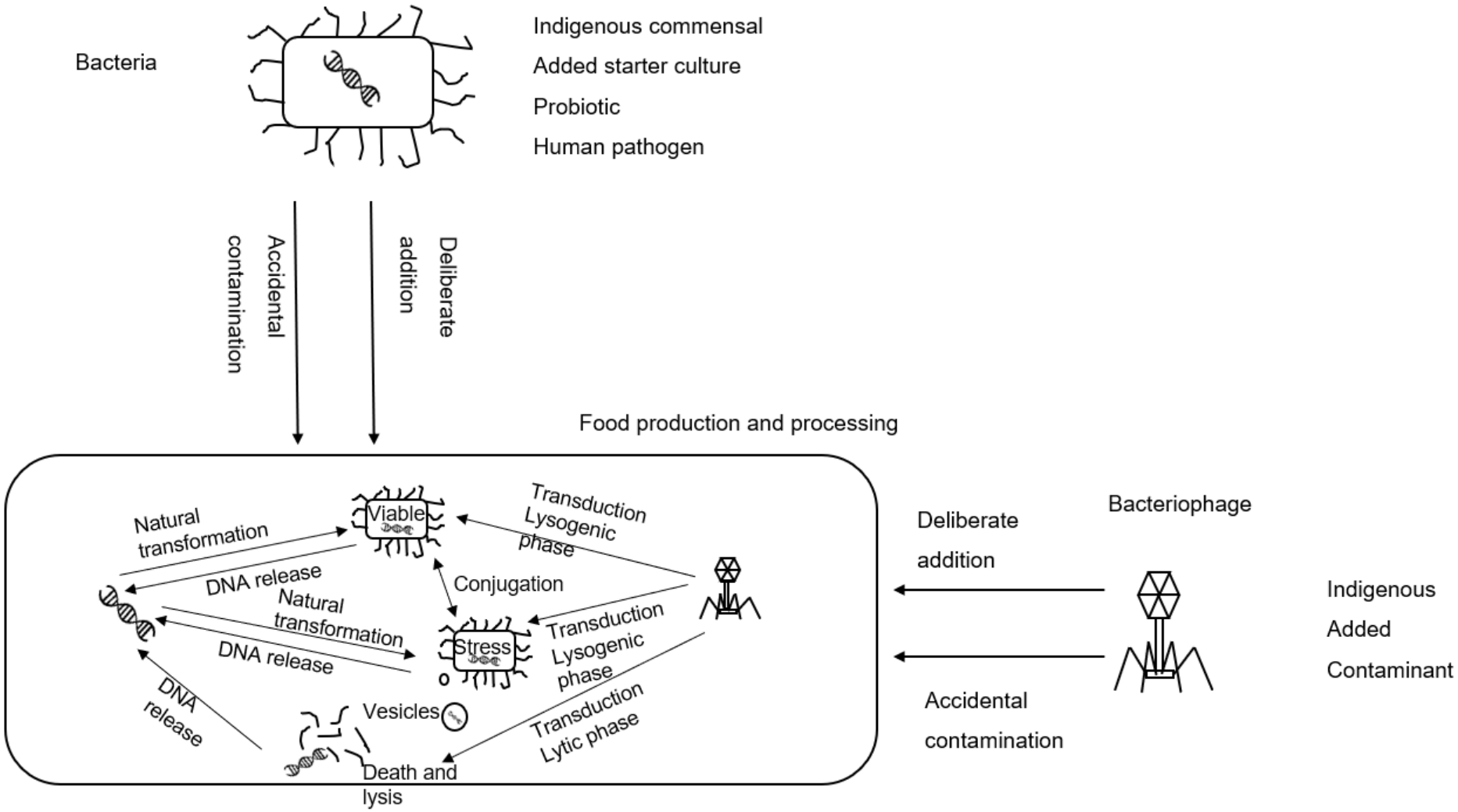
5. Introduction

Antimicrobial resistance (AMR) can be defined as an ability acquired by a microorganism to atypically resist an inhibition to growth or death due to exposure to an antimicrobial compound at a concentration that is higher than is typically required for the suppression of a specific bacterial species (Verraes et al. 2013).

To succeed, microorganisms must constantly adapt to become better suited to their environment. AMR is a natural, evolutionary process which is driven by a selective pressure applied to a susceptible population due to exposure to an antimicrobial (Michael et al. 2014). In a large number of cases, but not all, the basis of the emergence of AMR is genetic adaptation (White and McDermott 2009). The genome sizes of microorganisms can vary widely but are typically around 5 Mbp (Land et al. 2015). In addition, there can be large numbers of microorganisms, up to 10^8 cells per ml, in small volumes and mean generation times that can be as short as 20 minutes, e.g. for some enteric bacteria such as *E. coli* (Michael et al. 2014).

Taken together, these factors mean that if a gene conferring antimicrobial resistance is present within an organism, then the fact that it that is less susceptible to an antimicrobial, can allow it to quickly become the dominant phenotype within a heterogeneous population. Organisms can acquire such antimicrobial resistance genes (AMG) by a variety of genetic mechanisms including mutation of wild-type, susceptible genes or their products to create less susceptible ones (e.g. genomic point mutations); and the acquisition of new genetic material by horizontal transfer (Verraes et al. 2013; Munita and Arias 2016) through mechanisms such as conjugation, natural transformation and transduction (Figure 1 An overview of the horizontal gene transfer processes operating on bacteria during food processing that create AMR bacterial strains. Adapted from Verraes et al. (2013)).

Figure 1 An overview of the horizontal gene transfer processes operating on bacteria during food processing that create AMR bacterial strains. Adapted from Verraes et al. (2013).



Horizontal gene transfer (HGT) is enhanced by recognised DNA structures and organisational motifs such as plasmids, integrons and transposons (Bennett 2008), which facilitate the movements of genes. Far less commonly, cellular fusion of different organisms and the fusion of vesicles to a new host may transfer resistance genes and create new AMR organisms (Verraes et al. 2013). The HGT processes of conjugation, transformation and transduction tend to occur at low frequencies (Michael et al. 2014) and it is more common for spontaneous point mutations to arise. Although overall, a change to a single organism's genome may be quite rare, the very large numbers of bacteria commonly contained within a population mean that the occurrence of beneficial changes are not uncommon and thus an organism carrying AMR can quickly become the dominant clone within a population when driven by the selective pressure applied by an antimicrobial (McDermott et al. 2002; White and McDermott 2009; Michael et al. 2014).

AMR affects the chemotherapeutic treatment of human infections and so, in 1969, the UK government published a report on the use of antibiotics in animal husbandry and veterinary medicine (Swann 1969) and the impact these may have on human clinical treatments. The Swann report was one of the first to consider the relationship between foods of animal origin and antimicrobial-resistant bacteria and to flag AMR as a potentially serious, emerging problem. The Swann report also contained clear and well-thought out strategies to prevent a serious future AMR issue such as restricting the use of antimicrobials used for growth promotion, and in the treatment of livestock, to those not used for the treatment of human infections. The 'restricted use' advice proposed originally by Swann et al (1969) continues to be issued by influential governmental bodies (Codex 2005; Anonymous 2019) and recent peer reviewed papers (Nhung et al. 2018).

The AMR issue is global and affects the chemotherapeutic treatment of human infections (Prestinaci et al. 2015). A current estimate of the seriousness of the issue is that 700,000 die prematurely each year as a consequence of physicians being unable to effectively treat infections caused by AMR bacteria (O'Neill 2014). Responding to the threat of AMR bacterial populations, the UK prepared a five year antimicrobial resistance strategy (DoHSC 2013). The strategy aimed to slow the spread of AMR using a holistic approach that made consideration of medical and veterinary chemotherapies and also the wider use of antimicrobials (AM) in agriculture and the environment.

The first World Health Organisation (WHO) report describing global surveillance of AMR was published in April 2014 (WHO 2014). Like the Swann et al. report (1969) and the UK strategy (DoHSC 2013), the WHO activities used novel, innovative and unorthodox approaches to the issue. The WHO strategy involved collating international surveillance data for the first time. Previous efforts and advice relating to AMR from the WHO had been targeted globally, but used a

science basis gathered from only a few, mostly European and/or English-speaking, countries (WHO 2001). Although some of the Swann report recommendations were implemented in the UK and Europe, over the 40 years between the Swann and WHO reports, the collated WHO (2014) data showed AMR to be a global issue that had quietly risen to become a credible threat to the effective treatment of infected patients (Prestinaci et al. 2015). Bacterial AMR had become established as a global issue in the areas of medical treatment of infections, livestock husbandry and the processing of foods of animal origin (Gyles 2011; McDermott 2013; Figueiredo et al. 2015). In addition, the WHO (2014) report also made clear that although AMR was an issue predominantly affecting bacteria, it was an issue that also impacted on populations of viruses and fungi.

Although the Swann et al. (1969) and WHO (2014) reports were key assessment and strategy documents, there was considerable research and consequent publication activity in the timespan between these salient publications. For example Hoelzer et al. (2017) concluded that AMR bacteria were an increasingly significant problem, based on the facts that resistance mechanisms had been identified for all known antimicrobial classes. No significant differences in AMG were seen in classes that were available for both the treatment of human and animal infections. Hence AMR found in animals might potentially also be AMR should they infect humans, and vice versa, limiting treatment options. A number of key papers have also indicated that antibiotic use in agriculture, particularly in livestock production, was contributing to the growing AMR issue (Schroeder et al. 2002; van de Sande-Bruinsma et al. 2008; Schrijver et al. 2018).

Hoelzer et al. (2017) reviewed antimicrobial chemotherapy use in food-producing animals and attempted to assess the associated risks to human health. Despite the established advice to curtail antimicrobial use in livestock noted above, Hoelzer et al. (2017) reported that the link between AMR in livestock and resistance in strains causing human infection was complex and not yet clear cut. A main issue appeared to be that acquired AMR could be conferred by more than one genetic mechanism (Michael et al. 2014). Furthermore, resistance traits carried by bacteria commonly, but not exclusively, could come with the price of a significant metabolic overhead but the burden of the overhead could be different for the same resistance phenotype, as the AMR property could be achieved by different genetic mechanisms (Levy and Marshall 2004). A key barrier to the establishment of causality or even correlation between antimicrobial usage in livestock and resistance strains in human infection was that clones that had a resistance phenotype with a high metabolic overhead could be selected against in antimicrobial-free environments by the same mechanisms that selected for them in the presence of antimicrobials (Kang and Park 2010; Maher et al. 2012; Michael et al. 2014). Thus, the AMR carrying organisms could be disadvantaged during growth in the absence of the antimicrobial and constitute a

reducing proportion of the population. Unpredictable changes to the numbers of AMR bacteria within a larger population can complicate attempts to study the dynamics of AMR acquisition and spread (Michael et al. 2014).

The above hypothesis and its impact on the establishment of causality is an extension of one made originally by Levy and Marshall (2004). A summary of the current theory is that the metabolic overhead costs may be higher for chromosomally mediated resistance mechanisms compared with plasmid-mediated resistance and might be influenced by the nature of the resistance mechanism (Marshall and Levy 2011). There is evidence that modified metabolic pathways, enzymes that modify antimicrobials into harmless derivatives, multiple point mutations in chromosomal genes, and the use of membrane channels, all have considerable metabolic overheads in comparison to sensitive wild type organisms (Levy and Marshall 2004; Marshall and Levy 2011; Michael et al. 2014). The hypothesis may also partly explain why some bacteria acquire resistance, but others occupying the same niche do not, when exposed to an antimicrobial (van Boven et al. 2003).

However despite barriers of these types, a study based on information from seven European member states established that there was significant correlation between veterinary use of the eight antimicrobial classes; fluoroquinolones, amphenicols, third generation cephalosporins, sulphonamides, penicillins, fluoroquinolones, aminoglycosides and tetracycline, and the AMR resistance of commensal *E. coli* isolated from the gastrointestinal tracts of bovines, porcines and ovines (Chantziaras et al. 2013). The study of Chantziaras et al. (2013) was more robust than a significant body of similar work (Jacobs-Reitsma et al. 1996; Cameron-Veas et al. 2016; Gibbons et al. 2016; Jimenez-Belenguer et al. 2016; Nguyen et al. 2016; Ohta et al. 2017; Waldner et al. 2019; Xia et al. 2019; Zeineldin et al. 2019) because it used data obtained from seven countries as well as studying a wide range of antimicrobials. Taken collectively, there is a significant body of scientific evidence that supported the hypothesis that the veterinary use of antimicrobials can cause the emergence of AMR bacteria associated the treated livestock (Barlaam et al. 2019).

In addition to resistant bacteria contaminating foods of animal origin, the emergence of food-related and clinically-isolated bacteria that were resistant to widely used clinical and industrial sanitisers such as quaternary ammonium compounds (QAC) had become a focus for study over the last 20 years of the 20th century (Sundheim et al. 1998). Bacteria present in a processing plant are subject to regular, frequently daily, exposure to sanitising chemicals intended to eliminate them. Resistance to such sanitisers had been reported and was not only confined to QAC and included other sanitiser classes including iodophors, peroxides, phenols and chlorine (Loughlin et al. 2002; Chapman 2003; Gupta et al. 2018). Early studies by Aase et al. (2000) revealed that one

resistance mechanism employed by QAC-resistant bacteria was an ABC-type efflux pump of the type able to export antibiotics such as ciprofloxacins from bacterial cytosols (Webber and Piddock 2003), and hence the transporter conferred resistance to these antibiotics. Furthermore, the transporter reported by Aase et al. (2000) had a low substrate specificity that it could additionally remove toxic chemicals such as ethidium bromide from the interior of *L. monocytogenes* cells, again conferring resistance.

In a review of disinfectant resistance mechanisms, cross-resistance, and co-resistance Chapman (2003) presented evidence that AMR to sanitising chemicals might not exclusively have a genetic basis, an assertion that has been repeated subsequently by other authors (Munita and Arias 2016). Bacterial niches, and in particular, bacterial growth in the form of biofilms contributed to disinfectant resistance because their structure provided a physical mechanism that could result in some microorganisms only being exposed to a sub lethal level of sanitising compounds. There are several reports that have shown resistance to sanitisers is more prevalent among food-borne and food processing environment *L. monocytogenes* isolates compared with isolates from human, animal, faecal and environmental (e.g. soil) sources (Gkana et al. 2017; Kim et al. 2017; Madden et al. 2018; Donaghy et al. 2019).

Thus, the use of sanitisers such as QAC during the routine cleaning and sanitation of processing environments may be important for increasing both the number and degree of resistance of AMR strains. One key factor that is important to note is that most QAC-based sanitisers are not required to be rinsed off surfaces before the commencement of processing. Such procedures are marketed as a strategy to maximise both the contact time between bacteria and the QAC and the value of the monies spent on the sanitiser (Fraise 2002). Long-term exposure to a QAC, with a lowered chemical reactivity and impaired ability to kill bacteria, possibly as a consequence of biofilm growth, could favour the survival of clones with higher minimum inhibitory concentrations (MICs) to various antimicrobial agents (Hutchison et al. 2014). It is therefore now well established that bacteria can become adapted to resist a range of sanitising chemicals in food processing areas, although the importance of sanitiser AMR isolates to the treatment of human infections remains to be established.

Given that the use of sanitising chemicals is important during processing and that foods of animal origin are an important potential source of AMR microorganisms, there have been a number of reviews that have focussed on these topics and attempted to draw conclusions regarding any impact made to the effective treatment of human illness. However, one area that appears to have escaped scrutiny is any contribution made to clinical infections by the secondary processing of meat and meat products. Consequently, this review attempts to assess the impact that established

and novel secondary meat processes have on AMR bacteria and also its impact on the transfer of antimicrobial resistance genes (AMG). Included in this is a consideration of the effects of bacterial stress responses and the use of sub-lethal food processing technologies on the potential transfer of resistance genes.

5.1 Definition of secondary processing and the focus of the review

This literature review was concerned with the impact of secondary processes on populations of antimicrobial resistant (AMR) bacteria, the genetic material conferring resistance and the mechanisms of transfer of these genetic materials. Secondary processes for the purposes of this review are any process outputs that are undertaken after the slaughter, dressing and primary chilling of red or white meat carcasses. Secondary processes include maturation chilling, cutting of carcasses into primals or portions, deboning and activities such as comminution that result in red meat or poultry meat preparations. These are defined as:

- Post-chill fresh meat, including meat that has been reduced to fragments that has had foodstuffs, seasonings or additives added to it or which has undergone processes insufficient to modify the internal muscle fibre structure of the meat and thus to eliminate the characteristics of fresh meat. An example of a meat preparation is minced meat.
- Meat products, which are defined as the products of further processing such that the cut surface shows that the product no longer has the characteristics of fresh meat. An example of a meat product is a fermented sausage such as salami.
- Poultry meat and poultry meat preparations, which are defined as products containing post-chill poultry meat with muscle fibre characteristics of fresh poultry meat.
- Mechanically separated meat (MSM) i.e. products obtained by removing red meat from flesh-bearing bones after boning or from poultry carcases, using mechanical means resulting in the loss or modification of the muscle fibre structure.

This review was concerned with the literature identified from reproducible searches of bibliographic databases followed by a favourable systematic appraisal, by at least two reviewers, to determine the relevance of each publication to the questions asked by the review. The review had an overall focus on any meat process that caused potentially changes to AMR in secondary processed meats and meat products (SPMMP), as defined above. We define AM to include both antibiotic chemotherapies and as well as antimicrobial cleaning and sanitising chemicals. Subject to the identification of suitable literature, the review also summarised surveillance of AMR in SPMMP. Areas of focus were changes in the viability of AMR bacterial populations consequent to stresses applied, the fate of genes responsible for conferring AMR and the impact of secondary processes on the transfer of antimicrobial resistance genes (AMG). The interaction of processing

with cellular processes such as plasmid conjugation, lysogenic phage activity and other aspects of genome plasticity were reviewed where possible to determine how extracellular genetic material survived outside the cell to potentially give rise to derivative AMR.

In more detail, this review strove to answer the following questions:

- Are the products of secondary meat processing known to be contaminated by AMR bacteria, or genetic materials that could give rise to AMR bacteria? The evidence is discussed within Section 1.3.

If such contamination can be confirmed, then the further questions to be answered are:

- Are the raw materials the only source of AMR bacteria, or materials conferring AMR properties to bacteria, in secondary processed meats? Discussions and supporting evidence relating to this question are provided in Sections 1.3.1.1 and 1.5.
- Does the transfer of genetic materials conferring antibiotic resistance occur between bacteria during secondary processes and, if so, which are the salient factors that influence such gene transfers? Evidence relating to this question is covered in Sections 1.4 and 1.6.
- Does any aspect of secondary processing (both traditional and novel) result in significant associations between antimicrobial resistance and the bacterial response to processing stresses which might impact the final population of AMR bacteria, discussed in Section 1.8?

6. Materials and methods

6.1 Database searching

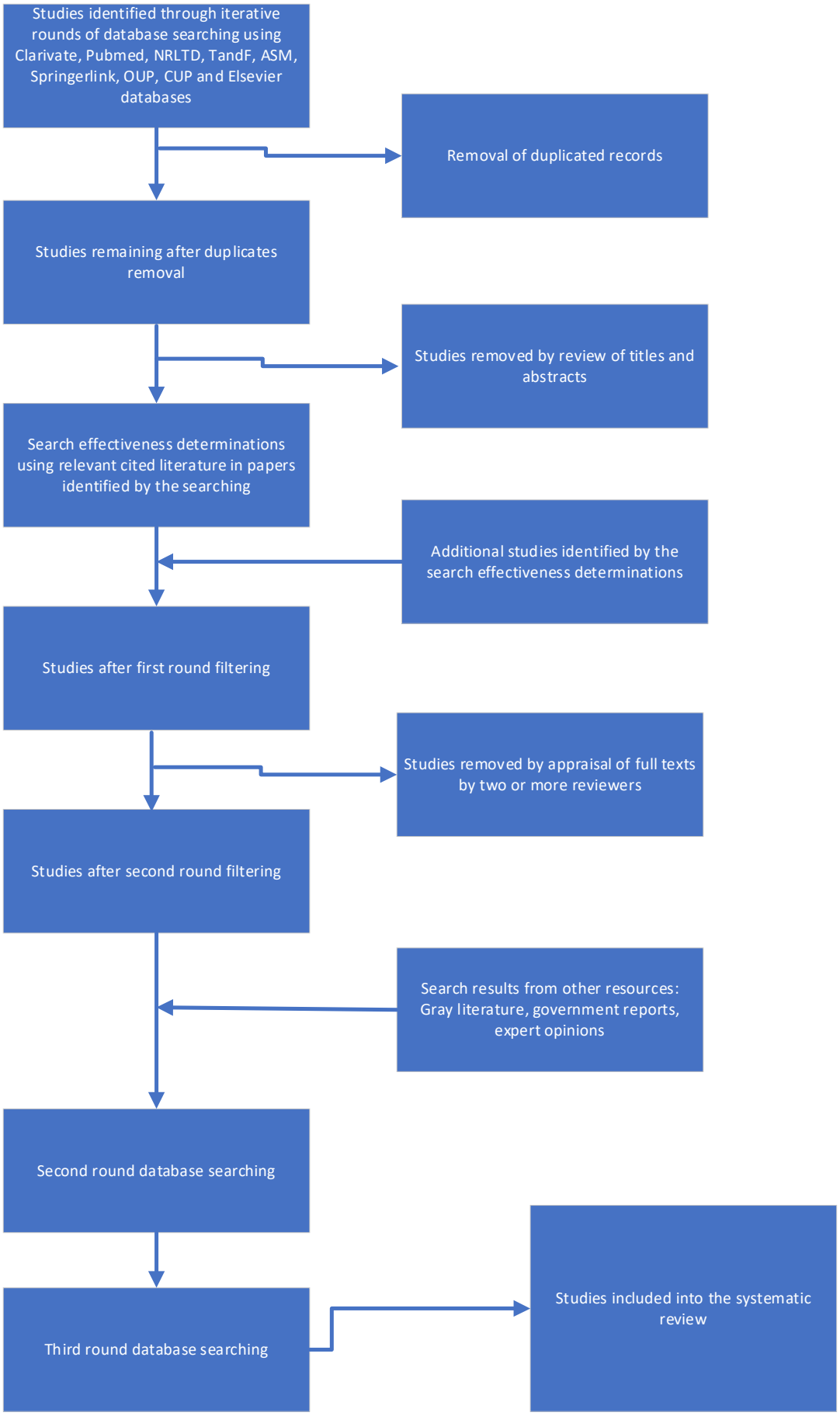
A detailed explanation of the search dates, strings used to search the databases, the versions of the databases used and the checks to help ensure no literature was missed are provided as Appendix 1. The source databases searched were the Clarivate Analytics (formerly Thomson-Reuters) Web of Science, the NDLTD (Networked Digital Library of Theses and Dissertations), the Taylor and Francis journals and eBooks databases, the American Society for Microbiology eBooks, SpringerLink, Cambridge and Oxford University Press publications, and Elsevier. There was some overlap between some of the databases, with some publications identified more than once. A workflow summary of the search processes is shown as **Error! Reference source not found.**. The search outputs are summarised in Table 1.

Table 1 A summary of the numbers of papers identified by the first round of database searching. WoS is web of science, NTCLTD is the networked digital library of theses and dissertations, TandF is Taylor and Francis, ASM is the American society for microbiology, OUP is Oxford university press, CUP is Cambridge University Press.

Database	Number of hits identified by the search (after removal of duplicates)	Number of relevant papers taken forward for further appraisal*
WoS	370	97
PubMed	124	28
NRCLTD	44	0
TandF	50	1
ASM	12	5
SpringerLink	266	0
OUP	845	3
CUP	247	7
Elsevier	475	2
Total	2433	143

*Papers were discarded if they were considered to be outside the scope of the review by both reviewers after reading the paper title and abstract.

Figure 2 A visualisation of the tasks undertaken to search for literature of relevance to AMR in SPMMP



7. A review of antimicrobial resistance in bacteria isolated from secondary processed meats and meat products.

The approach of Zhou et al. (2017) was adopted and two to three rounds of paper appraisals were undertaken and compared between reviewers. An initial round was undertaken after 10 pairs of papers had been appraised in order to detect gross differences in appraisal outcomes, with an intended strategy that any large differences between reviewers could be addressed and hence corrected at a comparatively early stage. The mean appraisal score from each reviewer was used to determine the acceptability of papers. There was no weighting applied to any of the PROMPT criteria or the questions contained within each section of the appraisal scoring form (Appendix 2). The cut-off score for paper acceptance for the initial round of comparisons was arbitrarily set initially at ≥3/5 for the average PROMPT score (≥60%).

There was an intention to review the acceptance cut off score after 50 papers had been evaluated. However, it was apparent after a short discussion between reviewers that the cut off score for acceptance into the review was set appropriately and seemed to be efficiently discarding papers that would not make a significant contribution to the review. There were two mechanisms used to fine-tune appraisal outcomes. The first was the value of the cut-off score itself, which was calculated from the appraisal sheets and could be reviewed if necessary. In addition, the opinion of the reviewers and how high or low the papers they appraised were scored could be adjusted. One reviewer, (R2) tended to give very low scores to specific aspects of papers leading to the exclusion of more papers than did R1 and R4. They were given guidance on this in order to allow them to alter their approach to appraising papers and hence to bring them towards equivalence with R1 and R4.

A summary of the comparisons results is shown as Table 2. Interpretation of κ was as previously described by Vierra (2005), with a κ of ≥0.61 being deemed as broadly equivalent agreement between pairs of reviewers (Table 3). After the initial round of ten appraisals, any reviewer that was scoring markedly different from the other three was informed of the fact and hence requested to adjust their scoring as appropriate.

A summary of the fate of the appraised papers, including those that were subject to arbitration review is shown as Table 4. Overall, 377 reviews were undertaken on the 163 papers, with each paper being reviewed at least twice. Papers 64 and 86 were appraised by all the reviewers. 46 papers (28.2%) were required to be arbitrated and hence subject to a third review. One paper (number 2) was reviewed in error by R4.

Table 2 A summary of statistical comparisons between reviewers using Cohen's kappa to determine the reliability of classifications into acceptance or rejection categories. An initial appraisal based on 10 papers was undertaken, with feedback provided to reviewers to ensure scoring was consistent across the review group. References 64 and 86 were selected randomly and appraised by all reviewers to facilitate discussions on scoring. Three sets of around 25 pairs of papers were appraised by each reviewer. Additional comparisons larger than 25 paper pairs have been included because hung appraisals (section 1.14.1), where one reviewer accepted and another rejected a paper, were settled by appraisal from a third reviewer.

First reviewer	Second reviewer	Paper identification numbers appraised by first reviewer	Number of papers appraised	Kappa (κ) on pairs of overlapping papers (n pairs)
R1	R2	3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 32, 33, 36, 39, 42, 45, 48, 49, 51, 54, 57, 60, 63, 64, 66, 69, 72, 75, 77, 83, 85, 86, 92, 93, 95, 97, 101, 112, 117, 118, 120, 122, 124, 154	44	0.70 (10) 0.52 (26) 0.43 (44)
R1	R3	2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 64, 65, 68, 71, 74, 86, 92, 112, 117	30	0.60 (10) 0.62 (25) 0.61 (30)
R1	R4	1, 2, 3, 4, 5, 6, 7, 10, 11, 12, 13, 15, 16, 17, 19, 21, 22, 23, 25, 27, 28, 30, 31, 33, 34, 36, 37, 38, 40, 42, 43, 44, 45, 46, 47, 49, 51, 52, 55, 58, 59, 61, 63, 64, 67, 69, 70, 73, 75, 77, 83, 85, 86, 93, 95, 97, 101, 118, 120, 122, 124, 149, 150, 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, 166, 167, 168, 169	81	0.80 (10) 0.88 (25) 0.86 (81)

First reviewer	Second reviewer	Paper identification numbers appraised by first reviewer	Number of papers appraised	Kappa (κ) on pairs of overlapping papers (n pairs)
R2	R3	32, 64, 76, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 100, 102, 104, 110, 112, 113, 115, 117, 119, 121, 123	24	0.60 (10) 0.75 (24)
R2	R4	3, 6, 12, 15, 21, 27, 30, 33, 36, 42, 45, 49, 51, 63, 64, 69, 75, 77, 79, 81, 83, 85, 86, 87, 88, 89, 91, 93, 94, 95, 97, 99, 101, 103, 105, 107, 109, 114, 115, 116, 118, 119, 120, 122, 124, 154	46	0.70 (10) 0.52 (25) 0.33 (46)
R4	R3	2, 5, 11, 17, 23, 38, 44, 47, 50, 59, 64, 86, 88, 94, 115, 119, 125, 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148	40	0.70 (10) 0.70 (25) 0.68 (40)

Table 3 The interpretation of calculated kappa values proposed by Vierra (2005)

Kappa value range (κ)	Degree of agreement
≤0	Less than chance
0.01 – 0.20	Slight
0.21 – 0.40	Fair
0.41 – 0.60	Moderate
0.61 – 0.80	Substantial
0.81 – 1.0	Almost perfect

Table 4 A summary of the fate of papers appraised for suitability into the review and the outcome when two reviewers disagreed on the suitability and a third review and majority decision was used to arbitrate.

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
1	R1	R4		Two scores ≥3	Accepted
2	R1	R2	R4	Three scores ≥3	Accepted
3	R1	R3	R4	Two/three scores ≥3	Accepted
4	R1	R4		Two scores ≥3	Accepted
5	R1	R2	R4	Two/three scores ≥3	Accepted
6	R1	R3	R4	Two/three scores ≥3	Accepted
7	R1	R4		Two scores ≥3	Accepted
8	R1	R2		Two scores <3	Rejected

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
9	R1	R3		Two scores <3	Rejected
10	R1	R4		Two scores ≥3	Accepted
11	R4	R2	R1	Two/three scores <3	Rejected
12	R1	R3	R4	Two/three scores ≥3	Accepted
13	R1	R4		Two scores <3	Rejected
14	R1	R2		Two scores <3	Rejected
15	R1	R3	R4	Two/three scores ≥3	Accepted
16	R1	R4		Two scores ≥3	Accepted
17	R1	R2	R4	Two/three scores ≥3	Accepted
18	R1	R3		Two scores <3	Rejected
19	R1	R4		Two scores <3	Rejected
20	R1	R2		Two scores ≥3	Accepted
21	R1	R3	R4	Two/three scores ≥3	Accepted
22	R1	R4		Two scores <3	Rejected
23	R1	R2	R4	Two/three scores ≥3	Accepted
24	R1	R3		Two scores ≥3	Accepted
25	R1	R4		Two scores ≥3	Accepted
26	R1	R2	R4	Two scores ≥3	Accepted
27	R1	R3	R4	Two/three scores <3	Rejected

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
28	R1	R4		Two scores ≥3	Accepted
29	R1	R2		Two scores ≥3	Accepted
30	R1	R3	R4	Two/three scores ≥3	Accepted
31	R1	R4		Two scores ≥3	Accepted
32	R1	R3	R2	Three scores <3	Rejected
33	R1	R3	R4	Two/three scores ≥3	Accepted
34	R1	R4		Two scores ≥3	Accepted
35	R1	R2		Two scores ≥3	Accepted
36	R1	R3	R4	Two/three scores ≥3	Accepted
37	R1	R4		Two scores <3	Rejected
38	R1	R2	R4	Two/three scores <3	Rejected
39	R1	R3		Two scores <3	Rejected
40	R1	R4		Two scores ≥3	Accepted
41	R1	R2		Two scores ≥3	Accepted
42	R1	R3	R4	Two/three scores ≥3	Accepted
43	R1	R4		Two scores <3	Rejected
44	R1	R2	R4	Two/three scores <3	Rejected
45	R1	R3		Two/three scores <3	Rejected
46	R1	R4		Two scores <3	Rejected

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
47	R1	R2	R4	Two/three scores <3	Rejected
48	R1	R3		Two scores <3	Rejected
49	R1	R4	R3	Two/three scores ≥3	Accepted
50	R1	R2	R4	Two/three scores <2	Rejected
51	R1	R3	R4	Two/three scores <3	Rejected
52	R1	R4		Two scores ≥3	Accepted
53	R1	R2		Two scores <3	Rejected
54	R1	R3		Two scores ≥3	Accepted
55	R1	R4		Two scores ≥3	Accepted
56	R1	R2		Two scores ≥3	Accepted
57	R1	R3		Two scores <3	Rejected
58	R1	R4		Two scores <3	Rejected
59	R1	R2	R4	Two/three scores <3	Rejected
60	R1	R3		Two scores <3	Rejected
61	R1	R4		Two scores <3	Rejected
62	R1	R2		Two scores <3	Rejected
63	R1	R3	R4	Two/three scores ≥3	Accepted
64	All four reviewers			Four scores <3	Rejected
65	R1	R2		Two scores <3	Rejected

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
66	R1	R3		Two scores <3	Rejected
67	R1	R4		Two scores <3	Rejected
68	R1	R2		Two scores <3	Rejected
69	R1	R3	R4	Two/three scores ≥3	Accepted
70	R1	R4		Two scores <3	Rejected
71	R1	R2		Two scores <3	Rejected
72	R1	R3		Two scores <3	Rejected
73	R1	R4		Two scores <3	Rejected
74	R1	R2		Two scores <3	Rejected
75	R4	R3	R1	Two/three scores <3	Rejected
76	R3	R2		Two scores <3	Rejected
77	R4	R3	R1	Two/three scores ≥3	Accepted
78	R3	R2		Two scores <3	Rejected
79	R4	R3		Two scores <3	Rejected
80	R3	R2		Two scores <3	Rejected
81	R4	R3		Two scores <3	Rejected
82	R3	R2		Two scores <3	Rejected
83	R4	R3	R1	Two/three scores <3	Rejected
84	R3	R2		Two scores <3	Rejected

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
85	R4	R3	R1	Two/three scores ≥3	Accepted
86	All four reviewers			Three/four scores <3	Accepted
87	R4	R3		Two scores <3	Rejected
88	R3	R2		Two scores <3	Rejected
89	R4	R3		Two scores <3	Rejected
90	R3	R2		Two scores <3	Rejected
91	R4	R3		Two scores <3	Rejected
92	R3	R2	R1	Two/three scores ≥3	Accepted
93	R4	R3	R1	Two/three scores ≥3	Accepted
94	R3	R2	R4	Two/three scores ≥3	Accepted
95	R4	R3	R1	Two/three scores ≥3	Accepted
96	R3	R2		Two scores <3	Rejected
97	R4	R3	R1	Two/three scores <3	Rejected
98					Rejected duplicate paper
99	R4	R3		Two scores <3	Rejected
100	R3	R2		Two scores <3	Rejected
101	R4	R3	R1	Two/three scores ≥3	Accepted
102	R3	R2		Two scores <3	Rejected

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
103	R4	R3		Two scores <3	Rejected
104	R3	R2		Two scores <3	Rejected
105	R4	R3		Two scores <3	Rejected
106					Rejected duplicate paper
107	R4	R3		Two scores <3	Rejected
108					Rejected duplicate paper
109	R4	R3		Two scores <3	Rejected
110	R3	R2		Two scores <3	Rejected
111					Rejected duplicate paper
112	R3	R2	R1	Two/three scores ≥3	Accepted
113	R3	R2		Two scores <3	Rejected
114	R4	R3		Two scores ≥3	Accepted
115	R3	R2	R4	Two scores ≥3	Accepted
116	R4	R3		Two scores ≥3	Accepted
117	R3	R2	R1	Two/three scores ≥3	Accepted
118	R4	R3	R1	Two/three scores ≥3	Accepted
119	R3	R2	R4	Two/three scores ≥3	Accepted

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
120	R4	R3	R1	Two/three scores ≥3	Accepted
121	R3	R2		Two scores <3	Rejected
122	R4	R3	R1	Two/three scores <3	Rejected
123	R3	R2		Two scores <3	Rejected
124	R4	R3	R1	Two/three scores ≥3	Accepted
125	R4	R2		Two scores ≥3	Accepted
126	R4	R2		Two scores <3	Rejected
127	R4	R2		Two scores <3	Rejected
128	R4	R2		Two scores ≥3	Accepted
129	R4	R2		Two scores <3	Rejected
130	R4	R2		Two scores <3	Rejected
131	R4	R2		Two scores ≥3	Accepted
132	R4	R2		Two scores <3	Rejected
133	R4	R2		Two scores <3	Rejected
134	R4	R2		Two scores <3	Rejected
135	R4	R2		Two scores <3	Rejected
136	R4	R2		Two scores <3	Rejected
137	R4	R2		Two scores <3	Rejected
138	R4	R2		Two scores <3	Rejected

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
139	R4	R2		Two scores <3	Rejected
140	R4	R2		Two scores <3	Rejected
141	R4	R2		Two scores <3	Rejected
142	R4	R2	R1	Two/three scores <3	Rejected
143	R4	R2		Two scores <3	Rejected
144	R4	R2		Two scores <3	Rejected
145	R4	R2		Two scores ≥3	Accepted
146	R4	R2		Two scores <3	Rejected
147	R4	R2		Two scores ≥3	Accepted
148	R4	R2		Two scores ≥3	Accepted
July 2019 repeat search updates					
149	R4	R1		Two scores ≥3	Accepted
150	R4	R1		Two scores ≥3	Accepted
151	R4	R1		Two scores ≥3	Accepted
152	R4	R1		Two scores ≥3	Accepted
153	R4	R1		Two scores ≥3	Accepted
154	R4	R1	R2	Two/three scores ≥3	Accepted
155	R4	R1		Two scores ≥3	Accepted
156	R4	R1		Two scores ≥3	Accepted

Paper identification numbers	First reviewer	Second reviewer	Third reviewer (for a hung paper)	Appraisal outcome	Accepted, rejected or rejected as duplicate
157	R4	R1		Two scores ≥3	Accepted
December 2019 repeat search updates					
158	R1	R4		Two scores <3	Rejected
159	R1	R4		Two scores ≥3	Accepted
160	R1	R4		Two scores ≥3	Accepted
161	R1	R4		Two scores <3	Rejected
162	R1	R4		Two scores ≥3	Accepted
163	R1	R4		Two scores <3	Rejected
164	R1	R4		Two scores ≥3	Accepted
165	R1	R4		Two scores <3	Rejected
166	R1	R4		Two scores ≥3	Accepted
167	R1	R4		Two scores <3	Rejected
168	R1	R4		Two scores ≥3	Accepted
169	R1	R4		Two scores ≥3	Accepted

8. Review questions

This review was commissioned to provide insight into the impact that established and novel secondary meat processes may have on AMR bacteria and with a particular emphasis on whether these processes may influence the transfer of antimicrobial resistance genes (AMG). In order to accomplish these aims, the following questions relating to AMR and secondary meat processing must be answered, using the information contained in the identified literature.

- Are the products of secondary meat processing known to be contaminated by AMR bacteria, or genetic materials that could give rise to AMR bacteria? (Section 1.3)

If such contamination can be confirmed, then the further questions to be answered are:

- Are the raw materials the only source of AMR bacteria, or materials conferring AMR properties to bacteria, in secondary processed meats? (Sections 1.3.1.1, 1.5)
- Does the transfer of genetic materials conferring antibiotic resistance occur between bacteria during secondary processes and, if so, which are the salient factors that influence such gene transfers? (Sections 1.4, 1.6)
- Does any aspect of secondary processing (both traditional and novel) result in significant associations between antimicrobial resistance and the bacterial response to processing stresses which might impact the final population of AMR bacteria? (Section 1.8)

8.1 Are secondary processed meats and meat products contaminated with AMR bacteria?

Table 5, Table 6 and Table 7 are summaries of the evidence relating to AMR isolations from secondary processed meats and meat products (SPMMP) extracted from the identified literature. In brief, a total of 57 papers were identified that investigated AMR bacteria in SPMMP. The successful isolation of some form of AMR bacteria was reported by 56 papers. Isolations were reported from products of ovine (n=5), bovine (n=25) and porcine (n=27) origin, with some studies reporting multiple isolations from multiple species. In nine papers, the animal of origin was not specified and there were 11 reports of isolations from products containing a mixture of species. Isolations were from all stages of secondary processing. However, at some stage after or downstream of maturation chilling was particularly favoured for sample collections, with every paper identified collecting at least some samples at or after this processing stage. Also favoured were sample collections after meat comminution (mincing), a stage where there was some sample collection by 51.2% of the identified literature. Post fermentation was also popular for sample collections (29.3% of papers). There were some challenges with the analyses of AMR on the basis

of species or bacterial grouping. An example of the problem was that some authors reported ESBL *E. coli* and others reported ESBL *Enterobacteriaceae*. Part of the bacteria group *Enterobacteriaceae* is composed of *E. coli* and so the populations are not independent, which is a requirement for standard statistical analyses that can detect differences between different categories. There were similar issues for lactic acid bacteria, which are a bacterial group also composed of multiple species. Some species were members of more than one group. An analysis that attempted to circumvent these issues and determine if any AM that were significantly more commonly reported than other AMs on a bacterial group basis is provided in Section 1.3.2.

Only one paper (Fernandes et al. 2017) failed to find their target of AMR *Salmonella enterica* in a beef jerky product. However, Fernandes et al. (2017) reported that the target AMR *S. enterica* was present in the processing environment and isolated from racks used to dry the beef. Although it should be kept in mind that it is difficult to publish negative results for surveillance i.e. where no AMR isolations were obtained, the published evidence supports the conclusion that isolations of AMR bacteria are routine from SPMMP.

Thus, based on the available scientific evidence, the response to the question of “Are the products of secondary meat processing known to be contaminated by AMR bacteria, or genetic materials which could give rise to AMR?”, should be that there is a body of evidence that supports SPMMP contamination by AMR bacteria, but there is no evidence to either support or constrain an assertion that genetic materials capable of creating AMR bacteria were present.

Table 5 A summary of evidence relating to the presence of antimicrobial-resistant (AMR) bacteria in post-chill meats and meat products grouped by organism, country and point of surveillance and resistance types. MP is multiple premises, SP is single premises. Insufficient information was provided to calculate percentage isolations for Barbuti et al. (1992), Belgacem et al. (2010) and Gevers et al. (2003c). Only a percentage breakdown of isolations and not numbers of isolations were reported by some studies. Multiple isolations of different strains from single samples were reported by Barbuti et al. (1992), Gardini et al. (2003), Messi et al. (2006) and Yu et al. (2017) which made reporting percentages misleading for these papers. Yu et al. (2017) did not provide a breakdown for isolates by species or product type but report no significant differences in the prevalence of resistant bacteria between cooked meat from different species. LAB are a mixed genera, collectively termed lactic acid bacteria; Lm is Listeria monocytogenes; Li is Listeria innocua; VTEC are Verotoxin-producing E. coli; VRE are vancomycin-resistant Enterococcus; MRSA are methicillin-resistant Staphylococcus aureus; ESBL are extended spectrum β-lactamases. *freezing of meat was undertaken as part of the breadcrumbs coating process, even for raw product (Bucher et al. 2007). Resistance phenotypes reported were observed in some, but not necessarily all, of the isolates. Synonyms of antibiotics have been standardised to a single name (e.g. all instances of co-trimoxazole have been changed to Trimethoprim-sulfamethoxazole) for clarity.

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
E. coli O157:H7	5/180 (2.8%)	Yes	South Africa at retail (MP)	Ampicillin Erythromycin Gentamicin Nalidixic acid	Abong'o and Momba (2009)	1
Enterococcus spp.	26/40 (65.0%)	Yes (not likely)	Canada after commercial processing (MP)	Ciprofloxacin Lincomycin	Aslam et al. (2010)	2

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Quinupristin-dalfopristin Tetracycline		
LAB (multiple genera)	Lactobacillus sakei (74.0%) Lactobacillus curvatus (21.2%) Leuconostoc mesenteroides (4.8%)	Unlikely	Spain at retail (MP)	Ampicillin Benzylpenicillin Chloramphenicol Gentamicin Linezolid Quinupristin-dalfopristin Tetracycline Vancomycin	Aymerich et al. (2006)	3
Salmonella enterica	Overall 78/1468 (5.3%)	Yes	Italy at retail (MP)	Ampicillin Gentamicin	Bacci et al. (2014)	4

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
(multiple serovars)	*S.* Derby 24/1468 (1.6%) *S.* Typhimurium 21/1468 (1.4%) *S.* Bredeney 9/1468 (0.6%) *S.* London 7/1468 (0.5%) *S.* Anatum 4/1468 (0.3%) *S.* Agona 3/1468 (0.2%) *S.* Virchow 3/1468 (0.2%)			Sulfamethoxazole Tetracycline		

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
	S. Senftenberg 2/1468 (0.1%) *S.* Livingstone 1/1468 (0.1%) *S.* India 1/1468 (0.1%) *S.* Heidelberg 1/1468 (0.1%) *S.* Bovis-morbificans 1/1468 (0.07) *S.* Coeln 1/1468 (0.07)					
LAB (multiple genera)	168 isolates/ unreported number of	Unlikely	Ethiopia during processing, not specified	Gentamicin Kanamycin	Bacha et al. (2010)	5

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
	samples (10-15 colonies per plate)			Methicillin Streptomycin Vancomycin		
Listeria monocytogenes L. innocua	22 *Lm* 28 *Li* 0 Lm 4 Li 4 *Lm* 9 *Li* 19 *Lm* 49 *Li* 6 *Lm* 11 *Li* 13 *Lm* 1 *Li*	Yes	Italy, not specified	Ceftazidime Colistin Nalidixic acid Piperidimic acid	Barbuti et al. (1992)	6
Enterococcus faecium E. faecalis	E. faecium 42 E. faecalis 10 Enterococcus 11	Yes (not likely)	Tunisia after artisanal processing (MP)	Ciprofloxacin Erythromycin Levofloxacin	Belgacem et al. (2010)	7

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
Enterococcus of undetermined species				Nitrofurantoin Rifampicin		
Enterococcus faecium E. faecalis E. hirae E. durans E. mundtii E. thailandicus	60 isolations from 20 samples *E. faecium* 44/60 (73.3%) *E. faecalis* 7/60 (11.7%) *E. hirae* 5/60 (8.3%) *E. durans* 2/60 (3.3%) *E. mundtii* 1/60 (1.7%)	Yes (not likely)	Turkey after commercial processing (MP)	Chloramphenicol Ciprofloxacin-Gentamicin Doxycycline Erythromycin Nitrofurantoin Rifampicin Streptomycin Teicoplanin	Demirgul and Tuncer (2017)	10

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
	E. thailandicus 1/60 (1.7%)					
Thermotolerant coliforms Escherichia coli Salmonella spp.,	Up to 3 log cfu/g Not detected 3/35 (8.6%)	Unlikely Unlikely Yes	Brazil after commercial processing (SP)	For Salmonella enterica No resistance to 14 tested antibiotics for product isolates.	Fernandes et al. (2017)	12
Enterococcus faecium E. faecalis E. durans E. casseliflavus E. mundtii	E. faecium (56.0%) E. faecalis (17.0%) 27% composed of *E. durans* (unspecified numbers)	Yes (not likely)	Argentina after commercial processing (SP)	Erythromycin Rifampicin Tetracycline	Fontana et al. (2009) sampling information from Fontana et al. (2005)	15

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
	E. casseliflavus (unspecified numbers) *E. mundtii* (unspecified numbers)					
VTEC O157	7/300 (2.3%)	Yes	Spain after commercial processing (SP)	Ampicillin Chloramphenicol Florfenicol Gentamicin Kanamycin Nalidixic acid Streptomycin	Fontcuberta et al. (2016)	16

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Sulfonamide Trimethoprim-sulfamethoxazole		
Micrococcus (M) Kocuria (K) Staphylococcus (S)	(Sals) M 4/30, K 2/30, S 74/30 (Sopr)M 1/18, K 1/18, S 51/18 (MiSal)M /5, K /18, S 14/5	Unlikely Unlikely Unlikely	Italy during commercial/artisanal processing (MP)	Bacitracin Colistin Piperidimic acid Sulfonamide Tetracycline Trimethoprim-sulfamethoxazole	Gardini et al. (2003)	21
Lactobacillus plantarum	9/not reported (batch 1) 8/not reported (batch 2)	Unlikely	Belgium after commercial processing (SP, two batches)	Tetracycline	Gevers et al. (2003c)	24

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
Listeria monocytogenes L. innocua	134 L. monocytogenes 99 L. innocua Number of tested samples not reported	Yes	Spain after commercial processing and at retail (MP)	Clindamycin Oxacillin Tetracycline	Gomez et al. (2014)	25
L. monocytogenes	5 MDR isolates from historical surveillance	Yes	Brazil at retail (MP)	Clindamycin Erythromycin Meropenem Rifampicin Streptomycin Tetracycline Trimethoprim-sulfamethoxazole	Haubert et al. (2016)	26

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
E. coli (generic)	9 MDR isolates from unspecified number of samples tested	Unlikely	Canada at retail (MP)	Ampicillin Bacitracin Erythromycin Kanamycin Penicillin Streptomycin Tetracycline	Jayaratne et al. (1987b)	30
E. coli (generic)	Overall, 75/620 (12.1%) Roasted meats 23/199 (11.6%) Pot-stewed meats 38/230 (16.5%)	Unlikely	China at retail (MP)	Ampicillin Cefoperazone Cefotaxime Ceftazidime Chloramphenicol	Jiang et al. (2014)	33

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
	Sausages 5/102 (4.9%) Smoked meats 9/89 (10.1%)			Ciprofloxacin Gentamicin Nalidixic acid Streptomycin Tetracycline Trimethoprim-sulfamethoxazole		
VTEC O157:H7	2/100 (2.0%)	Yes	Turkey at retail (MP)	Ampicillin Clindamycin Enrofloxacin Florfenicol Penicillin Sulfadimethoxine	Kalender (2013)	34

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Tetracycline Tiamulin Tilmicosin Trimethoprim-sulfamethoxazole		
VRE	59/120 (29.2%)	Yes	Italy from unspecified source	Ampicillin Benzylpenicillin Erythromycin Gentamicin Kanamycin Streptomycin Teicoplanin Vancomycin	Messi et al. (2006)	40

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
ESBL-Enterobacteria	24 isolates from 20/100	Yes (not likely)	Austria at retail (MP)	Amoxicillin/clavulanic acid Ampicillin Cefepime Cefotaxime Cefoxitin Ceftazidime Cefuroxime Chloramphenicol Ciprofloxacin Gentamicin Imipenem Meropenem	Petternel et al. (2014)	42

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Moxifloxacin Nalidixic acid Piperacillin/tazobactam/cefalexin Tetracycline Trimethoprim-sulfamethoxazole		
MRSA	9/100 (9.0%)	Yes	Austria at retail (MP)	Cefoxitin Clindamycin Erythromycin Fusidic acid Gentamicin Linezolid Mupirocin	Petternel et al. (2014)	42

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Norfloxacin Penicillin Rifampicin Tetracycline Trimethoprim-sulfamethoxazole		
Enterococcus spp.	17/15 19/15 28/15 2/15 1/15	Yes (not likely)	Belgium after commercial processing (SP, 15 batches per product)	Amoxicillin-clavulanic acid Ampicillin Bacitracin Benzylpenicillin Chloramphenicol Ciprofloxacin Erythromycin	Santos et al. (2017)	52

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Gentamicin Nalidixic acid Nitrofurantoin Norfloxacin Quinupristin-dalfopristin Rifampicin Streptomycin Teicoplanin Tetracycline Trimethoprim/sulfamethoxazole Vancomycin		

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
Campylobacter spp.	35/229 (15.3%) 13/64 (20.3%) 10/87 (11.5%) 1/19 (5.3%)	Yes	Éire at retail from 149 MP	Ciprofloxacin Erythromycin Gentamicin Kanamycin Nalidixic acid Trimethoprim	Scanlon et al. (2013)	54
ESBL Enterobacteria	27/73 (37.0%)	Yes (not likely)	Single meat wholesaler supplied from Germany (70%), Netherlands, Poland, Belgium, Spain	Ampicillin Azithromycin; Cefepime Cefotaxime Cefoxitin Ceftazidime	Schill et al. (2017)	55

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Chloramphenicol Ciprofloxacin Colistin Ertapenem Gentamicin Meropenem Nalidixic acid Streptomycin Tetracycline Tigecycline Trimethoprim-sulfamethoxazole		

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
E. coli (generic)	136 streptomycin-resistant *E. coli* from 934 isolations.	Unlikely	Norwegian national surveillance, sources not reported	Streptomycin	Sunde and Norstrom (2005)	69
Campylobacter coli	15/1802 (0.8%) 1/624 (0.2%) 4/187 (2.1%)	Yes	Poland after commercial processing (MP)	Ciprofloxacin Erythromycin Gentamicin Nalidixic acid Streptomycin Tetracycline	Wieczorek et al. (2013)	85
E. coli (generic)	75/620 (12.1%)	Unlikely	China at retail (MP)	Ampicillin Nalidixic acid Streptomycin	Yu et al. (2016)	92

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Tetracycline Trimethoprim-sulfamethoxazole		
Enterobacteriaceae	24/51 (47.1%)	Yes (not likely)	China at retail (MP)	Ampicillin Cefotaxime Ceftazidime Chloramphenicol Ciprofloxacin Gentamicin Levofloxacin Streptomycin Sulfamethoxazole Tetracycline	Yu et al. (2017)	93

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
Pseudomonas	17/51 (33.3%)	Yes (not likely)	China at retail (MP)	Ampicillin Cefotaxime Ceftazidime Chloramphenicol Ciprofloxacin Gentamicin Levofloxacin Streptomycin Sulfamethoxazole Tetracycline	Yu et al. (2017)	93
Vibrio	13/51 (25.5%)	Yes	China at retail (MP)	Ampicillin Streptomycin	Yu et al. (2017)	93

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Sulfamethoxazole Tetracycline		
Gram positive	16/51 (31.4%)	Yes (not likely)	China at retail (MP)	Ampicillin Tetracycline	Yu et al. (2017)	93
ESBL *E. coli* (generic)	26/50 (52.0%)	Unlikely	Austria at retail (MP)	Cefoxitin Ceftazidime Chloramphenicol Nalidixic acid Tetracycline Trimethoprim-sulfamethoxazole	Zarfel et al. (2014)	94
Staphylococcus aureus	5/50 (10.0%)	Yes	Austria at retail (MP)	Cefoxitin Penicillin	Zarfel et al. (2014)	94

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
VRE	4/50 (8.0%)	Yes	Austria at retail (MP)	Ampicillin Teicoplanin	Zarfel et al. (2014)	94
Staphylococcus	39/ unspecified number of tests	Unlikely	Croatia at retail (MP)	Ampicillin Erythromycin Gentamicin Tetracycline	Zdolec et al. (2013)	95
Salmonella spp.	92 in total Retail 11/35 (31.4%) Wholesale 14/57 (24.6%)	Yes	Canada at retail and wholesale (MP)	Amikacin Amoxicillin-clavulanic acid Ampicillin Cefoxitin Ceftiofur Ceftriaxone	Bucher et al. (2007)	101

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Chloramphenicol Ciprofloxacin Gentamicin Kanamycin Nalidixic acid Streptomycin Sulfamethoxazole Tetracycline Trimethoprim-sulfamethoxazole		
Proteus mirabilis	52/178 (29.2%)	Yes (not likely)	China at retail (MP)	Benzalkonium chloride (QAC)	Jiang et al. (2017)	112
E. coli (generic)	64/720 (8.9%)	Unlikely	China at retail (MP)	Ampicillin	Li et al. (2017)	114

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Ampicillin-sulbactam Cefotaxime Cefoxitin Ceftazidime Chloramphenicol Ciprofloxacin Gentamicin Tetracycline Trimethoprim-sulfamethoxazole		
MRSA	10/240 (4.2%) 0/240 (0.0%)	Unlikely	China at retail (MP)	Penicillin Clindamycin Erythromycin	Li et al. (2016)	115

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Trimethoprim/sulfamethoxazole Tetracycline		
ESBL Enterobacteria	17/240 (7.1%) 1/240 (0.4%)	Yes (not likely)	China at retail (MP)	Ampicillin Cefotaxime Ceftazidime Chloramphenicol Ciprofloxacin Levofloxacin Tetracycline	Li et al. (2016)	115
Campylobacter spp.	92/1189 (7.7%)	Yes	Midwestern USA after commercial processing (MP)	Ciprofloxacin Erythromycin	Lutgen et al. (2009) sampling info Logue et al. (2003)	116

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
VTEC O157:H7	2/91 (2.2%)	Yes	Brazil at retail (MP)	Unable to differentiate	Rodolpho and Marin (2007)	49
E. coli (generic)	91/91 (100%)	Yes	Brazil at retail (MP)	Amikacin Amoxicillin Amoxicillin-clavulanic acid Ampicillin Cefalothin Ceftriaxone Ciprofloxacin Gentamicin Nalidixic acid Streptomycin	Rodolpho and Marin (2007)	49

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Tetracycline Trimethoprim/sulfamethoxazole		
Salmonella spp.	68/200 (34.0%) overall Carcasses 8/50 (16.0%) Drumsticks 14/50 (28.0%) Livers 16/50 (32.0%) Giblets 30/50 (60.0%)	Yes	Mansoura, Egypt at retail (MP)	Amoxicillin Ampicillin Erythromycin Nalidixic acid Sulfamethoxazole Oxytetracycline Penicillin	Abd-Elghany et al. (2015)	128
Staphylococcus aureus	5/90 (5.6%)	Yes	United Kingdom at retail (MP)	Ciprofloxacin Clindamycin Erythromycin	Dhup et al. (2015)	131

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Methicillin Oxacillin Tetracycline		
Listeria spp.	69/199 (34.7%)	Yes	Iran at retail (MP)	Ampicillin Ciprofloxacin Enrofloxacin Erythromycin Penicillin Tetracycline	Fallah et al. (2012)	145
Listeria spp.	38/115 (33.0%)	Yes	Iran at retail (MP)			
Listeria spp.	27/88 (30.7%)	Yes	Iran at retail (MP)			
Campylobacter spp.	Skin-on thighs 21/41 (51.2%)	Yes	Italy after commercial processing and 10d refrigeration (SP)	Ciprofloxacin Erythromycin Nalidixic acid	Casagrande Proietti et al. (2018)	148

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
	Skinless breast 1/37 (2.7%)			Tetracycline		
Salmonella enterica	Formal abattoir: 33/42 (79.0%) Informal abattoir: 15/71 (21.0%)	Yes	South Africa at the end of processing (MP)	Amoxicillin Ampicillin Cefotaxime Ceftriaxone Chloramphenicol Ciprofloxacin Gentamicin Imipenem Kanamycin Meropenem	Jaja et al. (2019)	150

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Neomycin Norfloxacin Streptomycin Sulfamethoxazole Tetracycline		
Salmonella spp.	67/69 (97.1%)	Yes	South Korea at retail (MP)	Ampicillin Chloramphenicol Florfenicol Nalidixic acid Neomycin Streptomycin Tetracycline	Shang et al. (2019)	152

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Trimethoprim/sulfamethoxazole		
Salmonella spp.	Pork 75/137 (54.7%) Chicken 43/91 (47.3%) Beef 34/99 (34.3%)	Yes	China at retail (MP)	Amoxycillin Ampicillin Ciprofloxacin Gentamicin Levofloxacin Oxytetracycline Trimethoprim	Deng et al. (2018)	153
E. coli ST101	140 isolates from an unspecified number of samples	Yes	Egypt at retail (MP)	Ampicillin Chloramphenicol Ciprofloxacin Colistin	Sadek et al. (2019)	159

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
				Gentamicin Kanamycin Nalidixic acid Tetracycline		
Salmonella spp.	95/300 (31.5%)	Yes	Brazil	Amoxicillin with clavulanic acid Ceftriaxone Ampicillin Ciprofloxacin Doxycycline Nalidixic acid tetracycline	Perin et al. (2019)	160

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
Campylobacter spp.	7/105 (6.7%) *C. jejuni* 4/105 (3.8%) *C. coli*	Yes	Brazil	Chloramphenicol Ciprofloxacin Erythromycin Gentamicin Nalidixic acid Streptomycin Tetracycline	Silva et al. (2019)	162
Staphylococcus aureus	58/230 (25.2%)	Yes	Algeria	Fosfomycin Oxacillin Penicillin G Tetracycline	Hachemi et al. (2019)	164

AMR organism	#isolations/ #samples tested (%)	Potential human pathogen	Surveillance country and sample collection location	Resistance phenotype(s) observed	Reference	Paper number
L. monocytogenes	296/2017 (14.7%)	Yes	South Africa	Clindamycin Fusidic acids Gentamicin Nitrofurantoin Streptomycin	Matle et al. (2019)	166

Table 6 A breakdown of the AMR bacteria isolated from meat products, food animal species (where specified) and the secondary processes applied to the product (where specified) for the isolations shown in Table 5. M-chill is maturation chilling, CSM is cooked sliced meat, RTC is ready-to-cook, RTE is ready-to-eat without further cooking; QAC are quaternary ammonium compounds. ^denotes a cooked meat product that contained other uncooked ingredients such as salad leaves. Species are bovines (cattle and buffalo), ovines (sheep and goat), porcine (pig) and galliformes (ground-based poultry).

AMR organism	Product	Species	Secondary processes	Reference
E. coli O157:H7	3x CSM 1x minced meat 1x jerky	Mixed Bovine Porcine	5x maturation chilling 1x comminution 4x dry thermal processing	Abong'o and Momba (2009)
Enterococcus spp.	Minced beef	Bovine	M-chill, comminution	Aslam et al. (2010)
LAB (multiple genera)	Fermented sausage	Porcine	M-chill, comminution dry fermentation	Aymerich et al. (2006)
Salmonella enterica (multiple serovars)	Meat and meat preparations	Porcine	M-chill; comminution	Bacci et al. (2014)
LAB (multiple genera)	Wakalim, (fermented sausage)	Bovine	M-chill, comminution, fermentation	Bacha et al. (2010)
Listeria monocytogenes	Fresh meat	Porcine	M-chill	Barbuti et al. (1992)

AMR organism	Product	Species	Secondary processes	Reference
L. innocua	Raw ham Sausages Salami Cured meat Cooked meat		M-chill M-chill, comminution M-chill, fermentation M-chill, curing M-chill, curing, wet or dry thermal processing	
Enterococcus faecium E. faecalis Enterococcus of undetermined species	Gueddid (fermented meat)	Not specified	M-chill, fermentation, drying	Belgacem et al. (2010)
Enterococcus faecium E. faecalis E. hirae E. durans	Sucuk (fermented sausage)	Mixture of bovine and ovine	M-chill, fermentation	Demirgul and Tuncer (2017)

AMR organism	Product	Species	Secondary processes	Reference
E. mundtii E. thailandicus				
Thermotolerant coliforms Escherichia coli Salmonella spp.,	Beef jerky	Bovine	M-chill, slicing, marinade, curing, drying	Fernandes et al. (2017)
Enterococcus faecium E. faecalis E. durans E. casseliflavus E. mundtii	Dry fermented sausage	Porcine	M-chill, comminution, fermentation	Fontana et al. (2009) sampling information from Fontana et al. (2005)
VTEC O157	Post chill carcasses	Bovine	M-chill	Fontcuberta et al. (2016)
Micrococcus (M) Kocuria (K) Staphylococcus (S)	Salsiccia Sopressata Milanese salami	Porcine	M-chill, comminution, fermentation	Gardini et al. (2003)

AMR organism	Product	Species	Secondary processes	Reference
Lactobacillus plantarum	Dry fermented sausage	Mixture of porcine and bovine	M-chill, comminution, fermentation	Gevers et al. (2003c)
Listeria monocytogenes L. innocua	Meat and meat products	Mixtures of unspecified species	M-chill, comminution, fermentation, wet or dry thermal processing	Gomez et al. (2014)
L. monocytogenes	Sausage	Unspecified species	M-chill, comminution	Haubert et al. (2016)
E. coli (generic)	Raw meats	Bovine Galliformes	M-chill	Jayaratne et al. (1987b)
E. coli (generic)	Cooked meats	Mixtures of unspecified species	M-chill, curing, wet or dry thermal processing	Jiang et al. (2014)
VTEC O157:H7	Minced beef	Bovine	M-chill, comminution	Kalender (2013)
VRE	Minced meat	Unspecified species	M-chill, comminution	Messi et al. (2006)
ESBL-Enterobacteria	Minced meat	Mixtures of bovine and porcine	M-chill, comminution	Petternel et al. (2014)

AMR organism	Product	Species	Secondary processes	Reference
MRSA	Minced meat	Mixtures of bovine and porcine	M-chill, comminution	Petternel et al. (2014)
Enterococcus spp.	Smoked fermented sausage Catalão Chouriço-preto Linguiça Salsichão Paio	Unspecified (Paio is exclusively porcine)	M-chill, comminution, fermentation, smoking	Santos et al. (2017)
Campylobacter spp.	Pork sausage Minced pork Pork chop Pork pieces	Porcine	M-chill, comminution M-chill, comminution M-chill M-chill	Scanlon et al. (2013)
ESBL Enterobacteria	Pork meat	Porcine	M-chill	Schill et al. (2017)

AMR organism	Product	Species	Secondary processes	Reference
E. coli (generic)	Assorted chilled meat	Mixtures of bovine, ovine, porcine, galliformes	M-chill	Sunde and Norstrom (2005)
Campylobacter coli	Post chill carcasses Chicken Beef Pork	Mixtures of bovine, porcine, galliformes	M-chill	Wieczorek et al. (2013)
E. coli (generic)	Cooked meats	Unspecified species	M-chill, comminution, smoking, wet or dry thermal processing	Yu et al. (2016)
Enterobacteriaceae	Cooked meats	Mixtures of bovine, porcine, galliformes	M-chill, curing/brining/seasoning, wet or dry thermal processing	Yu et al. (2017)
Pseudomonas	Cooked meats	Mixtures of bovine, porcine, galliformes	M-chill, curing/brining/seasoning, wet or dry thermal processing	Yu et al. (2017)
Vibrio	Cooked meats	Mixtures of bovine, porcine, galliformes	M-chill, curing/brining/seasoning, wet or dry thermal processing	Yu et al. (2017)

AMR organism	Product	Species	Secondary processes	Reference
Gram positive	Cooked meats	Mixtures of bovine, porcine, galliformes	M-chill, curing/brining/seasoning, wet or dry thermal processing	Yu et al. (2017)
ESBL *E. coli* (generic)	Raw chicken meat	Galliformes	M-chill	Zarfel et al. (2014)
Staphylococcus aureus	Raw chicken meat	Galliformes	M-chill	Zarfel et al. (2014)
VRE	Raw chicken meat	Galliformes	M-chill	Zarfel et al. (2014)
Staphylococcus	Fermented sausage Dry kulen sausage Slavonian sausage	Porcine	M-chill, comminution, fermentation	Zdolec et al. (2013)
Salmonella spp.	Raw/frozen chicken nuggets/strips	Galliformes	M-chill, comminution, freezing*, seasoning, dry thermal processing	Bucher et al. (2007)
Proteus mirabilis	Cooked meats	Unspecified species	M-chill, marinade, thermal processing	Jiang et al. (2017)
E. coli (generic)	Cooked meats	Unspecified species	M-chill, marinade (n=240), thermal processing (n=720)	Li et al. (2017)

AMR organism	Product	Species	Secondary processes	Reference
MRSA	Raw pork Cooked pork	Porcine	M-chill	Li et al. (2016)
ESBL Enterobacteria	Raw pork Cooked pork	Porcine	M-chill, thermal processing	Li et al. (2016)
Campylobacter spp.	Post-chill turkey carcasses	Galliformes	M-chill	Lutgen et al. (2009) sampling info Logue et al. (2003)
VTEC O157:H7	Minced beef	Bovine	M-chill, comminution	Rodolpho and Marin (2007)
E. coli (generic)	Minced beef	Bovine	M-chill, comminution	Rodolpho and Marin (2007)
Salmonella spp.	Raw poultry meat	Galliformes	M-chill	Abd-Elghany et al. (2015)
Staphylococcus aureus	Assorted chilled meat	Mixtures of bovine, porcine, galliformes	M-chill	Dhup et al. (2015)

AMR organism	Product	Species	Secondary processes	Reference
Listeria spp.	Raw poultry meat	Galliformes	M-chill	Fallah et al. (2012)
Listeria spp.	RTC poultry	Galliformes	M-chill, various including barbeque marinade, breadcrumbs	
Listeria spp.	ᗑRTE poultry	Galliformes	M-chill, thermal processing	
Campylobacter spp.	Raw poultry meat	Galliformes	M-chill (10 days)	Casagrande Proietti et al. (2018)
Salmonella enterica	Raw chilled carcasses	Bovine Ovine Porcine	M-chill	Jaja et al. (2019)
Salmonella spp.	Raw poultry meat	Galliformes	M-chill	Shang et al. (2019)
Salmonella spp.	Assorted chilled meat	Mixtures of bovine, porcine, galliformes	M-chill	Deng et al. (2018)
E. coli ST101	Fresh chilled beef Frozen beef Mutton	Mixtures of bovine, ovine, porcine,	M-chill, marinade, curing, brining, seasoning	Sadek et al. (2019)

AMR organism	Product	Species	Secondary processes	Reference
	Minced meat Burger Sausage Luncheon meat Kofta Pastrami			
Salmonella spp.	Frozen chicken portions	Galliformes	M-chill, freezing	Perin et al. (2019)
Campylobacter spp.	Raw chilled carcasses	Galliformes	M-chill	Silva et al. (2019)
Staphylococcus aureus	Raw sausage meat	Unspecified species	M-chill, seasoning, comminution	Hachemi et al. (2019)
L. monocytogenes	Raw intact meat RTE meat products Raw processed meat products	Bovine Ovine Porcine, Galliformes Game meat	M-chill, seasoning, comminution	Matle et al. (2019)

Table 7 Methodologies used for determination of AMR status for the organisms and products contained in Table 5. EUCAST is the European Committee on Antimicrobial Susceptibility Testing, CLSI is the Clinical Laboratory Standards Institute.

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
E. coli O157:H7	Ampicillin Erythromycin Gentamicin Nalidixic acid	Bauer and Kirby disk diffusion method using CLSI described breakpoint criteria	Abong'o and Momba (2009)
Enterococcus spp.	Ciprofloxacin Lincomycin Quinupristin-dalfopristin Tetracycline	Sensititre automated microdilution broth method	Aslam et al. (2010)
LAB (multiple genera)	Ampicillin Benzylpenicillin Chloramphenicol Gentamicin	Agar overlay disc diffusion method with modifications	Aymerich et al. (2006)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Linezolid Quinupristin-dalfopristin Tetracycline Vancomycin		
Salmonella enterica (multiple serovars)	Ampicillin Gentamicin Sulfamethoxazole Tetracycline	Unspecified disk diffusion method using CLSI described breakpoint criteria	Bacci et al. (2014)
LAB (multiple genera)	Gentamicin Kanamycin Methicillin Streptomycin	Unspecified disk diffusion method using unspecified breakpoint criteria	Bacha et al. (2010)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Vancomycin		
Listeria monocytogenes L. innocua	Ceftazidime Colistin Nalidixic acid Piperidimic acid	Unspecified disk diffusion method using unspecified breakpoint criteria	Barbuti et al. (1992)
Enterococcus faecium E. faecalis Enterococcus of undetermined species	Ciprofloxacin Erythromycin Levofloxacin Nitrofurantoin Rifampicin	ATB ENTEROC 5 strips using CLSI described breakpoint criteria	Belgacem et al. (2010)
Enterococcus faecium E. faecalis	Chloramphenicol Ciprofloxacin-Gentamicin	Unspecified disk diffusion method using CLSI described breakpoint criteria	Demirgul and Tuncer (2017)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
E. hirae E. durans E. mundtii E. thailandicus	Doxycycline Erythromycin Nitrofurantoin Rifampicin Streptomycin Teicoplanin		
Thermotolerant coliforms Escherichia coli Salmonella spp.,	For Salmonella enterica No resistance to 14 tested antibiotics for product isolates.	Agar disc diffusion method using breakpoints as described by CLSI, 2015	Fernandes et al. (2017)
Enterococcus faecium E. faecalis	Erythromycin Rifampicin	Microdilution method using breakpoints as described by CLSI, 2005	Fontana et al. (2009) sampling information from Fontana et al. (2005)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
E. durans E. casseliflavus E. mundtii	Tetracycline		
VTEC O157	Ampicillin Chloramphenicol Florfenicol Gentamicin Kanamycin Nalidixic acid Streptomycin Sulfonamide Trimethoprim-sulfamethoxazole	Agar disc diffusion method using breakpoints as described by CLSI, 2006	Fontcuberta et al. (2016)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
Micrococcus (M) Kocuria (K) Staphylococcus (S)	Bacitracin Colistin Piperidimic acid Sulfonamide Tetracycline Trimethoprim-sulfamethoxazole	Agar disc diffusion method as described by National Committee for Clinical Laboratory Standards (which evolved into CLSI), 1988	Gardini et al. (2003)
Lactobacillus plantarum	Tetracycline	Kirby-Bauer disc diffusion method (modified using MRS agar). Breakpoint criteria as described by AB Biodisk manufacturer's instructions	Gevers et al. (2003c)
Listeria monocytogenes L. innocua	Clindamycin Oxacillin Tetracycline	Agar disc diffusion method as described by EUCAST, 2012	Gomez et al. (2014)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
L. monocytogenes	Clindamycin Erythromycin Meropenem Rifampicin Streptomycin Tetracycline Trimethoprim-sulfamethoxazole	Agar disc diffusion method as described by EUCAST, 2014	Haubert et al. (2016)
E. coli (generic)	Ampicillin Bacitracin Erythromycin Kanamycin Penicillin	Bauer and Kirby disk diffusion method using unspecified breakpoint criteria	Jayaratne et al. (1987b)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Streptomycin Tetracycline		
E. coli (generic)	Ampicillin Cefoperazone Cefotaxime Ceftazidime Chloramphenicol Ciprofloxacin Gentamicin Nalidixic acid Streptomycin Tetracycline	AM dilution into agar and using CLSI described breakpoint criteria	Jiang et al. (2014)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Trimethoprim-sulfamethoxazole		
VTEC O157:H7	Ampicillin Clindamycin Enrofloxacin Florfenicol Penicillin Sulfadimethoxine Tetracycline Tiamulin Tilmicosin Trimethoprim-sulfamethoxazole	Sensititre Susceptibility System methodology using CLSI-described breakpoint criteria	Kalender (2013)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
VRE	Ampicillin Benzylpenicillin Erythromycin Gentamicin Kanamycin Streptomycin Teicoplanin Vancomycin	Unspecified CLSI (2000) agar-based methodology (modified using Mueller-Hinton agar supplemented with 5% sheep blood) using CLSI-described breakpoint criteria	Messi et al. (2006)
ESBL-Enterobacteria	Amoxicillin/clavulanic acid Ampicillin Cefepime Cefotaxime	Agar disc diffusion method as described by EUCAST (unspecified version)	Petternel et al. (2014)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Cefoxitin Ceftazidime Cefuroxime Chloramphenicol Ciprofloxacin Gentamicin Imipenem Meropenem Moxifloxacin Nalidixic acid Piperacillin/tazobactam/ cefalexin Tetracycline		

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Trimethoprim-sulfamethoxazole		
MRSA	Cefoxitin Clindamycin Erythromycin Fusidic acid Gentamicin Linezolid Mupirocin Norfloxacin Penicillin Rifampicin Tetracycline	Agar disc diffusion method as described by EUCAST (unspecified version)	Petternel et al. (2014)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Trimethoprim-sulfamethoxazole		
Enterococcus spp.	Amoxicillin-clavulanic acid Ampicillin Bacitracin Benzylpenicillin Chloramphenicol Ciprofloxacin Erythromycin Gentamicin Nalidixic acid Nitrofurantoin	Mixed methodologies, Vancomycin resistance testing using Oxoid E-test strips. Other AM using agar disc diffusion technique as described by CLSI, 2013	Santos et al. (2017)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Norfloxacin Quinupristin-dalfopristin Rifampicin Streptomycin Teicoplanin Tetracycline Trimethoprim/sulfamethoxazole Vancomycin		
Campylobacter spp.	Ciprofloxacin Erythromycin Gentamicin Kanamycin	Custom agar disc diffusion method using Mueller-Hinton agar supplemented with 5% lysed horse blood using CLSI (2004) described breakpoint criteria	Scanlon et al. (2013)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Nalidixic acid Trimethoprim		
ESBL Enterobacteria	Ampicillin Azithromycin; Cefepime Cefotaxime Cefoxitin Ceftazidime Chloramphenicol Ciprofloxacin Colistin Ertapenem	Microdilution method using ISO 20776- 1:2006	Schill et al. (2017)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Gentamicin Meropenem Nalidixic acid Streptomycin Tetracycline Tigecycline Trimethoprim-sulfamethoxazole		
E. coli (generic)	Streptomycin	Sensititre microdilution methodology using EUCAST-described breakpoint criteria (of unspecified year) (NB: Determined from the NORM-VET protocols used for original strain isolations)	Sunde and Norstrom (2005)
Campylobacter coli	Ciprofloxacin Erythromycin	Sensititre microdilution methodology using publication specific breakpoint criteria	Wieczorek et al. (2013)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Gentamicin Nalidixic acid Streptomycin Tetracycline		
E. coli (generic)	Ampicillin Nalidixic acid Streptomycin Tetracycline Trimethoprim-sulfamethoxazole	Methodology determined from Jiang et al., (2014) as unspecified CLSI (2011) agar-based methodology using CLSI-described breakpoint criteria	Yu et al. (2016)
Enterobacteriaceae	Ampicillin Cefotaxime Ceftazidime	Agar disc diffusion CLSI (2011, 2013) using publication specific breakpoint criteria	Yu et al. (2017)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Chloramphenicol Ciprofloxacin Gentamicin Levofloxacin Streptomycin Sulfamethoxazole Tetracycline		
Pseudomonas	Ampicillin Cefotaxime Ceftazidime Chloramphenicol Ciprofloxacin	Agar disc diffusion CLSI (2011, 2013) using publication specific breakpoint criteria	Yu et al. (2017)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Gentamicin Levofloxacin Streptomycin Sulfamethoxazole Tetracycline		
Vibrio	Ampicillin Streptomycin Sulfamethoxazole Tetracycline	Agar disc diffusion CLSI (2011, 2013) using publication specific breakpoint criteria	Yu et al. (2017)
Gram positive	Ampicillin Tetracycline	Agar disc diffusion CLSI (2011, 2013) using publication specific breakpoint criteria	Yu et al. (2017)
ESBL *E. coli* (generic)	Cefoxitin	Mixed methodologies. Mostly agar disc diffusion method as described by EUCAST (unspecified version). For tetracycline, chloramphenicol and	Zarfel et al. (2014)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Ceftazidime Chloramphenicol Nalidixic acid Tetracycline Trimethoprim-sulfamethoxazole	nalidixic acid, there was no EUCAST guidance and so CLSI breakpoint criteria were used	
Staphylococcus aureus	Cefoxitin Penicillin	Mixed methodologies. Mostly agar disc diffusion method as described by EUCAST (unspecified version). For tetracycline, chloramphenicol and nalidixic acid, there was no EUCAST guidance and so CLSI breakpoint criteria were used	Zarfel et al. (2014)
VRE	Ampicillin Teicoplanin	Mixed methodologies. Mostly agar disc diffusion method as described by EUCAST (unspecified version). For tetracycline, chloramphenicol and nalidixic acid, there was no EUCAST guidance and so CLSI breakpoint criteria were used	Zarfel et al. (2014)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
Staphylococcus	Ampicillin Erythromycin Gentamicin Tetracycline	Mostly agar disc diffusion method as described by CLSI, 2010. For vancomycin and oxacillin, an epsilometer-based test (E-test) on isosensitest agar was used.	Zdolec et al. (2013)
Salmonella spp.	Amikacin Amoxicillin-clavulanic acid Ampicillin Cefoxitin Ceftiofur Ceftriaxone Chloramphenicol Ciprofloxacin	Unspecified CLSI method using breakpoints from CLSI (2003)	Bucher et al. (2007)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Gentamicin Kanamycin Nalidixic acid Streptomycin Sulfamethoxazole Tetracycline Trimethoprim-sulfamethoxazole		
Proteus mirabilis	Benzalkonium chloride (QAC)	AM dilution into agar and using CLSI (2012) described breakpoint criteria	Jiang et al. (2017)
E. coli (generic)	Ampicillin Ampicillin-sulbactam Cefotaxime	AM dilution into agar using custom protocol and using CLSI (2012) described breakpoint criteria	Li et al. (2017)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Cefoxitin Ceftazidime Chloramphenicol Ciprofloxacin Gentamicin Tetracycline Trimethoprim-sulfamethoxazole		
MRSA	Penicillin Clindamycin Erythromycin Trimethoprim/sulfamethoxazole Tetracycline	Bauer and Kirby disk diffusion method using CLSI (2012) described breakpoint criteria	Li et al. (2016)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
ESBL Enterobacteria	Ampicillin Cefotaxime Ceftazidime Chloramphenicol Ciprofloxacin Levofloxacin Tetracycline	Bauer and Kirby disk diffusion method using CLSI (2012) described breakpoint criteria	Li et al. (2016)
Campylobacter spp.	Ciprofloxacin Erythromycin	AM dilution into agar and using a mixture of custom and CLSI (2002) described breakpoint criteria	Lutgen et al. (2009) sampling info taken from Logue et al. (2003)
VTEC O157:H7	Unable to differentiate	CLSI agar disc diffusion method using breakpoints as described by CLSI, 2000	Rodolpho and Marin (2007)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
E. coli (generic)	Amikacin Amoxicillin Amoxicillin-clavulanic acid Ampicillin Cefalothin Ceftriaxone Ciprofloxacin Gentamicin Nalidixic acid Streptomycin Tetracycline	CLSI agar disc diffusion method using breakpoints as described by CLSI, 2000	Rodolpho and Marin (2007)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Trimethoprim/sulfamethoxazole		
Salmonella spp.	Amoxicillin Ampicillin Erythromycin Nalidixic acid Sulfamethoxazole Oxytetracycline Penicillin	CLSI agar disc diffusion method using breakpoints as described by CLSI, 2000	Abd-Elghany et al. (2015)
Staphylococcus aureus	Ciprofloxacin Clindamycin Erythromycin Methicillin	Agar dilution using the British Society for Antimicrobial Chemotherapy Method and custom breakpoints (2011)	Dhup et al. (2015)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Oxacillin Tetracycline		
Listeria spp. Listeria spp. Listeria spp.	Ampicillin Ciprofloxacin Enrofloxacin Erythromycin Penicillin Tetracycline	CLSI agar disc diffusion method using breakpoints as described by CLSI, 2006	Fallah et al. (2012)
Campylobacter spp.	Ciprofloxacin Erythromycin Nalidixic acid Tetracycline	Custom microdilution method using breakpoints described by EUCAST, 2013	Casagrande Proietti et al. (2018)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
Salmonella enterica	Amoxicillin Ampicillin Cefotaxime Ceftriaxone Chloramphenicol Ciprofloxacin Gentamicin Imipenem Kanamycin Meropenem Neomycin Norfloxacin	CLSI agar disc diffusion method using breakpoints as described by CLSI, 2017	Jaja et al. (2019)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Streptomycin Sulfamethoxazole Tetracycline		
Salmonella spp.	Ampicillin Chloramphenicol Florfenicol Nalidixic acid Neomycin Streptomycin Tetracycline Trimethoprim/sulfamethoxazole	Mixed methodologies including the Sensititre system and agar dilution. Breakpoints as described by CLSI (2008, 2016) and also some breakpoints set by publication authors	Shang et al. (2019)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
Salmonella spp.	Amoxycillin Ampicillin Ciprofloxacin Gentamicin Levofloxacin Oxytetracycline Trimethoprim	AM dilution into agar and using CLSI (2016) described breakpoint criteria	Deng et al. (2018)
E. coli ST101	Ampicillin Chloramphenicol Ciprofloxacin Colistin Gentamicin	CLSI agar disc diffusion method using a mix of breakpoints. Some as described by CLSI and some by EUCAST (years not specified)	Sadek et al. (2019)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Kanamycin Nalidixic acid Tetracycline		
Salmonella spp.	Amoxicillin with clavulanic acid Ceftriaxone Ampicillin Ciprofloxacin Doxycycline Nalidixic acid tetracycline	Unspecified CLSI test method using breakpoints as described by CLSI, 2008, 2013	Perin et al. (2019)
Campylobacter spp.	Chloramphenicol Ciprofloxacin	Unspecified disk diffusion test method using breakpoints as described by CLSI, 2003	Silva et al. (2019)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Erythromycin Gentamicin Nalidixic acid Streptomycin Tetracycline		
Staphylococcus aureus	Fosfomycin Oxacillin Penicillin G Tetracycline	Unspecified disk diffusion test method using breakpoints as described by EUCAST, 2013, 2017	Hachemi et al. (2019)
L. monocytogenes	Clindamycin Fusidic acids Gentamicin	Kirby–Bauer disk diffusion method using Mueller–Hinton agar plates containing 5% sheep blood and breakpoint determinations as described by EUCAST, 2014	Matle et al. (2019)

AMR organism	Resistance phenotype(s) observed	AMR determination method	Reference
	Nitrofurantoin Streptomycin		

8.1.1 Considerations of AMR isolates from secondary processed meats and meat products

Although there seems little doubt that AMR bacteria can be routinely isolated from SPMMP, much of the surveillance literature was based on materials collected at retail. Most, (64%) of the isolations from the identified papers related exclusively to surveillance at retail, but there were three isolations of AMR (6%) where the sampled sources were unclear, and the remaining isolations were from a mixture of samples collected during processing combined with retail (4%) or collected during some stage of secondary meat processing (26%). Retail surveillance information is not ideal because, although it is what is bought by consumers; clear identification of the contamination source is not always possible. There exists the possibility of contamination after a process critical control point such as effective thermal processing (Gomez et al. 2014; Jiang et al. 2014) from the processing plant environment (Gomez et al. 2014; Fernandes et al. 2017). The issue is further complicated by the fact it can be difficult to differentiate between such contaminations and the carry forward from the meat used for current and previous meat batches, if it was sourced from livestock previously exposed to antimicrobials (Melero et al. 2012). For example, effective thermal processing (cooking) would be expected to kill any bacteria associated with meat. Cross contamination could occur however during retailing whilst undertaking operations such as store-slicing cooked meats, with the slicer acting as a fomite (Melero et al. 2012).

There is some evidence that contamination can be acquired during the later stages of SPMMP processing. Gevers et al. (2003c) reported both increased isolations of tetracycline-resistant *Lactobacillus* and the acquisition of new resistant species after slicing and packing compared with the same batch of fermented sausage sampled immediately after ripening and prior to slicing. Similarly, Li et al. (2017) considered that most AMR bacteria would be destroyed by the thermal processes applied during the manufacture of RTE cooked meat and that it was therefore probable that AMR *E. coli* contamination of finished cooked meat product occurred by cross-contamination from food handling by workers or the processing environment after cooking. Fernandes et al. (2017) did not detect *Salmonella* in raw beef used to manufacture jerky. However, the finished product acquired at least two different serovars of non-AMR *Salmonella* during the slicing, marinating and drying stages of processing, with AMR *S. enterica* isolated from the processing environment. Mateus-Vargas et al. (2017) reported a low prevalence of AMR in wild deer and boar meat and, although it is speculative, the paper's discussion makes it clear the authors consider it likely the AMR populations were acquired from the processing environment rather than from the GI tract of wild animals that had (probably) not been previously exposed to antibiotics.

A review of *L. monocytogenes* in cooked sliced meats (CSM; FSA project FS241045), which did not consider AMR status, concluded that during the processing and manufacture of CSM, *L. monocytogenes* typically entered the processing environment as a contaminant on process inputs, and then became persistently established in the processing environment, potentially contaminating the final product for extended periods of months and even years (Hutchison et al. 2014). A particular barrier to the study of AMR in *L. monocytogenes* is the common use of acriflavine, known to cure plasmids from Gram positive organisms, as a selective agent in enrichment media for *Listeria* (Axelsson et al. 1988; Mesas et al. 2004). Although the route of product contamination is well described for *L. monocytogenes*, how important such pathways are for other bacteria is currently less clear, although some progress has been made. Shang et al. (2019) reported that AMR resident *Salmonella* in a chicken slaughterhouse subsequently gave rise to the most common isolates found in chicken meat from the plant when products were sampled at retail.

None of the surveillance papers reporting AMR in fermented meat products reported the original source of the starter culture fermentation strains. Typically, products were purchased at retail and any isolates then phenotypically characterised for AMR. In some cases, there was an additional genetic characterisation to identify specific resistance or virulence loci. It is likely that for a high proportion of AMR isolates, the researchers did not take cognisance of the finer details of the manufacture process. Historically, traditional meat fermentation for products such as sausages exploited the indigenous microbiota present in the meat and processing environment (FAO 1985). Populations of bacteria that were favourable for flavour and food safety were selected for by controlling environmental conditions such as moisture, pH, salt content and temperature during the product aging. However, over the last 100 years, it became more difficult to use the traditional approach to the manufacture of fermented meats to achieve reliable results, for several reasons. These include better hygiene in food processing plants and the use of sanitisers that expunge resident bacterial populations within the processing environment (FAO 1985). The bacteria present within a processing environment transferring to meat destined for fermentation were an important historical source of fermentation bacteria. The FAO reports that in the 1940s it became common for industrial-scale manufacturers of fermented meat products to use starter cultures to more reliably achieve a final product with a consistent flavour and appearance (FAO 1985). It is reasonable to consider the deliberate addition of starter cultures to fermented meats as something distinct to the unintended, accidental contamination of products by commensals or potential enteric human pathogens.

8.1.1 AMR in fermentation starter cultures

The AMR bacteria isolated from SPMMP can be broadly categorised into three groups, which are:

- Cultures suitable for meat fermentation (Aymerich et al. 2006; Belgacem et al. 2010)
- Commensal contaminants naturally present as part of the microbiota that were unlikely to cause human illness, such as non-toxigenic *E. coli* (Rodolpho and Marin 2007; Li et al. 2017)
- Potential enteric human pathogens such as verocytotoxigenic *E. coli* (Fontcuberta et al. 2016) and *Salmonella* (Bucher et al. 2007).

Starter cultures differ from commensals and potential enteric pathogens because they are deliberate additions to a process and so can be controlled by FBOs. Commensals and potential pathogens are part of a natural biota associated with SPMMP, often despite the hygienic practices implemented by FBOs to help prevent product contamination. Starter cultures such as LAB can rapidly reduce the pH of a food matrix, reducing populations of spoilage organisms and halting the proliferation of potential human pathogens (Fontana et al. 2005; Abriouel et al. 2015; Fraqueza 2015). This study identified insufficient papers to undertake any analysis of AMR in fermentation starter cultures and noted that the majority of papers were skewed towards tetracycline resistance in LAB.

However, AMR genes have been identified in some starter cultures used for fermentation of SPMMP and at high concentrations: exceeding 10^8/g in finished products (Abriouel et al. 2015). Thus, AMR in starter cultures is a credible potential issue. In order to better assess the significance of the issue, more surveillance work characterising AMR genetic material in commercial starter cultures would be required. A summary of AMR in fermentation cultures in the identified literature is shown as Table 8.

Table 8 A summary of AMR genes in fermentation starter cultures used to manufacture fermented meat products Lb. is Lactobacillus, Pd is Pediococcus, Ec is Enterococcus, Lu is Leuconostoc, Tet is tetracycline, Ery is erythromycin, Chl is chloramphenicol, Van is vancomycin. Data summarises the AMR identified in products, but not all of the isolates necessarily harboured all of the AMR loci. Only organisms and papers where all of the resistance loci were identified are included.

Organism	Phenotype resistance	Resistance genes	Reference
Lb. alimentarius	Tet	tetM	Gevers et al. (2003a)
Lb. curvatus	Tet	tetM	Gevers et al. (2003a)
Lb. plantarum	Tet	ermB, tetM	Gevers et al. (2003a)
Lb. sakei	Tet	tetM	Gevers et al. (2003a)
Lb. brevis	Ery, Tet	ermB, tetM	Zonenschain et al. (2009)
Lb. curvatus	Ery, Tet	ermB, tetM, tetW	Zonenschain et al. (2009)
Lb. paracasei	Ery, Tet	ermB, tetM	Zonenschain et al. (2009)
Lb. plantarum	Ery, Tet	ermB, ermC, tetM, tetS, tetW	Zonenschain et al. (2009)
Lb. reuteri	Ery, Tet	ermB, tetM	Zonenschain et al. (2009)
Lb. rhamnosus	Ery, Tet	ermB, tetM	Zonenschain et al. (2009)
Lb. sakei	Ery, Tet	ermB, tetM, tetW	Zonenschain et al. (2009)
Lb. paracasei	Ery, Tet	ermB, tetM	Comunian et al. (2010)
Ec. faecalis	Ery, Tet, Chl, Van	ermB, pbp5, aac69-Ie-aph299, tetM, vanA, vanB	Ribeiro et al. (2011)
Lb. salivarius	Ery, Tet	ermB, tetL, tetM, tetO, tetW	Thumu and Halami (2012)
Pd. pentosaceus	Ery, Tet	ermB, tetM	Federici et al. (2014)

Organism	Phenotype resistance	Resistance genes	Reference
Lb. paraplantarum	Ery	ermB	Federici et al. (2014)
Ec. faecium	Tet	tetM	Landeta et al. (2013)
Lb. plantarum	Tet	tetM	Landeta et al. (2013)
Lb. sakei	Tet	tetM	Landeta et al. (2013)
Ec. faecalis	Ery	ermB	Federici et al. (2014)
Lactococcus	Tet	tetM	Federici et al. (2014)
Lb. sakei	Tet	tetM, tetK	Fraqueza (2015)

As yet, there are no apparent widespread clinical issues related to the treatment of LAB using antibiotics (Landeta et al. 2013; Campedelli et al. 2019), with a high percentage of strains susceptible to a wide range of clinically-relevant antibiotics (Landeta et al. 2013; dos Santos Cruxen et al. 2019). However, a number of authors have flagged AMR transfer from LAB as a potential issue (Laulund et al. 2017; Alvarez-Cisneros 2018). Typically, there are large numbers of LAB in final products at the end of fermentation (Fraqueza 2015) and as LAB are acid adapted they can potentially transit the stomach into the lower human GI tract where they might pass on the AMR to other bacteria (Tuohy et al. 2009). However, Federici et al. (2014) highlight the fact that there is a lack of information regarding the fate of LAB exposed to bile after passage through a stomach. This study identified only one paper that described the unequivocal transfer of AMR from *Lactobacillus* to *Enterococcus* in a mammalian GI tract, i.e. Thumu and Halami (2019). The theoretical potential hazard is historically well described however (Penders et al. 2013), as is the impact of high intestinal cell densities on the probability that comparatively rare events such as plasmid exchange might occur (Verraes et al. 2013).

In the EU, the laws which cover the use of starter cultures used for food fermentation EU Regulation No. 178/2002, have been recently reviewed and Laulund et al. (2017) considered this regulation to be the most important because in article 14 it states “Food shall not be placed on the market if it is unsafe and it is the food business operator’s responsibility for ensuring food safety”. However, regulation EU 2015/2283 covering novel foods may also be relevant because it states

“This regulation applies to foods and food ingredients in the following categories: […] foods and food ingredients which consist of microorganisms, fungi or algae”. It seems tenuous to consider that a chromosomal mutation or natural plasmid exchange creates a genetically modified organism (GMO), although if that is accepted, there are at least regulations that would apply to such bacteria (Laulund et al. 2017). Also of relevance when considering starter cultures is the EU Qualified Presumption of Safety (QPS) process (Herman et al. 2019), which consists of a flow chart-based risk assessment tool created by EFSA panels for the evaluation of food and feed products containing microorganisms. QPS would authorise the use of a bacterium with AMR based on a chromosomal mutation, but not a resistant organism reliant on a mobilisable AMR gene.

The authors concur with the general precautionary approach and recommendation from WHO (2015) that FBOs should be advised to use fermentation starter cultures that are free from anti-microbial resistance. As an interim measure towards full compliance, it might be reasonable to allow a time-restricted caveat to allow mobilisable AMR for historic strains before stricter implementation allowing only intrinsic resistance. As part of this study, three fermented meat manufactures were asked informally about AMR in their LAB starter cultures. None of the manufacturers had ever tested any of their strains for AMR. FBOs could test their starter cultures for AMR, or build this as a requirement into purchase specifications, using a recommended standard protocol. FBOs could be provided with validated methodologies designed to cure AMR plasmids from their strains.

8.1.2 Analyses of AMR classes in the identified literature

It is usual in systematic reviews for any identified information of the type summarised by Table 5 to be analysed so that further broad conclusions can be drawn. For example, metanalyses of the data from multiple studies which are broadly equivalent can be used to aggregate and increase confidence in study findings. An obvious use of the information collated by Table 5 would be to identify readily apparent, emergent trends of resistance to specific drugs in specific organisms/groups in secondary processed meat and meat products. Selected AMR bacteria that could be readily grouped are shown in Table 10, which abstracts the information from a selection of the identified scientific publications (n=45) reporting on organisms that were broadly compatible for grouping. Organisms were grouped as reported by the papers authors and there was generally insufficient detail reported such that consideration could be made for overlap between groups e.g. *Enterococcus* could not be differentiated from those papers reporting the prevalence of AMR LAB. For each of the bacterial groups shown in Table 10, the antimicrobials were classified according to their base chemistry or activity as presented in Table 24. Table 24 used a uniform approach to antibiotic names and classes in order to overcome the multiplicity of antibiotics reported due to the use of manufacturer product names and other synonyms for antibiotics in research papers.

Although a statistical analysis was attempted, as is usual in systematic critical reviews, the authors have some reservations regarding the robustness of any outcomes. The concerns arise from data that might show the same overall outcome, but that was collected in a diverse manner. Specifically, the plethora of non-standardised methodologies applied were of concern.

Overall, there was only limited standardisation of the laboratory methods used to isolate presumptive AMR bacteria. For example, Petternel et al. (2014) used two enrichment methods one with 1.0% peptone, the other with thioglycolate bouillon for the isolation of ESBL populations, including *Enterobacteriaceae.* Yu et al. (2017) however enriched for the same broad class of ESBL bacteria using only 0.1% peptone.

In addition, although a significant majority of the papers identified used the CLSI standard to assess isolates for resistance, not all papers followed the standard CLSI protocols in full. Furthermore, the CLSI standard itself allows for the reporting of a standardised selection of AMs. However, there additional antimicrobials that can be optionally added to the standard selections. Finally, how testing is reporting to the CLSI standard should be undertaken has been reviewed and updated on an annual basis since at least 2000. The majority of papers identified span the time interval since 2000, there are potential differences and breakpoint updates between revised CLSI protocol versions that are a further source of uncertainty. Thus, there may be differences between the isolation methodologies even between papers that report using a version of the CLSI standard. In addition, even when a version of the CLSI methodology was used, there is a choice of using either zones of inhibition around AMR-containing discs on agar, or a lack of growth in broth supplemented with antibiotics, to measure resistance. Overall, it is not clear how the results from these different methodologies relate to each other. Finally, some papers that used the CLSI protocol used additional antibiotics and concentrations outside the optional ranges for each organism and there were some papers reporting that researchers had set their own breakpoints.

To circumvent the majority of these issues, Table 10 summarises the assessments of resistance that were reported by the authors of the papers, without making consideration of how the laboratory protocols isolated presumptive AMR or measured resistance, i.e. Table 10 concentrates on the reported author outcomes rather than how the outcomes were achieved. The authors of this report acknowledge that there may be issues with the different isolation and resistance determination methodologies that may be difficult to correct for without obtaining the original isolates and retesting them under fully standardised conditions. The issue identified highlights why standardised protocols for isolation and AMR determination, such as those operated with EU member states, are important for the generation of high-quality information that is not subject to similar limitations.

For the reasons described above, we consider that the results of the statistical analyses should be considered advisory and indicative of areas where further investigation may be beneficial to confirm emergent, potentially problematic AMR.

Given the concerns with the quality of the underlying information, the statistical analysis chosen was very simple. The mean number of reported resistance instances by antibiotic class (Table 24) counted by organism group was calculated along with the standard deviation around the mean. High outliers representing potential future issues were identified as those antibiotic classes that exceeded the mean by more than two standard deviations.

The only group that contained a high outlier was the *Enterobacteriaceae*, where the analysis indicated that resistance to cephalosporins might be an area of upcoming concern in SPMMP. A high percentage of the resistance identified was to third generation cephalosporins, particularly cefotaxime and ceftazidime, which are CLSI group B and group C (i.e. reported selectively) drugs. There were four papers relating to AMR in the *Enterobacteriaceae* with genetic characterisation that identified the basis of resistance to the cephalosporins. A summary of these papers is shown as Table 9.

Table 9 A summary of the β-lactamase (bla) genes responsible for conferring cephalosporin resistance in AMR Enterobacteriaceae isolated from SPMMP.

Number of AMR isolates	Number of *bla* genes belonging to the CTX-M family	Percentage *bla* belonging to CTX-M	References
24	21	87.5%	Petternel et al. (2014)
19	5	26.3%	Li et al. (2016)
19	17	89.5%	Schill et al. (2017)
43	1	2.4%	Yu et al. (2017)

The Petternel and Schill papers made their observations in Austria and Germany respectively and report a high percentage of cephalosporin resistance was conferred by the CTX-M family of β-lactamases. The Li and Yu studies were both undertaken in China, where CTX-M was observed to be less prevalent. Whilst there are too few papers to draw any firm conclusions, we note that third

generation cephalosporin resistance in the *Enterobacteriaceae* (3GCR-E) has been reported as an important clinical issue for human infections over the last 15 years (Livermore and Woodford 2006; Potz et al. 2006; Asensio et al. 2011; Oliveira et al. 2015; Rohde et al. 2018). Potz et al. (2006) undertook a comprehensive characterisation of over 1200 *Enterobacteriaceae* isolates responsible for clinical infections in the UK over a three-month period and concluded “The predominant mechanism of cephalosporin resistance in isolates from both hospital and community settings was the production of CTX-M-type ESBLs”. Similar reports regarding the importance of the CTX-M family for the treatment of human infections have been widely made elsewhere too (Eckert et al. 2004; Tumbarello et al. 2006; Mendonça et al. 2007; Manyahi et al. 2017; Zeynudin et al. 2018). In addition, CTX-M is commonly isolated from livestock destined for food processing (Apostolakos et al. 2019; Zajac et al. 2019). The evidence that SPMMP might be an important clinical source of cephalosporin resistant *Enterobacteriaceae* is currently however only indicative and circumstantial.

8.1.3 In Summary

In order to unequivocally determine if SPMMP are important for human infections by 3GCR-E, a further series of detailed studies would be required to characterise SPMMP isolates, so they could be compared to those causing human illness and infections sources could be attributed.

Table 10 AMR organisms and frequency of antibiotic resistance and antibiotic class from selected reports (n=45) relating to secondary processed meat and meat products. The number of instances of antibiotic may not sum to the same number as the antibiotic class because mixtures such as Trimethoprim/sulfamethoxazole have been counted as both one instance of an amino oxymethyl benzyl pyrimidine and one instance of a sulfonamide. High outliers representing significantly elevated detections are highlighted in bold. Clavulanic acid is a β-lactamase inhibitor and was not assessed as an antimicrobial for the purposes of this analyses. SD is the standard deviation around a mean.

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
E. coli (all serovars)	Amikacin	1	Aminoglycoside	10	13.93
	Amoxicillin	2	Amino oxymethyl benzyl pyrimidine	7	
	Ampicillin	9	Cephalosporin	10	
	Bacitracin	1	Cyclic polypeptide	1	
	Cefalothin	1	Fluoroquinolone	10	
	Cefoperazone	1	Macrolide	3	
	Cefotaxime	2	Nitrobenzine	4	
	Cefoxitin	2	Nitrobenzine (derivative)	2	
			Penicillin	11	

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Ceftazidime	3	Pleuromutilin	1	
	Ceftriaxone	1	Sulfonamide	9	
	Chloramphenicol	4	Tetracycline	7	
	Ciprofloxacin	3			
	Clindamycin	1			
	Enrofloxacin	1			
	Erythromycin	1			
	Florfenicol	2			
	Gentamicin	5			
	Kanamycin	2			

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Nalidixic acid	6			
	Penicillin	2			
	Streptomycin	6			
	Sulfadimethoxine	1			
	Sulfonamide	1			
	Tetracycline	7			
	Tiamulin	1			
	Tilmicosin	1			
	Trimethoprim/sulfamethoxazole	7			
Listeria spp.	Ampicillin Ceftazidime	1 1	Aminoglycoside	3 1	3.68

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Ciprofloxacin	1	Amino oxymethyl benzyl pyrimidine	1	
	Clindamycin	2	Carbapenem	1	
	Colistin	1	Cephalosporin	1	
	Enrofloxacin	1	Cyclic polypeptide	3	
	Erythromycin	2	Fluoroquinolone	1	
	Meropenem	1	Heterocyclic amine carboxylic acid	2	
	Nalidixic acid	1	Macrolide	3	
	Oxacillin	1	Penicillin	1	
	Penicillin	1	Pyridopyrimidine	1	
	Piperidimic acid	1	Sulfonamide	3	
	Rifampicin	1	Tetracycline		
	Streptomycin	1			
	Tetracycline	3			

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Trimethoprim-sulfamethoxazole	1			
Enterococcus spp.	Amoxicillin-clavulanic acid	1	Aminoglycoside	8	9.2
	Ampicillin	3	Amino oxymethyl benzyl pyrimidine	1	
	Bacitracin	1	Cyclic polypeptide	4	
	Benzylpenicillin	2	Fluoroquinolone	7	
	Chloramphenicol	2	Glycopeptide	7	
	Ciprofloxacin	4	Macrolide	6	
	Doxycycline	1	Nitrobenzine	2	
	Erythromycin	5	Nitrofuran	3	
	Gentamicin	3	Penicillin	5	
	Kanamycin	1	Streptogramin	1	
	Levofloxacin	1	Sulfonamide	4	
	Lincomycin	1			

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Nalidixic acid	1	Tetracycline	4	
	Nitrofurantoin	3			
	Norfloxacin	1			
	Quinupristin-dalfopristin	2			
	Rifampicin	4			
	Streptomycin	3			
	Teicoplanin	4			
	Tetracycline	3			
	Trimethoprim/sulfamethoxazole	1			
	Vancomycin	3			
Staphylococcus spp.	Ampicillin	1	Aminoglycoside	5	5.66
	Cefoxitin	1	Amino oxymethyl benzyl pyrimidine	2	
	Ciprofloxacin	1			

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Clindamycin	3	Cephalosporin	1	
	Erythromycin	4	Cyclic polypeptide	1	
	Fusidic acid	1	Fluoroquinolone	2	
	Gentamicin	2	Fusidane	1	
	Linezolid	1	Macrolide	4	
	Methicillin	1	Oxazolidinone	1	
	Mupirocin	1	Penicillin	5	
	Norfloxacin	1	Polar fatty acid	1	
	Oxacillin	1	Sulfonamide	2	
	Penicillin	2	Tetracycline	4	
	Rifampicin	1			
	Tetracycline	4			
	Trimethoprim/sulfamethoxazole	2			

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
Enterobacteriaceae	Amoxicillin/clavulanic acid	1	Aminoglycoside	5	10.76
	Ampicillin	4	Amino oxymethyl benzyl pyrimidine	2	
	Azithromycin	1	β lactamase inhibitor	1	
	Cefepime	2	Carbapenem	4	
	Cefotaxime	4	**Cephalosporin**	13	
	Cefoxitin	2	Cyclic polypeptide	1	
	Ceftazidime	4	Fluoroquinolone	5	
	Cefuroxime	1	Macrolide	1	
	Chloramphenicol	4	Nitrobenzine	4	
	Ciprofloxacin	4	Penicillin	6	
	Colistin	1	Sulfonamide	3	
	Ertapenem	1	Tetracycline	5	
	Gentamicin	3			

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Imipenem	1			
	Levofloxacin	2			
	Meropenem	2			
	Moxifloxacin	1			
	Nalidixic acid	2			
	Piperacillin/tazobactam/cefalexin	1			
	Streptomycin	2			
	Sulfamethoxazole	1			
	Tetracycline	4			
	Tigecyclin	1			
	Trimethoprim-sulfamethoxazole	2			
Salmonella spp.	Amikacin	1	Aminoglycoside	15	15.56
	Amoxicillin	4		3	

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Amoxicillin-clavulanic acid	1	Amino oxymethyl benzyl pyrimidine	2	
	Ampicillin	7	Carbapenem	5	
	Cefoxitin	2	Cephalosporin	8	
	Ceftiofur	1	Fluoroquinolone	1	
	Ceftriaxone	2	Macrolide	5	
	Chloramphenicol	4	Nitrobenzine	13	
	Ciprofloxacin	4	Penicillin	7	
	Erythromycin	1	Sulfonamide	6	
	Florfenicol	1	Tetracycline		
	Gentamicin	5			
	Imipenem	1			
	Kanamycin	3			
	Levofloxacin	1			

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Meropenem	1			
	Nalidixic acid	3			
	Neomycin	2			
	Oxytetracycline	2			
	Penicillin	1			
	Streptomycin	4			
	Sulfamethoxazole	5			
	Tetracycline	4			
	Trimethoprim	1			
	Trimethoprim-sulfamethoxazole	2			
Campylobacter spp.	Ciprofloxacin	4	Amino oxymethyl benzyl pyrimidine	1	8.20
	Erythromycin	4			
	Gentamicin	2	Aminoglycoside	4	

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Kanamycin	1	Fluoroquinolone	7	
	Nalidixic acid	3	Macrolide	4	
	Streptomycin	1	Tetracycline	2	
	Tetracycline	2			
	Trimethoprim	1			
Lactic acid bacteria	Ampicillin	1	Aminoglycoside	4	4.28
	Benzylpenicillin	1	Glycopeptide	2	
	Chloramphenicol	1	Nitrobenzine	1	
	Gentamicin	2	Oxazolidinone	1	
	Kanamycin	1	Penicillin	3	
	Linezolid	1	Streptogramin	2	
	Methicillin	1	Tetracycline	2	
	Quinupristin-dalfopristin	1			

Organism	Antibiotic name	Number of reports	Antibiotic class	Number of reports	Threshold for high outlier (mean+2SD)
	Streptomycin	1			
	Tetracycline	2			
	Vancomycin	2			

8.1.4 Analysis to determine geographical influence

A geographical analysis was undertaken, with prior acknowledgement that any apparently significant findings should be interpreted cautiously, because of the previously discussed examination methodological issues (Section 1.3.2). Also, it was apparent from the literature reviewed that the countries reporting AMR in SPMMP did not represent a random selection from across the globe and, in addition, there were comparatively few AMR reports relating to SPMMP with, again with a lack of apparently random selection for the product types. Finally, it was considered that some countries were more likely to report AMR than others for technical or commercial reasons. Following consideration of these matters, it was felt that an efficient way of addressing the issues of non-random country selection and low numbers of reports might be to group the identified reports by continent before undertaking any analysis. During the initial groupings by continent, those papers that reported on more than one type of bacteria were counted once for every different bacterium that was reported. A summary of the reports by continent is shown as Table 11. As before, antibiotics were grouped into classes based on their chemistry and mechanism of action prior to analyses.

Table 11 Reports of AMR in SMMP grouped by reporting continent

Reporting continent	Number of papers
Europe	21
North America	4
South America	6
Africa	4
Asia	17

It was apparent that there was a considerable disparity in the numbers of reports, with significantly more studies arising in Asia and Europe than the other continents (Table 11). Initially, an analysis of variance comparison was undertaken between Europe and Asia, but this did not highlight any statistically significant differences. Similarly, no significant differences were found when North America, South America and Africa were compared. It was felt inappropriate to report a comparison between all five continents because the large differences in the numbers of reports would justifiably leave such a comparison open to criticism on the basis it could confound the

analysis. Therefore, we were unable to determine if there were differences in the AMR between continents because there was a lack of AMR reporting from North and South America and Africa.

Other ways of grouping the information were attempted including by northern or southern hemisphere, which again resulted in unevenly matched datasets. Using the prime meridian and 180th meridians resulted in better matched, but still less than ideal groups of reports, which did not show significant differences.

8.1.5 In summary:

We did not observe any differences in AMR on the basis of geography for SPMMP. However, the relatively small numbers of reports combined with the very different distribution in the numbers of papers between regions confounded balanced analysis, which may underlie the observed outcome of no significant differences. As before there would be benefit in repeating the analyses if more information became available, especially if a standardised examination protocol was used to generate the newer data.

8.2 Evidence for transfer of antibiotic resistance between bacteria

The evidence relating to AMR transfer with regard to SPMMP was extracted from the identified literature and is presented as Table 12.

The earliest identified papers relating to AMR transfer associated with SPMMP were by Caudry and Stanisich (1979) and Jayaratne et al. (1987a), who studied transfer from *E. coli* donor strains containing naturally-occurring R-plasmids, isolated originally from raw and frozen meats. Caudry and Stanisich (1979) described mating AMR donor *E. coli* with an *E. coli* K12 recipient and the transfer of R-plasmids, however the methodology was not well described and the matrix used for the matings was not clearly defined. More detail was reported in the study of Jayaratne et al. (1987a) who described similar transfers when the mating matrix was ground meat or summer sausage. Jayaratne et al. (1987a) observed typical transfer frequencies in the order of 10^{-3} or 10^{-4} transconjugants/recipient and transfer was lowered when the donor strains had to compete with the indigenous microflora of the matrix for nutrients. Overall, transfer frequencies were higher in ground beef compared with summer sausage. The composition of the sausage was not reported. The original study by Jayaratne et al. (1987a) was subsequently extended to include six additional donor *E. coli*, also isolated from commercially-available meats (Jayaratne et al. 1987b). Four of the nine donor strains were able to transfer R-plasmid into *E. coli* but only two of the nine donors could transfer R-plasmid into *Salmonella*. Transfer frequencies at 37^{o}C were similar to those reported in the initial work, being in the range 10^{-3} or 10^{-4} transconjugants/recipient. The extended Jayaratne

studies (1987a, 1987b) were some of the first evidence that intraspecies transfer can occur from SPMMP-related strains of AMR under *in vitro* conditions.

Similar, seminal work by Vogel et al. (1992) assessed the transfer of naturally-present R-plasmids in meat LAB under *in vitro* conditions as well as in sausage undergoing fermentation. *In vitro*, a 26.5 kb plasmid conferring resistance to erythromycin was conjugally transferred from *Enterococcus faecalis* to *Lactobacillus curvatus* LTH1432 and between strains of *L. curvatus* at frequencies of 2.1×10^{-7} and 8×10^{-6} transconjugants/recipient, respectively. Mixed beef and pork sausages were fermented under conditions of decreasing temperature and humidity that were typical of a commercial dry fermentation. The transfer rate between two *L. curvatus* strains was 1.3×10^{-6} and hence was similar to the rate observed *in vitro*. Although one of the first studies in the area of AMR in SPMMP, the work described by Vogel et al. (1992) is important. The conditions for plasmid exchange, which Vogel et al. describe as *in situ*, are a very close mimic of a commercial process. The starter culture numbers (1×10^7 cfu/g), sausage meat composition and the temperature and humidity accurately reproduced a typical fermentation of 21 days duration. Hence, the study of Vogel et al. (1992) was the first time that the transfer of natural plasmid DNA among starter cultures was observed for fermented meat under typical commercial processing conditions and the first evidence that AMR transfer can happen during commercial processing.

Table 12 A summary of the evidence relating to the transfer of AMR between bacteria associated with SPMMP

AMR donor(s)	AMR recipient(s)	Significant findings	Reference
AMR *E. coli* isolated from the liquid generated from thawing frozen chicken carcasses purchased at retail	E. coli K12	Freezing did not kill AMR *E. coli.* Overall, there was transfer of plasmid-based AMR genes from 14/129 (35%) tetracycline^r stains, 54/142 (38%) sulfathiazole^r strains, 45/76 (59%) streptomycin^r strains and 29/72 (40%) ampicillin^r strains.	Caudry and Stanisich (1979)
Three *E. coli* strains isolated from chilled raw meats	E. coli K12	*In vitro* transfer of tetracycline, streptomycin (3/3 donors) kanamycin, neomycin (1/3 donors) resistance to recipient via plasmid. Elevated transfer rates in ground beef compared with sausage or broth. Transfer was temperature dependent, operating at 10^{o}C; but not 5^{o}C.	Jayaratne et al. (1987a)
Nine *E. coli* strains isolated from chilled raw meats	*Salmonella enterica* subsp. *enterica* serovar Typhimurium E. coli K12	Frequencies of *in vitro* plasmid transfer were highest at 37^{o}C and declined with decreasing temperature. Two isolates conjugally transferred antibiotic resistance at 10^{o}C. The highest transfer frequency occurred between pH 6.5-7.0. The influence of [NaCl] and anaerobic conditions on plasmid transfer was variable with isolates from different sources.	Jayaratne et al. (1987b)
Lactobacillus curvatus from fermented sausage meat and Enterococcus faecalis	Lactobacillus curvatus	Transfer of erythromycin resistance from *E. faecalis* to LAB, and between LAB, by plasmid conjugation during sausage fermentation under commercial processing conditions.	Vogel et al. (1992)

AMR donor(s)	AMR recipient(s)	Significant findings	Reference
Enterococcus faecalis	Enterococcus faecalis	The most effective transfer for a plasmid conferring tetracycline resistance and a second plasmid conferring vancomycin resistance was when matings were performed in a fermenting sausage matrix	Cocconcelli et al. (2003)
14 *Lactobacillus* strains from fermented dry sausages	Enterococcus faecalis L. lactis subsp. lactis Staphylococcus aureus of unstated origins	Seven out of 14 tetracycline-resistant *Lactobacillus* isolates were able to transfer this resistance *in vitro* to *Enterococcus faecalis*. Two of the strains could also transfer their resistance to *Lactococcus lactis* subsp. *lactis.* No conjugal transfer to a *Staphylococcus aureus* recipient was observed.	Gevers et al. (2003b)
241 *E. coli* from meat and meat products	E. coli DH5α	Resistance genes were successfully transferred from 38% of the isolates. The transfer was more frequent from resistant isolates harbouring resistance genes inside class 1 integrons. Transfer was plasmid mediated. Resistance transfer for tetracycline, streptomycin, sulphonamides, trimethoprim and chloramphenicol conferred by a variety of genes for each antibiotic was observed.	Sunde and Norstrom (2006)
Salmonella enterica subsp. *enterica* serovar	*E. coli* K12 of unstated origins	Transfer of *bla*-harbouring plasmid at 37^{o}C and 25^{o}C in all tested media. At 15^{o}C plasmid transfer and ampicillin resistance occurred only in ground meat media. No transfer in any media at 4^{o}C	Walsh et al. (2008)

AMR donor(s)	AMR recipient(s)	Significant findings	Reference
Typhimurium DT104 of unstated origins			
Enterococcus faecium from commercial fermented dry sausage	Enterococcus faecium clinical isolates	*In vitro* transfer of tetracycline resistance gene *tetM* from a commercial sausage isolate of *E. faecium* into clinical isolates of *E. faecium*. First evidence of class 1 integrons in *Enterococcus* isolated from food.	Jahan et al. (2015)
Lactobacillus sakei subsp. s*akei*, a fermented sausage starter	Listeria monocytogenes from meat	The effect of temperature (7ºC to 37ºC) and MAP (modified atmosphere packaging); air, 50% CO_2–50% N_2, and 100% N_2) on *tet*M gene transfer was studied. At high donor concentrations, transfer was observed at all temperatures and MAPs. At low donor concentrations, transfer was only observed under 100% N_2 atmosphere at 7ºC on the surface of cooked ham. Low temperature and modified atmosphere packaging, do not necessarily prevent plasmid transfer.	Van Meervenne et al. (2015)
Enterococcus faecium from commercial fermented dry sausage	Listeria monocytogenes L. innocua	*L. monocytogenes and L. innocua* strains were able to acquire *tetM* via *in vitro* mating with *E. faecium*. Basis of resistance in *L. innocua* was something other than *tetM* transfer. Basis of *tetM* transfer unreported.	Jahan and Holley (2016)
E. coli from a cooked pork sample	*E. coli* J53 (a mutant of K12, originally	ESBL encoding genes could be transferred by conjugation from one isolate of ESBL *E. coli* carrying $bla_{CTX\text{-}M\text{-}9}$ to *E. coli* J53.	Li et al. (2016)

AMR donor(s)	AMR recipient(s)	Significant findings	Reference
	isolated from human faeces)		
Listeria monocytogenes from raw meat	E. coli DH5α	A *bcr*ABC cassette and *cad*AC genes conferring resistance to QAC and cadmium were identified on a plasmid in a meat isolate of *L. monocytogenes.* The plasmid was transmissible and conferred acquired tolerance in *E. coli* DH5α both by chemical and natural transformation.	Xu et al. (2016)
E. coli from cooked meat products	*E. coli* J53 (a mutant of K12, originally isolated from human faeces)	Four varieties of plasmid-based class 1 integrons encoding various combinations of tetracycline, trimethoprim/sulfamethoxazole, streptomycin, ampicillin, ceftazidime, Gentamicin, chloramphenicol, nalidixic acid, cefepime, cefoperazone and ciprofloxacin resistance could be transferred to recipient *E. coli* J53 by conjugation and natural transformation.	Yu et al. (2016)
Proteus mirabilis	*E. coli* J53 (a mutant of K12, originally isolated from human faeces)	QAC resistance was conferred by *qac*H and other genes inside non-classic, class 1 integrons located on conjugative plasmids. The creation of QAC-resistant recipient transconjugants showed that the transfer of QAC and antimicrobial resistance *in vitro* was possible. Questionable mimicking of processing conditions.	Jiang et al. (2017)

AMR donor(s)	AMR recipient(s)	Significant findings	Reference
E. coli isolates (n=64) from RTE meat samples	*E. coli* J53 (a mutant of K12, originally isolated from human faeces)	ESBLs and/or PMQRs with QACs can be co-localized (but that's not always the case). Antibiotic resistance and QAC resistance co-transferred *in vitro* via plasmid from RTE meat isolate *E. coli* to recipient *E. coli*.	Li et al. (2017)
Twenty-two species from 15 genera including *Enterobacteriaceae, Pseudomonas* spp., *Vibrio* spp., Gram positive cocci and bacilli all isolated from cooked meat	*E. coli* J53 (a mutant of K12, originally isolated from human faeces)	All the donors were screened for mating with the recipient. The three resulting transconjugants carried the same *qnr* and *bla* resistance genes as their donors. The genes were located on a plasmid and within a class 1 integron. Class 2 integrons were present in the donor isolates but did not transfer to the recipient.	Yu et al. (2017)
Enterococcus faecium Enterococcus faecalis	E. faecalis	The donor strains were isolated from cooked hamburger/cheeseburger. An *E. faecalis* recipient was able to acquire tetracycline and erythromycin via *in vitro* filter mating. The transfer of resistance was by the genes *erm*B, *tet*M, *int* (hamburger) and *erm*B, *tet*M, *int*, *aac(6')-Ie-aph(2'')-Ia* (cheeseburger) and was plasmid based.	Chajecka-Wierzchowska et al. (2019)
Staphylococcus xylosus	S. xylosus	Transfer of plasmid-borne tetracycline resistance was demonstrated, both *in vitro* using filter matings and *in situ* during a pilot sausage	(Leroy et al. 2019)

AMR donor(s)	AMR recipient(s)	Significant findings	Reference
		fermentation. Transconjugant numbers were higher from the fermentation compared with the filter mating.	

Gevers et al. (2003b) assembled a panel of 14 tetracycline resistant *Lactobacillus* isolates previously obtained from fermented sausage samples collected at retail (Gevers et al. 2000). The panel of donor strains was selected as being isolates that naturally harboured R-plasmids containing a *tetM* open reading frame (ORF). Determination of the ability of the strains to transfer tetracycline resistance into *Enterococcus faecalis, Lactococcus lactis* subsp. *lactis* or *Staphylococcus aureus* was the stated aim of the paper. In summary, seven of the *Lactobacillus* isolates were able to transfer resistance *in vitro* to *Enterococcus faecalis* at frequencies ranging from 10^{-4} to 10^{-6} transconjugants/recipient using a filter mating protocol. Two of the seven strains could also transfer *tetM* resistance to *Lactococcus lactis* subsp. *lactis* at frequencies ranging from 10^{-5} to 10^{-7} transconjugants/recipient. There was no conjugal transfer of *tetM* to *Staphylococcus aureus*. The authors concluded that transfer to *Enterococcus* and *Lactococcus* could occur at high frequencies *in vitro* when there were high numbers of donor and recipient cells. The study also produced some potentially interesting results that were never further investigated, e.g. the presence of *tetM* was confirmed by southern blotting in the new transconjugants, but the *tetM* probe hybridised to a band that was a different size to the *tetM* on the plasmid in the donor strains in six of the newly created transconjugants. The *tetM* signal was the same size as the transconjugant chromosome, which may indicate either chromosomal incorporation or a change (i.e. increase) in plasmid size.

Cocconcelli et al. (2003) assessed conjugation in *Enterococcus* spp. in a fermented sausage matrix. The donor strains for tetracycline resistance were *E. faecalis* OG1rf, the origin of which was not described by either Cocconcelli et al. (2003), or the original isolation study (Dunny et al. 1978); and *E. faecalis* A256 which carried vancomycin resistance (*vanA*) on a plasmid and was originally isolated from a human patient with a urinary tract infection. The recipient strain was *E. faecalis* BF3098c, a raw milk cheese isolate. Transfer of tetracycline resistance from *E. faecalis* OG1rf cells to the recipient *E. faecalis* BF3098c was demonstrated, even in the absence of a selective tetracycline pressure, the transferred plasmid persisted in the food isolate recipient. A high frequency of transfer and extended AMR gene persistence was observed in the sausage matrix (10^{-3} recipients/donor) compared with a cheese matrix (10^{-6} recipients/donor) and mating on M17 agar (10^{-4} recipients/donor). *VanA* transfer frequency was similar to that observed for the tetracycline resistance transfer at around 10^{-3} recipients/donor. The main conclusion of Cocconcelli et al. (2003) was that significantly higher conjugation frequencies were observed for both resistance genes studied during the ripening of fermented sausages compared with other matrices, and this was the second study to report such a finding.

Sunde and Norstrom (2006) made use of a Norwegian national surveillance programme to assemble a large panel of 241 AMR *E. coli* isolated from meat and meat products. The AMR

resistance genes within the isolates were detected and identified by PCR using a limited range of 11 primer sets. AMR transfer was assessed by *in vitro* conjugation with the resistant isolates from meat as donor strains and *E. coli* DH5α as a recipient. There were extensive and valuable statistical analyses of transconjugants and the AMR genes that were transferred. Overall, plasmid transfer was observed from 38% of the donors and the primer sets used identified the resistance loci in approximately 2/3 of the strains. There were both expected and unexpected relationships demonstrated by the statistical analyses. Linkage between the detection of *strA-strB* genes (streptomycin resistance) and *sul2* (sulphonamide resistance) was expected since these resistance genes are known to co-reside on broad-host-range plasmids that are widely distributed among coliform bacteria (Sundin and Bender 1996). Of greater interest were the unexpected positive associations between *tetA* (tetracycline resistance) and the genetic elements *sul1* (sulphonamide resistance), *dfrA1* (a trimethoprim-insensitive dihydrofolate reductase), *aadA1* (streptomycin and spectinomycin resistance) and *int1* (class 1 integron integrase) that were found.

Collectively, the linkages were strongly indicative of an association between *tetA* and class 1 integrons, suggesting transfer of *tetA* might be integron mediated in significant numbers of meat isolates of *E. coli*. More generally, AMR transfer was reported as more frequent from resistant donors that harboured class 1 integron components. An association was also found between *tetB* and the *sul2* gene with the presence of one locus making it more likely that the other would be detected. There were similar positive associations for the *cat* gene (chloramphenicol resistance) and both the *sul2* and the *strA-strB* genes. The authors caution that although the association was significant, there were too few isolates positive for the *cat* gene for the finding to be reliable. Negative associations were found between resistance genes encoding for resistance to the same antimicrobial agent which was interpreted by Sunde and Norstrom (2006) as once one resistance loci to an antibiotic was acquired, there was no need to incur the metabolic overhead associated with an analogue. The main conclusions of Sunde and Norstrom (2006) were that plasmid transfer played a significant role in conferring new resistance phenotypes among the isolates investigated *in vitro*. The possibility of transferring resistance increased if the donor *E. coli* was multi-AMR and/or if the donor contained class 1 integron components. The conjugation experiment results suggest that *tetA* and class 1 integrons are commonly co-located on the same conjugative plasmids.

Walsh et al. (2008) also undertook *in vitro* studies to assess the conjugal transfer of bla_{TEM} (conferring ampicillin resistance) into *E. coli* K12 from *Salmonella* Typhimurium DT104 isolated from a pre-chilled, dressed beef carcass (Hutchison-Duffy, personal communication, 30/08/2019). Mating experiments were conducted in broth, milk and thawed, previously frozen, minced beef over a range of temperatures (Section 1.4.2.1). Ampicillin-resistance transfer was observed at

similar frequencies of 10^{-4} to 10^{-5} transconjugants/recipient in all of the assessed media at 25ºC and 37ºC when a mating duration of 18 h was allowed. However, ampicillin-resistance transfer frequencies in ground meat were lower than in broth or milk when the bacteria were exposed to a stress temperature of 15ºC. There were no significant differences among transfer frequencies in ground meat, broth or milk at near optimal" temperatures of 25ºC or 30ºC.

Jahan et al. (2015) and Jahan and Holley (2016) undertook related *in vitro* studies to determine if there was transfer of AMR genes from a donor strain of *Enterococcus faecium* originally isolated from fermented sausage. The two studies assessed transfer into clinical *Enterococcus* isolates (Jahan et al. 2015) and meat plant isolates of *Listeria monocytogenes* and *L. innocua* (Jahan and Holley 2016). *In vitro* transfer of *tetM* by plate mating was demonstrated for two out of the nine assessed strains of donor *Enterococcus*. Both AMR recipients of the *tetM* gene were isolated originally from human blood. The transfer frequencies were 1.1×10^{-6} and 2.1×10^{-5} transconjugants/donor. A short investigation was undertaken by Jahan et al. (2015) in an attempt determine the mechanism of the AMR transfer but, in brief, the results were not conclusive. However, the findings conclusively indicated that *tetM* was not transferred via plasmid. In addition, there was no integrase gene detected in the donor or recipient strains, making it unlikely the transfer was by the Tn916-1545 conjugative transposon; which is commonly associated with *tetM* transfer in the genus *Enterococcus* (Rizzotti et al. 2009). An adenylyltransferase gene (*aadA*) was detected by PCR in the donor strain and one of the recipient strains after mating, indicating that in at least one of the recipients a class 1 integron may have facilitated *tetM* transfer. Prior to the Jahan et al. (2015) study, the presence of class 1 integrons in *Enterococcus* was confined only to clinical isolates and this work presented the first evidence of integrons in food isolates of *Enterococcus*.

Related studies were undertaken by the same research group in which they assessed the possibility of *tetM* transfer into *Listeria* spp. using the same *E. faecium* donor (Jahan and Holley 2016). Resistance was successfully transferred to one *L. innocua* and one *L. monocytogenes* out of the nine isolates assessed with transfer frequencies of 6.3×10^{-8} and 3.8×10^{-8} transconjugants/donor respectively. For *L. monocytogenes*, *tetM* was transferred and no plasmids were present in the recipient strain (i.e. the transfer was not plasmid mediated). The tetracycline resistance basis was different for *L. innocua* (i.e. not *tet*M) compared with *L. monocytogenes* and the authors speculate that possibly *tetU* was the basis of the newly acquired resistance for *L. innocua*. However, the *tetU* gene was not tested for in the recipient *L. innocua* hence this, the second study by the Holley group, did not provide significant additional information on the mechanism of *tetM* AMR transfer from the donor strain.

Xu et al. (2016) investigated the transfer of a *bcrABC* cassette naturally contained on a plasmid in *L. monocytogenes* isolated from raw meat. The main focus of the paper was however characterisation of the plasmid and the genes contained within it and determination of its sequence. Plasmid curing demonstrated the role of the plasmid in conferring benzalkonium chloride (BC) and cadmium (Cd) tolerance in the *L. monocytogenes* strain. The *bcrABC* cassette and *cadAC* genes could be transferred to *E. coli* DH5α, by chemical and natural transformation. The transformed strains consequently acquired tolerance to BC and Cd. Although Xu et al. (2016) cite three papers as the source methodologies for the transformations, none of the papers provide details of how the chemical and natural transformations were achieved. However, one did cite another paper which gave rise to an extended and convoluted citation chain. It was apparent that the natural transformation was complex and involved. Xu et al. (2016) used biofilm cells, either grown on polystyrene balls or on glass rods in a chemostat. There were multiple rinses in media and buffers and plating was of cells liberated from sessile biofilms exposed to highly purified plasmid DNA and made planktonic by sonication. Although both Xu et al. (2016) and the original paper (Li et al. 2001) called the process natural transformation, it is difficult to see how some of the protocol stages could occur outside of a laboratory. Xu et al. (2016) did not report the efficiencies of the transformations.

Li et al. (2016) undertook studies that have limited relevance to this review. Transfer of selected chilled raw and cooked pork isolates (actual number not provided) carrying ESBL genes was studied by performing transfer experiments with *E. coli* J53. Li et al. (2016) work in the same department as Xu et al. (2016) and although there was no overlap in the authors, they use the same convoluted citation sequence for the natural transformation methodology. The $bla_{\text{CTX-M-9}}$ gene of one AMR *E. coli* isolate from cooked pork samples was observed to be transferred to *E. coli* J53 by the natural conjugation methodology that used microcolony biofilms described by Li et al. (2001).

Yu et al. (2016) isolated 75 *E. coli* from cooked meats at retail in China. The panel of strains was screened by PCR and 11 of the isolates (14.7%) were found to contain *intI1* (a class 1 integrase) and corresponding resistance cassettes consisting of dfrA17-aadA5 (n=7), dfrA1-aadA1 (n=2), dfrA12-orfF-aadA2 (n=1), and aacA4-catB8-aadA1 (n=1). The transfer frequencies for conjugation of the plasmids containing the integrons ranged from 10^{-6} to 10^{-4} resistant transconjugants/total recipients (NB a unit change from previous studies). An important result observed by Yu et al. (2016) was that extracellular, naked, integron-containing DNA and AMR resistance cassettes from the donors could transform an *E. coli* J53 recipient with a frequency of 10^{-7} to 10^{-5} transconjugants/recipient via *in vitro* transformation using naturally competent stationary-phase cells. The study authors considered that *E. coli* isolates from cooked meat products could acquire

environmental class 1 integrons facilitated by natural transformation, and that these isolates may potentially act as reservoirs of resistance genes or provide a mechanism for AMR access into the human food chain.

The study of Yu et al. (2017) described an extension of their previous studies published in 2016. In brief, 49 cooked meat samples were purchased at retail in China and 150 AMR isolates representing 22 species from 15 genera obtained. One or more of sixteen resistance loci; bla_{TEM-1} and $bla_{CTX-M-14}$ (β-lactams), *aac(3)-IIa* (Gentamicin), *qnrB* and *qnrS* (fluoroquinolones), *cat1* and *cat2* (chloramphenicol), *strA* and *strB* (streptomycin) *sul1*, *sul2*, and *sul3* (sulfamethoxazole), and *tetM*, *tetA*, *tetS*, and *tetB* (tetracycline), were detected in 54 of the isolates (36%) by PCR. Twelve isolates tested positive for *int1,* a class 1 integrase gene, and four isolates carried the class 2 integrase gene, which we believe is the first report of a class 2 integrase in a cooked meat isolate, although class 2 integrons have been reported in clinical *Proteus* isolates (Wei et al. 2013). The main focus of the study was the characterisation of prevalence and type of resistance genes and so only a limited amount of transfer experiments were undertaken. The donors were *Enterobacter cloacae* 11-1 with *qnrB* (quinolone) and $bla_{CTX-M-14}$ (β–lactam), *Proteus penneri* 7-2 with *qnrS* and bla_{TEM-1} and *Proteus mirabilis* 37-2 carrying an uncommon cassette array *dfrA32-ereA-aadA2* (trimethoprim, erythromycin, streptomycin). All three donors were able to pass their AMR genes to an *E. coli* J53 recipient using *in vitro* broth mating.

Li et al. (2017) isolated 64 AMR *E. coli* from 720 ready to eat meat products in China. Multidrug resistance was observed in 70.3% of the AMR isolates and all of them (100%) were resistant to benzalkonium chloride. Four analogue β-lactamase genes in 16 ESBL-producing *E. coli* were identified as bla_{SHV} (9.4%), bla_{TEM} (7.8%), $bla_{CTX-M-15}$ (1.6%), and $bla_{CTX-M-9}$ (1.6%). Plasmid-mediated quinolone resistance (PMQR) was present in nine isolates. Coexistence of ESBL and/or PMQR with QAC resistance was confirmed in 21 isolates (32.8%). The *aac(6')-Ib-cr* and $bla_{CTX-M-15}$ genes were observed to be co-transferred with *qacF* (quaternary ammonium resistance) by one isolate. Thus, this study indicates that it is possible for resistance to β-lactams and/or quinolones to be present on a plasmid along with resistance to QACs in *E. coli* isolates from RTE meat products. The study also provided evidence that these three sets of AMR genes can also be co-transferred.

Jiang et al. (2017) reported similar findings to Li et al. (2017) with *Proteus mirabilis* being detected in 29.2% of 178 retail cooked meat samples in China. The isolates MIC to benzalkonium chloride (BC) and antibiotics was determined, and the majority of strains had BC MICs of ≥24 mg/mL and were therefore considered resistant to QAC. PCR assays indicated that a variety of loci encoding multi-drug efflux pumps that could export intracellular QAC were common in the strains and these

were apparently the main mechanism of QAC resistance. A high proportion of the strains were also resistant to streptomycin and chloramphenicol. Five groups of integrons containing resistance gene cassettes were identified in 10 *intI*1-positive isolates, but the focus of the study was class 1 integrons containing the antibiotic resistance genes and *qacH* (a multi-drug [including QAC] efflux pump). Mating experiments created *E. coli* J53 transconjugants containing the integrons and resistance genes to streptomycin chloramphenicol and BC. The observation demonstrated that *qacH*-associated non-classic class 1 integrons were located on conjugative plasmids and therefore could facilitate the co-dissemination of disinfectant and antimicrobial resistance genes among bacteria. The transconjugants had increased resistance to streptomycin and chloramphenicol. However, although the transfer of *qacH* was confirmed by PCR the gene was apparently not expressed and the transconjugants did not show any change in their resistance to BC.

A second *in situ* study was undertaken using conditions indistinguishable from those used for a typical commercial fermentation process (Leroy et al. 2019). Three strains of *S. xylosus* resistant to tetracycline (*tetK*) were isolated from commercial fermented sausages. The *tetK* gene was plasmid based. Two different sets of experiments assessed the *in vitro* and *in situ* transfers of resistance between *S. xylosus* strains. Only one *S. xylosus* isolate was able to transfer tetracycline resistance to a recipient strain at a low frequency of $3.4x10^{-9}$ transconjugants/recipient using filter mating, i.e.*in vitro*. The three donor and recipient strains were tested in pilot-scale fermented sausage production, which used a realistic sausage meat composition and fermentation conditions. Transfer was also observed, at a rate of 1.4×10^{-7}; but only when a donor concentration of 10^8 cfu/g of meat was used. The authors concluded that plasmid-based *tetK* resistance was possible between strains of *S. xylosus*, but that the transfer frequencies were low. Nevertheless, since transfer was possible, the authors advise the use of AMR-free starter cultures as a hurdle to help prevent AMR genetic materials entering the human food chain.

There was an established body of evidence describing the *in vitro* AMR transfer on filters from and between LAB. However, Chajecka-Wierzchowska et al. (2019) reported an additional transfer that merits specific mention. Horizontal, conjugative, transfer of AMR located in *Enterococcus*, originally isolated from a range of ready-to-eat foods including cooked hamburger and cheeseburger in Poland, was observed. The *tetM* resistance gene was co-transferred with an *int* gene, which the authors considered as evidence that a transposon (Tn916/Tn1545) was involved in one of the transfer mechanisms and that more than one mechanism was operating (Chajecka-Wierzchowska et al. 2019). One of the two *E. faecium* isolates from cooked hamburger and the single isolate of *E. faecalis* from cheeseburger transferred their AMR to a donor *E. faecalis* at the frequencies described in Table 13.

Table 13 AMR loci and transfer frequencies for Enterococcus isolated from cooked minced beef at retail in Poland (Chajecka-Wierzchowska et al. 2019)

Donor strain	Source	AMR genes and transfer frequency (transconjugants/donor)	Resistance loci
E. faecium	Cooked hamburger	Tetracycline 3.5×10^{-6} Erythromycin: 6.2×10^{-7}	ermB, tetM, int
E. faecalis	Cooked cheeseburger	Chloramphenicol 1.7×10^{-6} Tetracycline 8.2×10^{-7} Erythromycin 2.9×10^{-7}	ermB, tetM, int, aac(6′)-Ie-aph(2″)-Ia

Thumu and Halami (2019) also undertook important studies relating to AMR donation, using two *Lactobacillus salivarius* strains and a *Lactobacillus reuteri* isolated from chilled chicken meat. *In vitro* filter matings were used to initially determine if the donors could transfer AMR to an *Enterococcus* recipient. *In situ* conjugations in fermenting sausage were assessed using a range of potential human pathogens as recipients. The paper is exceptionally novel because it also involved feeding an *Enterococcus* recipient and the chicken isolates to a rat, as an animal model, and assessing the plasmid transfer of AMR in rat faeces i.e. the study also assessed transfer during passage through a mammalian gut. The AMR transferred were plasmid borne erythromycin (*ermB*) and tetracycline (one or more of *tetL*, *tetW*, *tetM*, dependent on strain), with the resistance loci in adjacent locations, irrespective of plasmid.

For the *in-vitro* model, filter matings were undertaken. The two *L. saliatius* strains co-transferred erythromycin and tetracycline resistance to the *E. faecalis* recipient at frequencies of 1×10^{-4} and 3.8×10^{-3} recipients/donor and the *L. reuteri* transferred *in vitro* at 2×10^{-3} recipients/donor cell respectively. The findings add to an already robust weight of evidence that conjugative transfer of AMR can occur *in vitro* in SPMMP.

The *in situ* studies were undertaken in minced □-irradiated chicken meat. A potential criticism of the study is that the meat was confirmed as sterile prior to fermentation, which is not typical of a commercial process. However, the fermentation conditions and duration (27d) were typical of a commercial process. A range of recipients, some of which were potential human pathogens were used. The donors included *L. monocytogenes*, *Yersinia enterocolitica*, *Bacillus cereus*, *S. aureus*,

Micrococcus luteus, E. faecalis and *E. coli*. In brief, the initial growth kinetics examined showed that the chicken sausage food model supported the growth of both the donor and the recipient isolates. However, although the fermenting meat was sterile at the commencement of the experiment and despite an initial rise, the numbers of potential recipients declined in the food system as the fermentation proceeded. After 7 days of fermentation only *L. monocytogenes* and *Y. enterocolitica* could be isolated from the partly fermented sausage, along with the donor strains. Transconjugation frequencies *in situ* were in the range 10^{-6} to 10^{-9} with no discernible pattern as to what donor or recipient was most effective for transconjugation.

The *in vivo* studies involved rats that were inoculated with an *E. faecalis* recipient, which was shed in stable numbers for the duration of the experiment. After 7 days, either of the two donor *L. salivarius* or *L. reuteri* were fed to the rats. Two days after donor introduction, there were detectable numbers of AMR *E. faecalis* transconjugants in the animal faeces, typically between 10^2 to 10^3 cfu/g manure.

In summary, there was a significant body of information in the form of 18 peer-reviewed papers that supported the supposition that plasmid-mediated transfer of AMR from SPMMP isolates is possible. It was not uncommon for the transferred plasmids to contain integrons and the AMR genes to be contained within an integron cassette. Natural transformation was reported in two papers. No papers were identified that unequivocally described a phage-mediated transfer. The majority of demonstrations of transfer were *in vitro* under laboratory conditions, typically using matings on nitrocellulose filters. Some of the model systems made at least some effort at simulating relevant environmental conditions, such as placing filters on a cut ham surface (Van Meervenne et al. 2015). The use of microbiological growth media (Li et al. 2017) shows little, if any, consideration of processing conditions. There were four papers where the use of a transfer matrix derived from a SPMMP enhanced transconjugation effectiveness. The majority of the paper authors acknowledge the aim of their studies was show a proof of concept that transconjugative transfer was possible. There were very few papers where the mating conditions could be considered to be a good model for processing conditions. Two *in situ* papers described transfer under commercially relevant sausage dry fermentations. A third also used relevant fermentation conditions but used irradiated meat so there was no competition with indigenous microflora. There is evidence that both inter- and intra-species AMR transfer is possible (Table 12), although not all SPMMP isolates are capable of transferring plasmids to all recipients. There was insufficient evidence to unequivocally state the reasons for any inability to transconjugate plasmids between strains. However, the identified literature supported that some recipients appeared to be incompatible with some donors because some donors transferred plasmids but not to all assessed recipients. There was one paper where AMR gene transfer occurred but there was no change in

the recipient resistance to QAC. The transfer of the gene was confirmed, but the reasons for unchanged resistance were not fully investigated. Thus, it is possible that physically transferred loci might not always be expressed in the new host.

8.2.1 In summary:

In vivo transfer of resistance genes can occur in at least some SPMMP under commercial processing conditions. There is evidence in particular that transconjugative transfer of AMR could occur during a sausage fermentation of around 4 weeks with high concentrations of starter cultures (10^8 cfu/g). Currently, there is insufficient information in the literature to make definitive statements regarding how frequently such AMR transfers occur.

8.2.2 Factors influencing AMR transfer

8.2.2.1 Temperature

There were four papers identified that considered the effect of temperature on the transfer of AMR between bacteria using strains or environments of relevance to SPMMP. Jayaratne et al. (1987a) assessed R-plasmid transfer from three *E. coli* isolated from beef, chicken and turkey respectively into an *E. coli* K12 recipient. The plasmids were naturally present in the meat isolates and conferred resistance to tetracycline, streptomycin (all strains), and kanamycin and neomycin (turkey isolate). The media used to assess any transfer of relevance to this review were ground beef and summer sausage. The transfer of AMR was assessed over a range of temperatures. A potential criticism of the study is that different incubation times were used for different temperatures, with the two highest temperatures having the shortest mating times (6 h). Statistical analyses of the results made no consideration of the different mating times. In addition to any influence of mating temperature, the effect of the indigenous microbiota was assessed by comparing transfer in heat-sterilised and untreated meat-derived matrices. Transfer of AMR was observed from all three of the meat isolate *E. coli*, with the frequencies of R-plasmid transfer declining with decreasing temperatures. A typical transfer frequency was 7.5×10^{-3} transconjugants/donor at 30°C, which fell to 1.4×10^{-5} transconjugants/donor at 10°C. There was no detectable transfer of any R-plasmids at 5°C after 24 h mating in any of the meat-derived substrates assessed by the study. Thus, there is some evidence that the effective refrigeration required by law in the UK for meat and meat products (EU regulation 853/2004) may additionally provide a hurdle that helps restrict AMR dissemination between different bacteria. In general, there were higher numbers of resistant transconjugants in the sterilised matrices compared with the untreated matrices containing a background microflora, with significant differences for some of the treatment comparisons. The authors speculated that the sterilised environments allowed faster

growth of the organisms under study because there was no competition for nutrients with the indigenous microflora.

A second study by Jayaratne et al. (1987b) extended their initial observations whilst using the same three AMR *E. coli* strains isolated from beef, chicken and turkey meats as their previous study (Jayaratne et al. 1987a), plus an additional six poultry meat AMR *E. coli* isolates, to assess R-plasmid transfer into two potentially human pathogenic *S.* Typhimurium strains. The R-plasmids contained within the donor strains conferred resistance to tetracycline, streptomycin, kanamycin and neomycin. No explanation was provided for the additional AMR acquisition of the original beef and chicken isolates. This second study again used an *in vitro* model to measure transfer frequencies and the impact of several environmental factors, including temperature. The model was less relevant for the current review compared with the first study (Jayaratne et al. 1987a) because transfer was assessed in nutrient broth rather than meat matrices. Four *E. coli* donor strains transferred R-plasmids containing resistance genes to an *E. coli* K12 711 recipient. Two of the *E. coli* donors also transferred R-plasmids to the two *S.* Typhimurium recipients. As before, temperature influenced the plasmid transfer frequencies, with the highest transfers observed at 37^{o}C. Transformation effectiveness declined with decreasing temperature. Higher temperature transfer frequencies were similar for *E. coli* and *Salmonella* recipients and in the order 5.0 x 10^{-6} transconjugants/donor. In general agreement with the previous study, lower temperature matings (10^{o}C) either did not transfer R-plasmids or had fewer effective transfers.

Walsh et al. (2008) investigated transfer of plasmid borne *bla* from *S.* Typhimurium DT104 into *E. coli* K12 using methodology broadly equivalent to that of Jayaratne et al. (1987a). The mating experiments most relevant to this review were conducted in minced beef. A range of incubation temperatures; 4, 15, 25 and 37^{o}C, were assessed at mating times of 18 or 36 h. Transfer of the *bla* gene was assessed by the acquisition of ampicillin resistance by the donor *E. coli*. Transfer was observed in ground beef at 25 and 37^{o}C and at these higher temperatures, frequencies were typically 10^{-4}-10^{-5}. Compared with the other matrices, transfer was more effective in ground beef, which was also the only matrix where transfer was observed at 15^{o}C, with an observed frequency of approximately 10^{-6} transconjugants/donor. At 4^{o}C, transfer was not observed in ground beef or any other matrix. Thus, the findings of Walsh et al. (2008) are in broad agreement with the findings of Jayaratne et al (1987a, 1987b) whereby at lower temperatures the transfer of plasmids harbouring AMR is less frequent. At refrigeration temperatures no transfer was measurable, even after an extended mating period of 36h. Walsh et al. (2008) discussed the reasons for transfer occurring in the minced beef matrix at 15^{o}C, but not the other two matrices assessed, concluding that it was not yet clear which aspects of the meat matrix are important to facilitate gene transfer. Hirt et al. (2002) reported that the presence of blood plasma in the transfer matrix environment can

elevate transconjugation frequencies to 10^{-2} transconjugants/donor, which are amongst the highest frequencies that have ever been observed. Enhanced aggregation is a virulence factor for *Enterococcus faecalis* causing nosocomial infections in patients with cardiac issues and blood plasma can upregulate genes that cause enhanced aggregation of *E. faecalis*, thereby increasing transconjugation effectiveness for the transfer of tetracycline resistance *in vitro* (Hirt et al. 2002).

Van Meervenne et al. (2015) also assessed AMR transfer and the influence of temperature using *Lactobacillus sakei* as the donor and *L. monocytogenes* as the recipient. Temperatures in the range 7-37°C were assessed and the AMR marker was plasmid borne *tetM*. Transfer was studied using filter matings on either a yeast extract agar or slices of cut ham. Two cell densities were studied: high-density (10^7 cfu/ml donors and 10^8 cfu/ml potential recipients) or low-density (5.27 x 10^2 cfu/ml donors and 4.60 x10^2 cfu/ml potential recipients).

- When using high densities of cells on yeast extract, plasmid transfer was observed from 10°C to 37°C when an overnight mating was undertaken. Typical transfer frequencies were 2.0 x 10^{-6} transconjugants/donor. Using an overnight mating, plasmid transfer was not observed at 7°C, and at 10°C only one of the four replicates showed a single transconjugant colony on selective agar.
- When high densities of cells were mated and the filters placed on cut ham at 7°C for either 5 or 10 days, *tetM* was transferred. The number of transconjugants was typically around 5.0 x 10^2 cfu/ml after 5 days (transfer frequency 5.0 x 10^{-5} transconjugants/donor) and this increased to 9.3 x 10^2 cfu/ml after 10 days (transfer frequency 9.3 x 10^{-5} transconjugants/donor). The finding was important because it showed that under conditions that were a good model of ham packed in air, there was plasmid transfer after an extended incubation period. In the UK, major retailers allow a shelf life of 2-3 days (including the day of slicing) for pre-cooked meats sliced in store (Hutchison et al. 2014). However, the shelf life of pre-packed cooked sliced meat (CSM) can exceed the timings assessed by Van Meervenne et al. (2015) and is set largely to prevent *L. monocytogenes* numbers from exceeding legally permitted limits in ready-to-eat foods (EU regulation 2073/2005) and without consideration of AMR transfer.
- When low density numbers of cells were mated in air on cut ham at 7°C, no transconjugants were observed for both 5- and 10-day incubations.

As previously noted in the other sections of this report, there is a lack of information that makes the provision of robust summaries backed by robust scientific evidence difficult. The results for the four identified studies all broadly agree that temperature can be an effective hurdle that reduces or prevents the exchange of plasmid borne genetic material that confers AMR between bacteria.

However, the following caveats apply. Firstly, the papers only cover donations by *E. coli*, *Lactobacillus* and *Salmonella*. Whether any inhibitory effect of refrigeration temperatures extends into other bacteria is unknown. We note that some of the natural microbiota of secondary processed meats such as *L. monocytogenes* and some *Lactobacillus* are psychrotrophic and with these organisms there was some evidence for low temperature plasmid exchange for high numbers of donor and recipient cells, following an extended mating period. Whether similar exchanges occur between enteric bacteria is not clear currently. An additional caveat is that the studies identified were all laboratory-based models of real-life conditions. Some of the studies used commercial bacterial growth media, thereby demonstrating proof of concept, but such studies may not be an effective model of real-world processing conditions.

It should also be kept in mind that, aside from one paper where the mechanism of AMR exchange was not clearly determined, the remaining identified literature related only to plasmid exchange. Although it is the most commonly reported method for AMR dissemination, other mechanisms exist (Figure 1 An overview of the horizontal gene transfer processes operating on bacteria during food processing that create AMR bacterial strains. Adapted from Verraes et al. (2013)) and we did not identify any information that would allow comment to be made on any influence of temperature for any of the other dissemination mechanisms.

Given that the law already states meat and meat products should be refrigerated to ≤4°C or ≤7°C (depending on species and product), our conclusion from this section of the review is:

8.2.3 In summary

Effective refrigeration of meat and meat products is a legal requirement in the UK. Although the intent is to limit the growth of human pathogens and spoilage organisms, thereby maintaining safety and extending product shelf life; there is information in the scientific literature that shows that effective chilling has additional merit *in vitro* because low temperatures can reduce or prevent the spread of AMR genes in SPMMP based on plasmid exchange from some bacterial donors to some recipients.

8.2.3.1 Other factors

Jayaratne et al. (1987b) assessed the effects of pH, salt concentration and atmosphere composition on R-plasmid transfer from strains of *E. coli* isolated from meat into a recipient *E. coli* K12.

- The influence of [NaCl] in combination with temperature was investigated. As noted in section 1.4.2.1 there was a significant decrease in plasmid transfer when the temperature

was reduced from 37°C to 10°C. The same observation was reproduced in a different isolate, which also showed a significant decrease in plasmid transfer as the salt concentration was increased in 0.5% increments from 0.5% to 3.0% (w/v). However, the effect salt concentration appeared to be strain specific with *E. coli* ECH 21 showing a statistically significant increase in plasmid transfer frequency with an increasing NaCl concentration. Jayaratne et al. (1987b) concluded that the influence of NaCl concentration on R-plasmid transfer was variable and strain specific.

- Conjugation was assessed under aerobic and anaerobic conditions for three donor strains with plasmid exchange being observed under both sets of conditions. The influence of atmosphere was again strain specific, with one donor strain having an increased transformation frequency under anaerobic conditions, one having a decreased efficiency and the other strain showing no significant difference.
- The compared strains exchanged plasmids differently at different pH and temperatures. The frequencies of plasmid transfer were highest at 37°C and declined with decreasing temperatures. Culture of donor strains at 48°C caused plasmid curing. Two of the nine *E. coli* assessed were however still able to donate plasmids at 10°C. The highest observed transfer frequencies of around 3.5×10^{-1} occurred in the pH range 6.5 to 7.0. A general conclusion based on the study results was that enteric temperatures and neutral pH were optimal for conjugation.

Van Meervenne et al. (2015) assessed the impact of modified atmosphere packing (MAP) on plasmid transfer from a *Lactobacillus sakei* donor to a *L. monocytogenes* recipient at 7°C on filters placed on cut ham for extended mating periods of 5 days or 10 days. The MAPs assessed were air, 50% CO_2/50% N_2 and 100% N_2.

- When 10^7 cfu/ml donors and 10^8 cfu/ml potential recipients were mated, transconjugants were measured as being approximately 5.0×10^2 cfu/ml (transfer frequency 5.0×10^{-5}) with no statistically significant differences between the different MAPs after 5 days. The authors noted there was a small decrease in the numbers of *L. monocytogenes* recipients over the duration of the mating for all three MAPs. There were no results reported for the 10 days mating.
- When a lower density of cells (5.27×10^2 cfu/ml donors and 4.60×10^2 cfu/ml potential recipients) was mated on filters placed on cut ham, no transconjugants were detected for any MAP after 5 days. After 10 days, there were no transconjugants for the air or 50% CO_2/50% N_2 treatments. For the 100% N_2 atmosphere, two single transconjugants were detected on two plates (transfer frequency 1.0×10^{-5}). Over the duration of the matings, the

numbers of untransformed *L. monocytogenes* recipients had increased to 10^3-10^4 cfu/ml (cells were counted in washings from filters) for all the MAPs. The donor strain had increased to 10^4 to 10^6 cfu/ml after 5 days and reached a density of 10^7 to 10^8 cfu/ml after 10 days.

The authors concluded that the risk of plasmid transfer increased with increasing temperature, which underscored the importance of preserving the cold chain. The authors also noted that transfer was only observed under densities that exceeded the EU food safety criteria (EU regulation 2073/2005) for *L. monocytogenes*, noting that if these limits were respected by good process hygiene and good manufacturing practices, the chance of AMR transfer would be minimised, although still possible. MAP for products such as CSM is a complex issue, with a variety of gas mixes commonly employed for retailing SPMMP in the UK (Hutchison et al. 2014). Studying the effects of MAP is further complicated by the fact that the gas composition changes over the shelf life duration, which is a function of bacterial metabolism inside the pack and the fact that some of the packing films used are designed to be permeable to some of the gases in the original packed atmosphere (Arvanitoyannis and Stratakos 2012).

8.3 AMR and the processing environment

8.3.1 *Listeria monocytogenes* persistence and quaternary ammonium-based sanitisers

There were 25 research papers identified that described some aspect of AMR in the processing environment. A large proportion of the identified papers reported some aspect of the survival of *Listeria* spp. after exposure to quaternary ammonium compounds that are commonly used for the sanitation of cleaned food contact surfaces in meat processing plants (Hutchison et al. 2014; Cherifi et al. 2018). An important issue with *L. monocytogenes* is its ability to colonise processing environments and remain *in situ*, contaminating product, for extended periods. Gelbíčová et al. (2018) reported that a strain of *L. monocytogenes* that caused a foodborne disease outbreak probably persisted in a plant manufacturing turkey CSM for at least four years, a length of time that is not exceptional for plant resident *L. monocytogenes* strains (Tompkin 2002; Heir et al. 2004; Ferreira et al. 2014; Rodríguez-López et al. 2018).

L. monocytogenes persistence can be associated with QAC resistance (Aase et al. 2000; Cherifi et al. 2018), although that is not universally the case (Lourenço et al. 2009; Cherifi et al. 2018). Some authors report that there are niches in processing plants that are colonised by *L. monocytogenes*, which do not get full exposure to QAC (Zhang et al. 2018) and that a sublethal exposure of bacteria in general can increase intrinsic QAC resistance (Sundheim et al. 1998; Gantzhorn et al. 2014). A review by Chapman (2003) also summarised evidence that biofilms make a contribution to disinfectant resistance because they provide a potential mechanism

whereby microorganisms are only exposed to a sub-lethal concentration of sanitising compounds, but this assertion was not fully supported by later studies (Lourenço et al. 2009). There are however, classic locations often described as “nooks and crannies” that can harbour *L. monocytogenes*; e.g. behind blade guards on slicer equipment, the threads of screws, damaged conveyor surfaces and cracked concrete (Tompkin 2002; Hutchison et al. 2014). Such locations can all persistently harbour *L. monocytogenes*, and hollow conveyor rollers, ball bearing assemblies and floor drains have also been reported as particularly problematic (Tompkin 2002).

The purpose of this section of the review was to investigate and summarise the identified evidence regarding the relative importance of mobilizable loci conferring resistance to QAC, increases in intrinsic (chromosomal) resistance as a consequence of sublethal exposure to QAC, and any relationship between biofilms and AMR (section 1.8). The primary focus was dictated by the predominant subject discussed in the relevant literature, which was *L. monocytogenes* and QAC; although other organisms and sanitisers are discussed below, where appropriate literature was identified. A summary of the identified papers relating to *L. monocytogenes* is provided as Table 14. It has been established that genes conferring resistance to QAC can be plasmid borne and transferred from resistant *L. monocytogenes* isolated from foods of animal origin to other bacteria (Xu et al. 2016).

A brief summary of published literature on QAC tolerance in *L. monocytogenes* is that there are main two resistance genes, which are named *bcrABC* and *qacH*. *bcrABC* is an ABC-style efflux pump that is commonly harboured on an 80kb mobilizable plasmid called pLM80 and less commonly is chromosomally integrated (Dutta et al. 2013). The pLM80 plasmid was first characterised in clinical isolates of *L. monocytogenes* from an outbreak caused by contaminated hot dogs in the USA in the late 1990s (Nelson et al. 2004; Elhanafi et al. 2010). *qacH* also codes for a multidrug exporter, a member of the drug/metabolite transporter (DMT) class of membrane proteins and a homologue of the same gene originally described in *Staphylococcus* spp. (Xu et al. 2014). There were some issues of concern with the genetic aspects of some studies. For example, transposon Tn6188 has been fully sequenced (Müller et al. 2013) and reported to contain *qacH*. However, some studies report the presence of the transposon rather than confirming the presence of *qacH* (Ortiz et al. 2016; Zuber et al. 2019). Two additional efflux pumps, *mdrL* and *lde* have also been implicated in induced QAC resistance in some food-related *L. monocytogenes* strains by Romanova et al. (2006).

Table 14 A summary of the identified papers relating to L. monocytogenes and QAC sanitiser resistance in commercial processing plants.

Sanitiser chemistry	Study conclusion	Reference
1. QAC 2. Alkali chlorine 3. Alkali hydroxide	Plasmid-mediated resistance to commercial disinfectants was not the basis of persistent strains in a poultry processing environment.	Earnshaw and Lawrence (1998)
QAC	QAC-resistant meat or meat plant isolates (n=20) harboured the *bcrABC* gene cluster. *bcrABC* was not detected amongst QAC-susceptible *L. monocytogenes* isolates. The *bcrABC* sequences were highly conserved across the majority of the resistant strains. *brcABC* was most commonly carried on a large plasmid, pLM80, which also contained *qacH* as part of transposon Tn6188. There was however, at least one instance of *bcrABC* integration into the *L. monocytogenes* chromosome.	Dutta et al. (2013)
QAC	*L. monocytogenes* isolates were obtained in Switzerland (n=142) from different food matrices and production environments, including meat-associated samples. Strains were characterised for genotype and phenotype. QAC resistance was detected and genetic determinants *qacH* and *bcrABC* were detected in 80% and 12% of the strains, respectively. Most of the strains isolated were poor biofilm formers. There were no correlations between strain serotype, genotype and biofilm quantity.	Ebner et al. (2015)

Sanitiser chemistry	Study conclusion	Reference
QAC	Five persistent QAC resistant *L. monocytogenes* strains were isolated from a newly commissioned plant after disinfection. Four *L. monocytogenes* isolates contained transposon Tn6188 and *brcABC*. Tn6188 includes *qacH*. The remaining strain lacked both the transposon and *brcABC*.	Ortiz et al. (2016)
QAC	A *bcrABC* cassette located on a plasmid was sequenced and characterized. Transferring the plasmid to a QAC sensitive *E. coli* conferred QAC resistance, confirming the efflux pump played a role in plasmid-mediated tolerance to BC in *L. monocytogenes*	Xu et al. (2016)
QAC	At [QAC] below 13μg/ml, *L. monocytogenes* isolates with *qacH* or *bcrABC* were not more tolerant to QAC in bactericidal tests in suspension or in biofilms compared with isolates lacking the genes. At ≥14 μg/ml strains of *L. monocytogenes* not containing *bcrABC* or *qacH*, showed inhibition, compared to strains with these genes.	Moretro et al. (2017)
QAC	Significant correlation of QAC tolerance with plasmid borne *bcrABC.* Increased sensitivity to QAC observed after plasmid curing. Expression of *bcrABC* genes was QAC-induced. No *qacH* detected in 77 meat plant environmental strains, *mdrL* and *lde* detected in all of these strains.	Minarovicova et al. (2018)

Sanitiser chemistry	Study conclusion	Reference
QAC	19/25 persistent *L. monocytogenes* strains in a porcine slaughterhouse harboured *bcrABC* genes on a mobilizable plasmid. The presence of QAC at 3.125μg/ml inhibited *L. monocytogenes* biofilm formation *in vitro*.	Cherifi et al. (2018)
QAC	Studying isolates from small scale meat processing plants, 0/20 *L. monocytogenes* strains assessed carried *bcrABC*, whereas 6/20 *L. monocytogenes* strains carried Tn6188	Zuber et al. (2019)
QAC	106/124 strains of *L. monocytogenes* from a deli meat plant were resistant to 10μg/ml QAC. 20 strains were subjected to WGS, 12/20 contained *bcrABC*, 4/20 contained *qacH,* none contained *emrE*.	Stoller et al. (2019)

There were insufficient papers identified to determine whether *bcrABC* was more commonly reported than *qacH*, with conflicting reports in the small numbers of papers identified. For example, Zuber et al. (2019) report 0/20 strains assessed contained *bcrABC*, whereas 6/20 contained Tn6188 (and hence, presumably *qacH*). Ebner et al. (2015) reported 80% of 142 strains from Swiss foods and processing plants contained *qacH* compared with only 12% of strains harbouring *bcrABC*. In contrast, Minarovicova et al. (2018) reported no *qacH* detected in 77 meat plant environmental *L. monocytogenes* strains but *bcrABC* was detected in 17/18 *L. monocytogenes* serotype IIa plant environment isolates examined. There was insufficient information identified to undertake statistical analyses to determine if there was any correlation between *L. monocytogenes* serotypes and resistance genes, because not all studies undertook classic serotyping of their isolates, rather PFGE was used to differentiate strains. Based on a lack of PCR products from the pLM80 plasmid and *bcrABC* amplicons, Dutta et al. (2013) believed they had isolated *L. monocytogenes* with chromosomal insertions for *bcrABC*. All of the suspected chromosomal insertions were from serotype 1/2a or 3a isolates. There was, however, no other evidence that serotype might influence AMR genes or their location in the genome in *L. monocytogenes*.

Both Heir et al. (2004) and Ebner et al. (2015) similarly reported that they found no correlations between QAC resistance and PFGE profile/serotype or QAC resistance and persistence.

The presence of the *qacH* analogue, *emrE* was rarely determined in any of the identified papers although Stoller et al. (2019) reported that *emrE* was not present in any of the 20-deli meat plant environment isolates they characterised. Two efflux pumps (*mdrL* and *lde*) were described as contributing to induced QAC resistance in some food-related *L. monocytogenes* strains by Romanova et al. (2006), who observed increased *mdrL* expression but no change to the regulation of *lde* on exposure to QAC. Two additional papers were identified that considered *mdrL* in the context of *L. monocytogenes* and SPMMP products or processing environments. Moretro et al. (2017) report 27/293 *L. monocytogenes* meat plant environment strains contained *mdrL*, whereas Minarovicova et al. (2018) reported detecting *mdrL*, and also *lde*, in 77/77 *L. monocytogenes* isolates.

It is important to make clear that although increased resistance has been observed towards compounds such as QAC, *L. monocytogenes* where no AMR loci were identified have been reported to survive exposures up to 3μg/ml (Cherifi et al. 2018) and up to 12μg/ml (Moretro et al. 2017) respectively. *L. monocytogenes* harbouring efflux pumps could survive [QAC] ≥13μg/ml, but were unable to survive a 100μg/ml *in vitro* exposure (Moretro et al. 2017). Martínez-Suárez et al. (2016) assembled the reported QAC MICs for *L. monocytogenes* from the literature (Table 15) and concluded it was rare for *L. monocytogenes* to withstand a QAC exposure exceeding 16μg/ml. QAC effectiveness is, in part, dependent on the length of a hydrophobic, acyl tail. The lowest concentration of a typical C14-C18 tailed commercial QAC application would be of the order of 200μg/ml (Frank and Chmielewski 1997; Hutchison et al. 2014), although some sanitisers are used at up to 1000μg/ml (Martínez-Suárez et al. 2016) and so *full exposure* to a manufacturer-recommended dose of QAC would be more than sufficient to eliminate planktonic *L. monocytogenes* populations, including all those strains reported as being resistant to QAC and containing AMR loci (Rodríguez-López et al. 2018).

8.3.2 *L. monocytogenes* biofilms and sanitisers

Six papers were identified that reported some aspect of biofilm growth in AMR *L. monocytogenes* related to SPMMP, which was considered insufficient for robust analysis (Table 15). However, there was a larger body of information related to food processing generally. Much of it focussed on *L. monocytogenes* and the manufacture of cold smoked fish, presumably because cold smoking lacks a process critical control point (CCP). Non-AMR strains on cold smoked fish were considered outside of the scope of this review and have been reviewed previously (FSA project FS425012).

Table 15 Changes in the MICs to quaternary ammonium sanitisers reported for susceptible and resistant strains of planktonic *L. monocytogenes* isolated from SPMMP. *denotes isolations from a disinfected surface before the commencement of processing. Adapted from Martínez-Suárez et al. (2016).

No. isolates tested	Number of resistant isolates (% of total)	MIC of susceptible isolates (μg/ml)	MIC of resistant isolates (μg/ml)	Processing plant type (country/ies)	Reference
132	12 (9%)	2-8	16	Poultry (France)	Lemaitre et al. (1998)
19	5 (26%)	<2	4-7	Beef (Canada, USA)	Romanova et al. (2002)
112	17 (15%)	2.0-3.0	4.0-8.0	Beef (Norway)	Heir et al. (2004)
123	57 (46%)	≤10.0	>10.0	Beef (Turkey, USA)	Mullapudi et al. (2008)
29	3(10%)	≤2.5	≥10.0	Pork (Spain)	Ortiz et al. (2014)
14*	11 (79%)	≤2.5	≥10.0	Pork (Spain)	Ortiz et al. (2016)

In general, *L. monocytogenes* is exposed to a range of sanitising chemicals in food processing areas, sometimes at concentrations below those recommended by manufacturers (Allen et al. 2016). Allen et al. (2016) also considered that sublethal exposures may last for long periods, are frequently repeated, and potentially a route that may promote increased resistance over time to quaternary ammonium and phenolic based sanitizers along with the promotion of plant residency

and persistence. However, the issue is not clear cut. In general, sanitiser resistance does not correlate with the ability of *L. monocytogenes* strains to persist in a processing plant (Ferreira et al. 2011). Carpentier and Cerf (2011) asserted that *L. monocytogenes* persistence is primarily a random accident because there have been no loci identified within *L. monocytogenes* that confer persistence, but niches exist in food industry premises and equipment where *L. monocytogenes* persist (Wang et al. 2015). Having comprehensively reviewed a large body of pertinent literature, Martínez-Suárez et al. (2016) concluded that local environmental conditions in plants create persistent niches fortuitously by blocking full exposure to disinfectants. One of the blocking mechanisms can be microorganisms forming biofilms that restrict disinfectant exposure. Repeated imperfect cleaning, which leaves organic residues, can also interfere with effective sanitation (Martínez-Suárez et al. 2016). A number of papers reported that sanitiser resistance created by biofilm growth should be considered a form of transient resistance induced by a physiological adaptation to a biofilm lifestyle that is lost when cells leave the biofilm and revert to being planktonic organisms (Pan et al. 2006; Bridier et al. 2011).

Pan et al. (2006) evaluated resistance to peroxide and QAC sanitisers by *L. monocytogenes* growing as a biofilm under *in vitro* conditions, designed to mimic a food processing environment. Biofilms were created on small stainless steel or Teflon tiles using a five-strain cocktail of *L. monocytogenes*. Three of the five strains were isolated from hot dog and turkey processing plants. The biofilms were subjected to repeated daily treatments comprising of a 60 s sublethal exposure to a sanitiser, storage of the biofilms without nutrients or water for 15 h and incubation of the biofilms in diluted growth medium for 8 h. The cycles were repeated daily for three weeks to mimic the conditions and stresses experienced by *L. monocytogenes* in a natural processing environment. Sessile *L. monocytogenes* numbers reduced over the course of the first week before the biofilm adapted and populations increased despite the repeated exposures to peroxide, chlorine or QAC. This study is particularly notable because it established that planktonic cells derived from biofilms with induced sanitiser resistance did not have any increased tolerance compared with the pre-biofilm planktonic cells. The results suggest that the apparently induced resistance of the treated biofilms to sanitisers may be due to extracellular polymers in the biofilm and not any altered regulation of resistance genes within the cells of the biofilm (Pan et al. 2006).

Whilst Adriao et al. (2008) reported differences in biofilm formation of non-meat isolate *L. monocytogenes* of unknown AMR status, dependent on the adhesion surface, we did not find similar papers relating to AMR *L. monocytogenes* from SPMMP environments. It also should be noted that several studies have shown a range of attachment capabilities when attempting to find correlation between attachment and persistence. However, the majority of papers related to *L. monocytogenes* isolates of unknown AMR status (Borucki et al. 2003). There is certainly evidence

that culture media might have an influence on attachment and biofilm density when using *in vitro* models of commercial processing conditions, which might confound any correlations (Carpentier and Cerf 2011). For example, some authors reported persistent strains were better at achieving higher sessile cell densities after growth in TSBYE (Borucki et al. 2003) or conversely that persistent and transient *L. monocytogenes* did not significantly differ in cell densities after growth in a chemically defined broth (Djordjevic et al. 2002).

One potential criticism of studies such as Pan et al. (2006) is that their model used a cocktail of *L. monocytogenes* to form a biofilm. In contrast, Kalmokoff et al. (2001) reported that *L. monocytogenes* is not particularly adept at forming biofilms, whilst Giaouris et al. (2015) suggested that an improved biofilm model would be one composed of mixed species, as happens in commercial processing. Mixed biofilm community members are involved in competitive, cooperative or neutral intracellular interactions. Norwood and Gilmour (2001) reported that mixed species biofilms contained reduced numbers of *L. monocytogenes* cells, compared to monocultures. Similarly, Rodríguez-López et al. (2018) explored the interactions between ten different accompanying strains and *L. monocytogenes* when forming dual-species biofilms. They observed a deleterious effect of several accompanying strains on the *L. monocytogenes* populations in the biofilms in 4 out of the 10 different combinations studied. However, there are papers that concluded the opposite and demonstrated that multi species companion strains increased the numbers of *L. monocytogenes* in a mixed biofilm (Bremer et al. 2001; Carpentier and Chassaing 2004). In summary, the current literature highlights that there are issues with the setup of model systems, the consequences of which are difficult to predict. These include the impact of growth media on cell density and the variable outcomes when species mixtures are used to create artificial biofilms. It is difficult to draw any firm conclusions until there have been sufficient additional studies undertaken to define appropriate culture media, strain mixtures and conditions that can create a mimic for a natural biofilm so that further studies on the effective removal of such biofilms can be undertaken. One barrier to those studies might be a perception that they are not required because *in vitro* studies have demonstrated QAC resistance in *L. monocytogenes* would have to increase by one order of magnitude before QAC was unable to sanitise *L. monocytogenes*. However, the majority of studies relating to *L. monocytogenes* have been undertaken in a laboratory, which might not be an ideal mimic of commercial conditions. Gantzhorn et al. (2014)isolated MDR *Salmonella* in six different commercial pork processing plants in Denmark, immediately after standard cleaning and disinfection using a variety of different acids and chlorides. The surfaces sampled included meat trays which do not appear to be especially challenging to sanitise effectively. The fact that Gantzhorn et al. (2014) also recovered

viable *L. monocytogenes* after disinfection raises some questions about the effectiveness of the sanitisers when used in processing environments.

Cherifi et al. (2018) compared 30 *L. monocytogenes* genomes and showed that the persistent strains contained a *bcrABC* efflux pump that mediated QAC resistance. Although Cherifi et al. (2018) claimed a relationship between *bcrABC* and persistence in the slaughterhouse environment, the claim is probably premature because it is based on a small number of strains. However, it is of note that Wang et al. (2015) report similar findings from environmental *L. monocytogenes* isolates from a delicatessen. Of greater interest potentially, is their observation that BC-resistant strains produced lower amounts of biofilm in the presence of sublethal concentrations of QAC, which is the first report of QAC reducing biofilm mass. The explanation might be as simple as the inhibition of growth in the early stages of biofilm formation. Cherifi et al. (2018) acknowledged that there might not be sufficient numbers of isolations to draw firm conclusions and advise further work "using a greater number of unrelated persistent and non-persistent strains should be conducted in different conditions … which mimic a slaughterhouse environment."

Zhang et al. (2018) reported that *L. monocytogenes* strain SZ08 (serovar 1/2b) isolated from a raw meat processing environment was persistent and harboured a 25kb plasmid, designated as pLMSZ08. In the wild type strain, biofilm formation was significantly higher at 30^{o}C compared to 20^{o}C. Biofilm formation by SZ08*, a strain cured of pLMSZ08, reduced significantly compared with wild type SZ08 after a 48hr incubation. Investigation revealed that the transcription levels of biofilm-associated genes *prfA* and *argB* located on the *L. monocytogenes* chromosome were significantly elevated after plasmid curing. This observation suggests that the plasmid may carry some other key factors that can down-regulate biofilm-associated gene expression and influence biofilm formation (Zhang et al. 2018).

A study by Stoller et al. (2019) attempted to circumvent some of the issues outlined above. The general approach of their study was to identify a meat processing plant that harboured persistent *L. monocytogenes* then to use the plant as an ongoing source of persistent *L. monocytogenes* for characterisation. Over a period of four years, 124 strains of *L. monocytogenes* were collected. Four of the strains were repeatedly isolated and presumed plant residents, persisting for at least four years. The study undertook serotyping, MLST and WGS of isolates. Of relevance to this review, the strains' ability to form biofilms and their resistance to QAC disinfectants and peracetic acid (PAA) was assessed. At 22^{o}C, all of the persistent strains showed biofilm formation comparable with a known high biofilm forming control strain. Biofilm growth was curtailed at 8^{o}C, even for the high biofilm control strain. Three persistent strains contained the *brcABC* or *qacH*, but

QAC resistance above 20 μg/ml was not observed. 106/124 *L. monocytogenes* strains could tolerate 10 μg/ml QAC. For PAA, minimal bactericidal concentrations of 1.2–1.6% with a 20 min exposure were required and *in vitro* MICs between 0.1 and 0.2% PAA were observed. The bactericidal concentrations required for some strains exceeded the 0.5–1% manufacturer recommended concentration, suggesting that PAA might no longer be effective at controlling *L. monocytogenes* in meat processing plants.

8.3.2.1 Prophages

A number of papers note that the genome of *L. monocytogenes* commonly contains prophage sequences. Hurley et al. (2019) undertook WGS of 100 *L. monocytogenes* processing environment isolates and reported that 62% of their strains contained QAC resistance genes, and 73% contained prophage sequences, but there was no evidence uncovered by this review that suggested phages had any significant role in the dissemination of AMR genes in *L. monocytogenes*. However, there is some evidence that *L. monocytogenes* prophages may contribute to plant persistence. Prophage insertions within *comK*, a gene which is involved in cell competence and DNA uptake, has previously been implicated as important for biofilm formation, persistence in food processing facilities, and virulence (Verghese et al. 2011)

8.3.3 In summary:

- QAC breakpoints for resistant *L. monocytogenes* continue to increase slowly (Aase et al. 2000; Romanova et al. 2002; Romanova et al. 2006).
- There is evidence that repeated exposure to sublethal concentrations of QAC sanitiser increases tolerance and resistance in some strains (Heir et al. 2004; Ortiz et al. 2014; Ortiz et al. 2016).
- Full exposure to a commercial-strength QAC-based sanitiser can be effective at removing *L. monocytogenes* under *in vitro* conditions, even when the *L. monocytogenes* contains AMR genes that help protect against QAC (Martínez-Suárez et al. 2016; Cherifi et al. 2018; Stoller et al. 2019).
- *L. monocytogenes* can persistently colonise processing environments and *L. monocytogenes* has been isolated immediately after cleaning and QAC disinfection (Ortiz et al. 2014; Ortiz et al. 2016). There are papers that report the ability to persist is correlated with biofilm formation (Borucki et al. 2003; Moretro et al. 2017) and/or AMR genes encoding efflux pumps conferring protection against QAC sanitisers (Aase et al. 2000; Martínez-Suárez et al. 2016). However, there are also reports of no correlations between an ability to form biofilms and the presence genes encoding efflux pumps and persistence (Heir et al. 2004; Ferreira et al. 2014).

- There is a single novel report that plasmid born loci can control the expression of chromosomally-located genes that control biofilm formation in *L. monocytogenes* (Zhang et al. 2018).
- Some of the contradictory reports are likely to be the result of methodological issues. The strains used, how they are grown, and the presence of additional species have all been reported to impact on biofilms used to assess *in vitro* sanitiser effectiveness and strain persistence. There would be benefit in undertaking work that creates standardised strains and conditions that are a good model for natural biofilms so that the relative roles of AMR and biofilms can be quantified in terms of strain, persistence and effective sanitation.
- There are a small number of papers that suggest PAA might no longer effectively remove some strains of *L. monocytogenes* (Stoller et al. 2019) or *Salmonella* (Gantzhorn et al. 2014) at the manufacturer-recommended concentrations from meat processing environments.

8.3.4 Sanitiser resistance in bacteria other than *L. monocytogenes*

8.3.4.1 Lactic acid bacteria

Lactic acid bacteria (LAB) used for the fermentation of foods mainly comprise *Lactococcus*, *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* (Sidhu et al. 2001). Early investigations by Sidhu et al. (2001) looked at QAC resistance in lactic acid bacteria. The viewpoint of the study was that imperfect processing plant environmental disinfection could impact on product shelf life because LAB can cause spoilage of some products. Just as QAC is used for the effective control of *L. monocytogenes*, it is also used also to expunge plant environment LAB. Overall, 320 LAB isolates from food industry and meat were screened for resistance to QAC. Five strains (1.5%) were considered to be QAC resistant (≥30μg/ml QAC) and 56 (17.5%) were assessed as QAC tolerant (≥10μg and <30μg/ml QAC), with the remainder of strains (81%) assessed as sensitive (<10 μg/ml QAC). It should be noted that the resistant strains were isolated from food processing equipment after normal cleaning and disinfection in commercial processing plants hence showing that LAB can survive an apparently adequate QAC treatment. Sidhu et al. (2001) did not investigate LAB survival subsequent to QAC treatment but did look for any correlation between QAC resistance and AMR. There was little cross-resistance (Section 3) observed for QAC resistance and that to any of a wide range of antibiotics assessed. However, there was potential evidence for QAC cross-resistance with Gentamicin and chlorhexidine, which are commonly used as skin sanitiser ingredients (Waldow et al. 2018). Although there was no in-depth investigation of survival after QAC treatment, a significant conclusion from the Sidhu study

was that it was comparatively easy for QAC-tolerant strains to adapt to higher levels of BC and achieve resistance, compared with QAC sensitive strains.

Messi et al. (2006) investigated the spread of vancomycin-resistant *Enterococcus* (VRE) in isolates from 59 meat samples and 119 processing plant environmental isolates. Glycopeptide resistance was conferred by *vanA* in around 1% of the isolates, *vanB* in around 15% of the isolates, and 11% of strains harboured *vanC*. This study was noteworthy because it established that all *vanA* genes were plasmid borne. Messi et al. (2006) concluded that plant environments, as well as food, are reservoirs of resistance determinants and there was a need for molecular studies to evaluate the mobility of glycopeptide AMR genes.

Aslam et al. (2010) collected swab samples from conveyers used for moving chilled carcasses prior to the start of daily processing operations (CC, 52 samples), with further samples being collected 2 h after the commencement of operations (DC, 52 samples). Ground beef (GB, 40 samples) prepared in the plant was also sampled for AMR. Of relevance to this review, 25% (n=13) of the CC samples contained AMR-resistant bacteria. *E. faecalis* (87.03%) was the most common species isolated followed by *E. faecium* (10.6%). The study also provided further evidence that routine cleaning and sanitation might not completely expunge LAB from processing environments. Isolations doubled for the DC samples indicating meat might be a source of AMR LAB (Aslam et al. 2010). No investigation was undertaken to explain why 25% of pre-processing conveyor surface samples yielded AMR *Enterococcus*. Unfortunately, the sanitiser used was not named, no correspondence email was included with the paper, and both the first and corresponding authors have moved on from Guelph, where the work was undertaken; hence no further details could be obtained.

Rizzotti et al. (2016) investigated nine strains of *Enterococcus faecalis* and 12 strains of *Enterococcus faecium*, isolated from different pork-related samples which had been characterised previously for the presence of AMR genes and were examined for phenotypic tolerance to seven biocides (chlorhexidine, QAC, triclosan, sodium hypochlorite, 2-propanol, formaldehyde and hydrogen peroxide). The presence of efflux systems encoded by the genes *qacA*/*B*, *qacC*, *qacE*, *qacED1* and *emeA* were tested for. Most strains were sensitive to the disinfectants and no strong correlations were found between sanitiser and antibiotic resistance. One *E. faecalis* strain was assessed as tolerant to triclosan and another *E. faecium* strain had higher tolerance to chlorhexidine than the other strains tested, explained by harbouring the *qacA*/*B* loci. The study conclusion was that phenotypic resistance to antibiotics was common in enterococcal isolates from the pork chain, but phenotypic tolerance to sanitisers was infrequent.

8.3.5 In summary:

There was evidence that some plant environmental LABs were resistant to routine commercial disinfectants. Although *in vitro*-determined resistance in some LABs was as low as 30μg/ml QAC, strains were able to survive in-plant treatment of >200□g/ml by currently uninvestigated mechanisms. One study concluded that in LAB phenotypic tolerance to disinfectants was relatively infrequent compared to antibiotics.

8.3.5.1 Miscellaneous bacteria including those with potential to be human enteric pathogens

Heir et al. (1995) made one of the earliest reports of meat-associated *Staphylococcus* that were resistant to QAC and found that the presence of the plasmid named pST827 was the basis of the resistance. An open reading frame on the plasmid coded for a gene product with a high degree of homology to *qacC*, an emergent issue at the time with clinical *Staphylococcus* strains. A strain bank of 191 food and meat plant isolates was screened for genes in the *qac* family and 25 were assessed as resistant to QAC. Five of these resistant strains did not hybridise to probes specific for *qacA-C* causing Heir et al. (1995) to speculate there might be alternative, unknown, resistance loci and concluded that QAC resistance was likely to be widespread within *Staphylococcus* and hence an emergent issue for food processing.

Fouladkhah et al. (2013) created biofilms using four-strain mixtures of *E. coli* O157:H7, six-strain mixtures of *E. coli* O26, O45, O103, O111, O121, and O145 and either two strain mixes of MDR *Salmonella* Newport or three of *S.* Typhimurium. The descriptions of the original isolate sources were incomplete, with the papers cited as the strain sources containing only the names of the researchers that supplied the strains. It was apparent that at least some of the strains were isolated from cattle manure. The study formed biofilms from the four different mixtures separately on small pieces of stainless steel under laboratory conditions. The target biofilm densities were 2 cfu/cm^2 after 7 days. Three growth temperatures were studied and, in general, larger quantities of biofilms were generated at the two higher temperatures, 15ºC and 25ºC, compared with 4ºC; the latter being an adequate refrigeration temperature for foods. Similar temperature effects have been reported previously for *E. coli* biofilms (Ryu et al. 2004). At seven days, the biofilms were exposed to QAC, and or acid-based (AB) sanitiser and after sanitation, there were larger numbers of survivors observed after growth at 15ºC and 25ºC compared with the 4ºC growth. There were no significant differences between the different pathogen mixtures. The QAC treatments were able to reduce one week mature biofilm to less than the detection limit following incubation at 4ºC, while the same treatment against biofilms formed at 25ºC left behind over 100 pathogenic cells/cm^2 on the surface of stainless steel. Fouladkhah et al. (2013) concluded there was poor efficiency for the peroxyacetic-based sanitizer for inactivation of one-week mature biofilm, at the

highest manufacturers recommended concentration. QAC, especially at the higher temperatures of biofilm formation, was assessed as better but not capable of the complete sanitation of the surfaces when also tested at the manufacturer's maximum recommended concentration for general disinfection.

After a limited review of the literature, Gantzhorn et al. (2014) noted that biocide resistant strains have been proposed as having a higher risk of developing antibiotic resistance hence they examined the prevalence of biocide resistant *Salmonella* before and after cleaning and sanitation in Danish pig slaughterhouses and evaluated if there was any correlation between resistance to biocides and antibiotics. Gantzhorn et al. (2014) also explored if cleaning and sanitation changed susceptibility toward biocides or antibiotics by determining *Salmonella* susceptibility to triclosan, a QAC sanitiser and an acid disinfectant. They reported no resistance towards the biocides tested but noted that the isolates obtained after cleaning and disinfection had higher MICs towards the acid-based sanitiser, which could indicate the selection of strains with higher tolerances due to cleaning and sanitation. No evidence was found to support the hypothesis that biocide resistance might correlate with increased risk of antibiotic resistance.

Wang et al. (2016) investigated *E. coli* O157:H7 isolated from beef plants that were experiencing atypically high product contaminations. The investigation focussed on the genetic basis for any biofilm-forming ability and atypically elevated sanitiser resistance. The results showed that compared with *E. coli* O157:H7 isolated during periods of normal contamination, the high product contamination strains had a significantly higher biofilm-forming ability on contact surfaces and an increased tolerance to common commercial disinfectants. However, there were no differences in the presence of disinfectant resistance genes or AMR genes between the high and usual product contamination strains, leading the authors to conclude that biofilm formation itself was protective against sanitisers. One observed difference between the high and usual product contamination strains was significantly higher copy numbers of a plasmid named pO157 in the high contamination strains. Further, there was a positive correlation between plasmid copy number, biofilm formation and sanitiser tolerance and high survival and recovery capability after sanitation. Wang et al. (2016) determined that the QAC resistance genes (*emrE*, *mdfA*, *sugE(c)* and *ydgE*/*ydgF*) were present on the O157 chromosome but did not find any plasmid-based resistance loci. pO157 was not further characterised as part of the study and so it is unknown if the plasmid is a second example [in terms of order discussed in this review; we note Wang et al. (2016) precedes (Zhang et al. 2018)] of chromosomal genes being highjacked and their expression controlled by a plasmid.

Jiang et al. (2017) isolated 52 strains of *Proteus mirabilis,* an opportunistic human pathogen (Drzewiecka 2016), from 178 samples of cooked meat, purchased at retail. MICs to QAC ranged from 4–32 μg/ml, with a breakpoint of 24μg/ml most commonly observed. PCR was used to screen for ten QAC resistance genes in the isolates with the *mdfA* gene being found in all of the isolates, *ydgE*/*ydgF* in 90.4% and *qacE* in 53.8%. Five other QAC resistance genes were identified as was the presence of an integrase in some strains. The Jiang study is notable because one strain contained a 100kb conjugative plasmid that contained the *intI1* integrase and *qacH* inside the integron cassette. The plasmid was shown to be mobilisable and transferred QAC resistance from *P. mirabilis* to *E. coli* (Jiang et al. 2017) and could therefore constitute an effective method for the co-dissemination of AMR and disinfectant resistance genes.

Wong et al. (2018) studied the molecular responses induced in *Vibrio parahaemolyticus* when exposed to peracetic acid-based sanitiser. The results implicated the *katE* family of genes in the protection mechanisms that are activated against acid sanitisers in this *Vibrio* and this provides additional evidence that acid-based sanitisers may not be reliably effective against environmental meat processing plant isolates.

8.3.5.2 In summary

- There is a growing body of evidence that cooler temperatures reduce or inhibit biofilm growth in multiple bacterial species (Fouladkhah et al. 2013; Zhang et al. 2018; Stoller et al. 2019)
- QAC may not be able to effectively sanitise *E. coli* and *Salmonella* biofilms, especially at cooler (4°C) temperatures. There is some evidence that higher temperatures result in better disinfection.
- However, in general; although QAC can effectively control environmental bacteria when there is full exposure, a number of authors report acid-based sanitisers are not effective at removing contamination from processing surfaces.
- It remains unclear whether biofilms help curtail sanitiser exposure, with some papers supporting protection and some claiming no protective effect.
- Resistance to QAC conferred by *qacH* in a class one integron cassette on a plasmid in *P. mirabilis* has been shown in one paper to be transferable to *E. coli* by transconjugation.

8.3.6 The processing plant as a source of contamination for SPMMP

A number of papers have concluded that processing environments can be a source of contamination for SPMMP, particularly for *L. monocytogenes* (Chmielewski and Frank 2003; Endrikat et al. 2010; Hoelzer et al. 2012; Chaitiemwong et al. 2014; Martin et al. 2014). The classic

approach is to undertake typing of plant environmental isolates and product isolates and to use matching of these types as evidence for a cross contamination event (Rodríguez-López et al. 2018). However, whilst the approach is commonplace, the direction of transfer is not always clear (Hudson et al. 2017). Whilst environment-to-product seems most likely in the majority of cases, product-to-environment can seldom be completely discounted. A summary of the small number of identified papers that reported some aspect of cross contamination in a processing plant environment is shown as Table 16.

Table 16 A summary of papers that have reported AMR bacteria in processing environments that may have contaminated products.

AMR bacteria	Product processed	Source(s)	Significant findings	Reference
Enterococcus spp.	Minced meat	Air, surfaces, water	No important difference in resistance profiles from plant environmental isolates compared with minced meat isolates processed in the same plant (direction of cross contamination was not clear).	Messi et al. (2006)
Enterococcus spp.	Chilled raw beef	Product conveyor belts	AMR environmental detections increased two-fold at 2h compared with the start of processing, which may be evidence of product contaminating the environment.	Aslam et al. (2010)
Salmonella enterica	Beef jerky	Drying rack	Same serovar detected in product at drying as was on the drying rack (direction of cross contamination was not clear).	Fernandes et al. (2017)

The intended outcome for this section of the report was to determine if the processing environment can act as a contamination source for AMR bacteria isolated from final SPMMP. As a strategy to circumvent any uncertainty of transfer direction for contamination, our intended approach was to collate the papers describing persistent processing plant AMR strains, either in the context of a foodborne disease outbreak, or as explanation for repeated isolations of indistinguishable bacteria from different batches of final product across extended periods of time.

There is a large body of robust information that shows persistent plant environment strains of undetermined AMR status are the main contamination source for finished products. These reports are typified by comprehensive investigations over several years of the type undertaken by Ortiz et al. (2010) and Gelbíčová et al. (2018). There was no information identified that related to antibiotic or sanitiser resistance, persistence in processing environments and extended, repeated contaminations of final products or outbreaks. There was one paper describing a single AMR persistent environmental strain with a corresponding AMR strain in SPMMP products (Ortiz et al. 2016). How widespread an issue AMR persistent environmental strains are in SPMMP is not known and represents a large gap in the literature. Levy and Marshall (2004) report that there may be an increased metabolic overhead for the operation and preservation of some resistance genes. If such cells are not exposed to an AM, they can struggle to compete with non-AMR counterparts because they expend some of their metabolic outputs on a resistance mechanism that does not confer benefit in the absence of an AM. Under such circumstances, the percentage of a population with the AMR phenotype will diminish over time. For that reason, it may not be appropriate to assume that what has been established for SPMMP contaminants of unknown AMR status is the same for contaminants with an AMR phenotype.

8.3.7 In summary:

We consider it may be prudent to undertake studies that clarify whether persistently colonised processing environments can act as a contamination source for AMR bacteria isolated from final SPMMP

8.4 Novel ABC transporter genes

During the appraisal of this report, one of the reviewers expressed concern that any influence of secondary processing on relatively recently identified novel ABC transporter genes such as *optrA*, *cfr* and *optA* may have been overlooked. Our original targeted searches did not identify any significant information regarding these specific loci. However, in order to make sure we had fully addressed the perceived shortcoming, we undertook a custom search of the WoS database in July 2020, specifically targeting the loci listed above. This search identified 54 papers, with nine being

considered of possible relevance to this study after initial screening. The text below is included to specifically address the review comment.

Cavaco et al. (2017) reported there were three *Enterococcus faecalis* isolates resistant to linezolid from chicken carcass samples collected at retail in 2010-11. The original, historic examination of the sample failed to detect AMR strains. However, in 2016, a reanalysis of the original WGS and SNP sequence data was undertaken, that considered the reporting of the novel resistance gene *optrA*. The *optrA* gene regions of the three plasmids contained within the resistant strains showed high similarity to the originally reported *optrA*-carrying plasmid pE349. The study established a low-level contamination of *E. faecalis* harbouring the novel ABC transporter *optrA* in chicken meat in Columbia.

Choi and Choi (2017) also investigated ABC transporters in *E. faecalis* in meats sampled at retail in Seoul, Korea. The study did not report any evidence for the *optrA*, *cfr* or *optA* loci in any of the isolates obtained. Elghaieb et al. (2019) undertook general surveillance of *E. faecalis* from faecal, wastewater, milk and meat samples in Tunisia and reported isolations from chicken meat and beef, although the product types were not specifically listed, hence the secondary processing that the samples had undergone could not be determined. The exact numbers of isolates obtained were challenging to determine because they were grouped by species rather than sample type, and so numbers of faecal and meat isolates were combined. Nineteen bovine meat samples were examined, one ovine meat sample and nine galliform (chicken) meat samples. AMR determinations of isolates used the EUCAST protocols (v 19, Jan2019). Overall, 287 samples were collected and *optrA* was detected in four samples but most of the detections were in faecal or wastewater samples, with only a single meat sample (an unspecified chicken meat product) yielding *optrA*.

The single *optrA*-harbouring chicken product sample reported originally Elghaieb et al. (2019) was further studied by Elghaieb et al. (2020) who obtained four isolates from the sample in which *optrA* was located in a transferable, chromosomal transposon, Tn6674. As part of the strain characterisations, *in vitro* assessments of *optrA* stability were undertaken. The AMR gene was stably preserved in the *E. faecalis* populations for several hundred generations, without selection.

Kim et al. (2019) also characterised historical surveillance isolates from chicken meat sampled at retail, but in Korea. The surveillance scheme was different to that reported by Choi and Choi (2017), above. AMR phenotypes were determined using the CLSI protocols (2013) and PCR characterisation of chloramphenicol resistant strains for a range of loci including *optrA* and *cfr* was undertaken. Four chloramphenicol resistant strains were identified out of the 345 *Enterococcus*

isolates obtained from chicken meat. All four chloramphenicol-resistant isolates carried phenicol efflux pumps; one isolate harboured *cfr* only, one harboured both *cfr* and *fexA*, and two isolates harboured both *fexA* and *optrA*. The *tndX* gene, which is transposon-associated, was not detected in any chloramphenicol-resistant *Enterococcus* isolate.

Osman et al. (2016a) undertook surveillance for *cfr* in *Staphylococcus* isolated from ready to eat chicken samples in Cairo, Egypt. Fifty chickens were sampled and examined, yielding 50 *Staphylococcus* isolates. A PCR-based methodology was used to screen for the *cfr* gene but none of the isolates carried it. A subsequent study by Osman et al. (2016b) collected 100 beef samples at retail, which were also examined for AMR *Staphylococcus*. There were 27 isolates, nine of which were *S. aureus*. Only two of the nine *S. aureus* harboured *cfr*, which was not detected in any of the other isolates.

Pokharel et al. (2016) investigated the fate of AMR *Salmonella* Typhimurium in vacuum massaged sirloin strip steaks. However, the AMR *Salmonella* was a cultured type strain, resistant to rifampicin, due to a point mutation that can be induced in *Salmonella*. The strain was cultured, inoculated onto the steaks, which were then vacuum massaged and survival determined. This study is notable, because vacuum massage is not well described in the literature and the authors observed survival of what is technically AMR *Salmonella* after exposure to a strong vacuum during brine massage.

A total of 220 samples of ready-to-eat foods were collected in tourist areas in India by Rana et al. (2020). Forty five of the samples were meat related and tested for AMR *Bacillus cereus*, which was detected in chicken momo (2/25 samples; 8%), mutton momo (1/15 samples; 6.7%), with no detections from chicken soup (0/15; 0%). All of the foods contained multiple ingredients and so it was not certain the meat was the contamination source. Overall, high levels (> 50%) of antimicrobial resistance were recorded for penicillin, amoxicillin, ampicillin, cefixime and ceftazidime in the *B. cereus* isolates but no information relating to *optrA* and *cfr* was determined by the study. The manner in which the information was presented by the study was of limited use to this review.

8.4.1 In summary:

Based on the papers identified by this review, the *optrA*, *cfr* and *optA* subgroups of ABC transporters do not appear to be widespread in SPMMP. Typically, the literature reports these AMR loci to be present only in a low (single digit) percentage of samples and multiple-species isolates. A single paper reported that *optrA* located in transposon Tn6674 persisted in a population without selection for hundreds of generations. However, it may be prudent to begin monitoring to

detect any changes in the prevalence of these loci, particularly in enterococci isolated from chicken meat, where the majority of authors have focussed their efforts.

8.5 Comparisons showing an effect for secondary processes on AMR populations

Work in this area can be classified into two main categories. The first is the effect of a secondary processing stage on the populations of AMR bacteria, ideally compared with the numbers before the processing stage. In addition, there were a very small number of papers that focussed on reduced resistance caused plasmid-mediated genes being lost by mechanisms such as plasmid curing. A summary of the identified literature is provided as Table 17.

Table 17 A summary of the effects of secondary processing stages on AMR bacteria in SPMMP

AMR Organism/group	Product	Secondary process	Pre treatment	Post-treatment	Treatment effect	Reference
E. coli	Liquid from freeze-thawed chicken	Freeze-thaw	Not determined	$1x10^2$ - $5x10^2$ cfu/ml liquid	Freezing does not kill all AMR *E. coli*	Caudry and Stanisich (1979)
Enterobacter cloacae	Comminuted beef	20 cycles of freeze-thaw	$8x10^6$ cfu/g	$5x10^5$ cfu/g	>1 log population reduction. No change to plasmid stability.	Jayaratne et al. (1990a)
LAB	Fermented dry sausages	Principally fermentation	TetS and tetM in Lactococcus, Lactobacillus, Streptococcus, Enterococcus	TetM in Lactobacillus,	Fermentation changed the LAB composition with *Lactococcus*, *Streptococcus*, and *Enterococcus* harbouring *tetS* being undetectable leaving only *Lactobacillus* harbouring *tetM*	(Gevers et al. 2003c)
C. coli	Skinless chicken breast meat	10 days refrigeration	7.3% detections	2.4% detections	Reduction in AMR bacterial detections	

	Skin on chicken thigh meat	10 days refrigeration	16.2% detections	2.7% detections		(Casagrande Proietti et al. 2018)
C. jejuni	Skinless chicken breast meat	10 days refrigeration	10.8 % detections	0.0% detections		
	Skin on chicken thigh meat	10 days refrigeration	46.3 % detections	36.6% detections		
Thermotolerant coliforms of unassessed AMR status	Chilled beef	Slicing	1.7 log cfu/g	2.4 log cfu/g	<1 log population increase	(Fernandes et al. 2017)
		Marinating	2.4 log cfu/g	3.0 log cfu/g	<1 log population increase	
		Curing	3.0 log cfu/g	2.9 log cfu/g	No significant change	
		Drying	2.9 log cfu/g	1.7 log cfu/g	<1 log population decrease	

8.5.1 The effect of secondary process stages on AMR populations or prevalence of AMR bacteria

8.5.1.1 Freezing

Early work undertaken by Caudry and Stanisich (1979) showed that AMR *E. coli* resistant to heavy metals, or at least one from a range of eight tested antibiotics, could be isolated from the liquid created by thawing frozen chicken carcasses. Although the work was basic, the finding was valuable because it indicated that freezing did not kill AMR *E. coli*.

Jayaratne et al. (1990a) used a historical AMR meat isolate of *Enterobacter cloacae* for laboratory-based investigations of the effects of repeated freeze-thawing. The strain carried resistance to kanamycin, conferred by a plasmid; pRPJ24. The study aimed to assess the effects of repeated freeze-thawing on the numbers of AMR *E. cloacae* and the stability of the plasmid and was undertaken using *in vitro* conditions of *E. cloacae* cultured in Luria broth containing raw minced beef. Four rounds of freeze-thaw caused a slight reduction in *E. cloacae* numbers from $8x10^6$ cfu/g to $4x10^6$ cfu/g. After 20 rounds of freeze-thaw, populations had declined to $5x10^5$ cfu/g. Plasmid stability was unaffected by the repeated freeze-thaw procedures. After the freeze thawing cycles had been completed, the minced beef was stored at 4°C. In contrast to the indigenous microflora in the meat which increased, the number of viable *E. cloacae* cells containing the pRPJ24 plasmid declined significantly during 14 days of refrigerated storage, post-freeze-thaw, again indicating the benefits of appropriate chilled storage to controlling AMR.

8.5.1.2 Refrigeration

Casagrande Proietti et al. (2018) enumerated AMR campylobacters in portioned chicken meat at end of slaughter (T1) and after 10 days of refrigerated storage at 4°C (T2). The campylobacter AMR profiles were different between different batches of chicken portions, however, there was a high proportion of campylobacters resistant to streptomycin, ciprofloxacin and tetracycline throughout the study. Casagrande Proietti et al. (2018) observed that the skin-on thigh samples had higher numbers and detections for campylobacters compared with skinless breast meat. There was a global reduction of *Campylobacter* spp. in both thigh and breast samples at T2 ($P <$

0.001), showing that chilling was able to reduce the AMR *Campylobacter* counts. Unfortunately, no comparisons of AMR resistance profiles before and after storage were reported.

8.5.1.3 Other processing stages

Gevers et al. (2003c) reported the prevalence and diversity of lactic acid bacteria, along a single processing line used for the manufacture of two different types of fermented dry sausages. The focus of the study was tetracycline resistance and how any tetracycline resistance genes present changed during processing. Samples were collected from the raw meat, the sausage batter and the fermented end product and were examined by a culture-based methodology, with PCR detection of tet^R genes. The diversity of tet^R LAB reduced along the processing line. There was a higher diversity of LAB genera in raw meat, which contained *Lactococcus*, *Lactobacillus, Streptococcus*, and *Enterococcus*, whilst only *Lactobacillus* spp. were present after fermentation. Furthermore, both the *tetM* and *tetS* genes were present in isolates from the raw meat, whereas only *tetM* was present after the fermentation. However, it should be noted that *tetS* was not present in *Lactobacillus* only in *Lactococcus*, *Streptococcus*, and *Enterococcus*, and only *Lactobacillus* spp. could be isolated after fermentation.

Fernandes et al. (2017) reported the analyses of samples collected on five different days during the manufacture of beef jerky. On each day samples were taken from one single product batch at the following points: raw chilled meat, meat after marinating, meat after cure, meat during drying on racks, meat after drying and final product after packing. In addition, associated food contact surfaces were sampled. Thermotolerant coliforms (of undetermined resistance phenotype), *E. coli* and *Salmonella* spp. were assessed for resistance to a range of antibiotics including sulphonamides, ampicillin and tetracycline. No critical control points for the process stages were identified. Overall, the value of the study is questionable because there was a lack of detection of AMR potential human pathogens, and the study was limited to five batches of product. *E. coli* was detected in 6.7% (2/30) of the samples collected from food contact surfaces in the plant however, AMR *E. coli* was not isolated from raw beef samples, or from the final product. Similarly, *Salmonella* spp. were detected only in 3.3% (1/30) of the processing surface samples and in 8.6%

(3/35) of the raw material samples; however, *Salmonella* was not isolated from the final product. Hence overall, no meaningful conclusions with regard to AMR could be drawn from the Fernandes et al. (2017) study.

8.5.1.4 In summary:

- No secondary processing stages that served as critical control points for AMR bacteria were reported in the identified literature.
- However, there were no reports describing the fate of AMR bacteria after thermal processing (i.e. cooking) identified. We note there were reports of cooked meats contaminated by AMR bacteria in the literature, primarily from China (Jiang et al. 2014; Li et al. 2016; Yu et al. 2016; Jiang et al. 2017; Yu et al. 2017), but most samples were collected at retail and whether the contamination had occurred after effective cooking was not determined.
- Refrigeration can reduce both the prevalence and numbers of AMR campylobacters in chilled chicken portions (Casagrande Proietti et al. 2018). Refrigeration for 14 days after 20 rounds of freeze-thawing significantly reduced numbers of *Enterobacter* in comminuted beef (Jayaratne et al. 1990a).
- Freezing, similarly, reduced but did not destroy populations of AMR *E. coli* (Caudry and Stanisich 1979) and AMR *Enterobacter* (Jayaratne et al. 1990a). In *Enterobacter*, the stability of plasmids harbouring AMR was not affected by 20 rounds of freeze thaw.

8.6 AMR linkages and associations between virulence determinants and stress responses

This section reviews any evidence of any association between AMR genes, enhanced virulence and stress responses, and improved bacterial survival. There were 11 papers identified that made some consideration of AMR, stress responses and survival.

Early work by Logue et al. (1998) compared the fate of streptomycin-resistant *Yersinia* containing a virulence plasmid with the same strain that had been cured of the plasmid. Using the same meat-derived growth media, growth rates with and without the plasmid were similar at refrigeration temperatures, suggesting that

carriage of the plasmid did not affect growth rates. Later work from this research group, described by Walsh et al. (2001) assessed if streptomycin-resistant *L. monocytogenes* were any more resistant to heat compared with wild type *L. monocytogenes*. In brief, decimal reduction times (D values, DRT) for AMR *L. monocytogenes* and the non-AMR wild type equivalent *L. monocytogenes* were not significantly different. Thus, both studies found no evidence for the possession of AMR properties conferring enhanced growth or survival with regard to temperature stress for the assessed strains. A potential issue with both the Logue et al. (1998) and Walsh et al. (2001) papers, was that antibiotic resistance to streptomycin was achieved by artificially creating resistant mutants. The growth rate measurements for the Logue study were assessed on meat sections or ground meat; however, the original source of the *Yersinia* was not specified. The *L. monocytogenes* experiments were based on one of two strains; that were isolated respectively from soft cheese and a clinical infection of unknown origin. The Logue study appears to have been inspired by the original paper that described how to artificially create the streptomycin resistant strains (de W. Blackburn and Davies 1994) that showed a difference between growth rates for AMR *Salmonella* created using the methodology, and wild type strains, at low temperatures. The approach used by these papers is therefore probably not optimal for inclusion into this review. A brief description of the studies' findings has been included to avoid discarding studies that were initially scored as suitable, without explanation. Again, it should be emphasised that comments on the unsuitability of the papers are in no way a comment on the scientific integrity of the work, only the suitability of the studies for inclusion into this review.

Hirt et al. (2002) and Cocconcelli et al. (2003) independently investigated *Enterococcus faecalis* harbouring plasmid pCF10, described by Hirt et al. (2002) as a sex pheromone plasmid. The plasmid encodes a variety of genes including an aggregation substance (AS) that is upregulated in response to the detection of 7- to 8-amino-acid-long hydrophobic peptides encoded on chromosomes and secreted by potential recipient cells. AS causes cellular aggregation, thereby facilitating efficient pCF10 transfer between compatible enterococci. AS secretion represents an expensive metabolic overhead and so it is tightly regulated (Hirt et al. 2002). Genes encoding for AMR, including tetracycline resistance, are also present on pCF10. *E. faecalis* is an increasingly important nosocomial (hospital-acquired) pathogen. In

addition, Hirt et al. (2002) showed that AS is also a virulence determinant for *E. faecalis*, which is upregulated in the presence of blood plasma and, in mammals, causes an increase in blood-borne fibrin and platelet masses, called vegetation; the cause of infective endocarditis resulting from infection by *E. faecalis*.

Cocconcelli et al. (2003) investigated the fate of pCF10 in *E. faecalis* and its transfer during the fermentation of sausage. In brief, Cocconcelli et al. (2003) observed transfer of pCF10 and tetracycline resistance into *Enterococcus*, isolated from food, during fermentation. The exchanges were notable because the food isolate *Enterococcus* did not secrete the pheromone peptides. AS was generated however, and plasmid transfer still occurred. Although Cocconcelli et al. (2003) discuss at length the fact that the basis of AS upregulation was plasma present in the sausage meat triggering upregulation of AS, concentrations of plasma protein were not determined by the study. The Cocconcelli et al. (2003) study is important because it is an example of an AMR gene being linked with a locus that increases virulence of a human pathogen and that also promotes the spread of AMR by virtue of both loci being co-located on the same plasmid. Further, an intrinsic property of the foodstuff apparently acts to stimulate the dissemination of the AMR.

8.6.1 Linkage between QAC resistance and other AMR

Mullapudi et al. (2008) investigated the prevalence of heavy metal (cadmium and arsenic) resistance among *L. monocytogenes* isolates from processing plant environments with the aim of determining if resistance to heavy metals was associated with resistance to quaternary ammonium disinfectants. *L. monocytogenes* isolates (n=192) from the environments of turkey processing plants in North America were assessed for resistance to cadmium, arsenic and QAC. There was significantly increased cadmium resistance between some *L. monocytogenes* serotypes 1/2a, 1/2b, 3a and 3b compared with strains of the serotype 4b complex. In addition, resistance to QAC was observed in 51-60% of *L. monocytogenes* serotypes 1/2a, 1/2b, 3a and 3b and among 7% of the serotype 4b strains. All the QAC-resistant strains were co-resistant to cadmium, although the converse was not always true. There were no correlations identified between resistance to arsenic and QAC resistance. It is speculative from the results presented, but Mullapudi et al. (2008) suggest processing plants as reservoirs of heavy metal and QAC resistant

L. monocytogenes and the possibility that the association of cadmium and QAC resistance is a consequence of a common mechanism(s) mediating resistance to QAC and to cadmium in *L. monocytogenes.* However, no further investigation was undertaken to determine if there was evidence to support the supposition.

Rakic-Martinez et al. (2011) undertook multiple resistance studies using *L. monocytogenes* strains containing the plasmid pLM80. The strains used were derived from an outbreak traced back to hot dogs, a second outbreak traced to sliced turkey meat, and a clinical isolate from a patient with listeriosis. The parental *L. monocytogenes* were cultured on ciprofloxacin (2 μg/ml) or QAC (10 μg/ml) resulting in derivatives with increased MICs to several other AMs, including Gentamicin, ethidium bromide, and tetraphenylphosphonium chloride. The MICs to ciprofloxacin and Gentamicin did not differ between parental and plasmid-cured strains. However, ciprofloxacin-selected derivatives of pLM80-harbouring strains had higher MICs than those derived from the plasmid-cured strains, suggesting the presence of genes conferring ciprofloxacin resistance on the plasmid. Several studies have shown pLM80 contained an ABC efflux transporter (Dutta et al. 2013) and susceptibility to the antimicrobials was partially restored in the presence of the efflux inhibitor, reserpine. Rakic-Martinez et al. (2011) concluded that mutations in efflux systems were the cause of the induced multidrug resistance phenotype of strains selected for on media containing ciprofloxacin or QAC. The latter study suffers from many of the shortcomings of previously discussed work (Hirt et al. 2002; Cocconcelli et al. 2003) in that the strain derivatives were created under artificial conditions and so it was not clear how relevant the study findings were to *in vivo* conditions.

Xu et al. (2016) extended the work of Mullapudi et al. (2008) and sequenced the *bcrABC* cassette contained on a plasmid in *L. monocytogenes* strain 11GZL18, isolated from maturation-chilled raw meat. The *bcrABC* cassette exhibited 100% identity to the pLM80 plasmid, which is harboured by *L. monocytogenes* strains H7550 and H7858; the strains that caused hot dog and turkey meat foodborne disease outbreaks. An additional 20kb of sequence from the regions adjacent to the efflux pump showed 99% identity with plasmid pLM80 and the presence of *cadA* (a cation-transporting ATPase) and *cadC* (a cadmium efflux system component).

Comparisons of the resistance to QAC and cadmium for the 11GZL18 strain containing, and cured of the plasmid, showed that the plasmid played a role in conferring tolerance to QAC and heavy metal. Using reserpine to inhibit the efflux pumps reduced the tolerance thresholds but did not completely eliminate tolerance. The *bcrABC* cassette and *cadAC* genes contained on pLM80 were seen to be transmissible, transferring QAC and cadmium resistance to *E. coli* DH5α. Based on the evidence reported by Xu et al. (2016) *L. monocytogenes* containing pLM80 is an example of cross resistance because the *bcrABC* cassette removes cadmium and QAC from the bacterial cytoplasm. A case can also be made that pLM80 is also an example of co-transfer because the *cadAC* genes are also transferred with the plasmid and contribute to cadmium tolerance in combination with *bcrABC*.

Ortiz et al. (2016) undertook studies into persistent *L. monocytogenes* colonisation by investigating a new processing plant that manufactured ready-to-eat (RTE) pork products. In the plant, QAC were repeatedly used as surface disinfectants. The study lasted 27 months and *L. monocytogenes* was only isolated from product manufactured in the plant after 12 months, and from the plant environment after 13 months. Five different pulsed-field gel electrophoresis (PFGE) types were identified from the 14 isolations. In addition, *in silico* multi-locus sequence typing (MLST) was used to characterise the strains following whole genome sequencing (WGS). Four of the pulsotypes were found to belong to MLST ST121, a persistent *L. monocytogenes* isolated from plants in several countries (Ortiz et al. 2016). The ST121 strains contained the BAC resistance transposon Tn6188, which contains the *qacH* gene (a multidrug exporter). PCR testing confirmed only the presence of *qacH* and a lack of *bcrABC* in the isolates. Resistance to several antibiotics was assessed, although no plasmid curing experiments were undertaken. Some increased tolerance to antibiotics such as Gentamicin was shown, compared with other strains, but since curing the plasmids was not undertaken it is difficult to draw firm conclusions as to the basis of the tolerance. The authors concluded that the resistances to the antibiotics found in QAC resistant strains of *L. monocytogenes* were typically at a low level and that QAC-resistant *L. monocytogenes* strains harbouring *qacH* may not show cross-resistance to antibiotics at a clinically relevant level.

AMR and any association with disinfectant and heavy metal resistance was investigated in 152 *Salmonella* isolates from retail food of animal origins by Deng et al. (2018). Twenty one *Salmonella* serovars were identified by the study and *bla*$_{TEM}$ and *tetA* genes conferring resistance to β-lactams and tetracycline respectively were present in around 45% of the isolates. The *qacF* and *qacEΔ1* genes conferring protection to QAC were detected in 18% and 9% of the isolates. Copper resistance genes *pcoR*, *pcoC*, and *pcoA* were present in the highest proportion of isolates (20-40%), followed by a mercury resistance gene *merA* present in 18% of isolates. Arsenic resistance was least common with *arsB* being present in less than 7% of the isolates. Antibiotic resistance was significantly associated with disinfectant, and some of the heavy metal resistance, genes. There was a significant statistical correlation between a copper resistance genotype conferred by *pcoC*, *pcoR*; disinfectant resistance conferred by *qacF* and *qacEΔ1*; and tetracycline and sulphonamide resistance conferred by the *tet* and *sul* gene families. The physical organisation and location of the resistance loci was investigated by Deng et al. (2018), who concluded that the heavy metal resistance genes (HMRGs), antibiotic resistance genes (ARGs), and the QAC genes were mostly located on mobile genetic elements such as plasmid pRJ1004 and transposons Tn4380 and Tn501. The Deng et al. (2018) study concluded that retail meats may be a reservoir for the dissemination of AMR *Salmonella* and that using disinfectants for decontamination, or metals in livestock farming, may provide a pressure for the co-selection of strains with acquired resistance to other antimicrobials.

The expression of stress response genes, the formation of biofilms and AMR were investigated in *L. monocytogenes* SZ08, isolated from a meat processing environment and harbouring pLMSZ08; a 25kb plasmid (Zhang et al. 2018). Comparative experiments were undertaken using SZ08 containing, and cured from, pLMSZ08. Compared to the wild type strain containing pLMSZ08, the cured strain designated SZ08*, was not able to grow as rapidly when exposed to high concentrations (5.5% w/v) of sodium chloride. Furthermore, SZ08* was more motile compared with SZ08, with the expression of flagella-associated genes increased in the cured strain. There were no significant growth rate differences between SZ08 and SZ08* under a range of different temperatures and pH conditions. SZ08* had a significantly reduced ability to form biofilms compared with SZ08, following 48hr of

incubation and was more resistant to cadmium and QAC. Sequencing of pLMSZ08 revealed genes encoding transcriptional regulators and metal (*copY, tcrY*) and drug (*matE*) efflux transporters. The basis of the increased tolerance to QAC and cadmium and the pLMSZ08 genes conferring protection was not investigated further. The authors concluded that the genes encoded by pLMSZ08 were associated with heavy metal and QAC tolerance, motility and biofilm formation in *L. monocytogenes* SZ08 harbouring the plasmid. The genes were associated by virtue of the fact that they were present on the same genetic structure and so co-transferred when the plasmid was mobilised.

A summary of the papers identified relating to QAC and co- or cross resistance are shown as Table 18.

Table 18 A summary of the identified evidence relating to QAC and co or cross resistance

Basis of QAC resistance	Associated AMR	Mechanism of association	Reference(s)
Not determined	Cadmium	Cross resistance (speculative)	Mullapudi et al. (2008)
bcrABC	Ciprofloxacin	Mutation in *bcrABC*	Rakic-Martinez et al. (2011)
bcrABC	Cadmium	Cross resistance	Xu et al. (2016)
bcrABC	Cadmium	Co resistance	
qacH	Multiple AM	No clinically relevant increase in breakpoints	Ortiz et al. (2016)
qacF and qacED1	Copper Tetracycline Sulfonamide	Co resistance	Deng et al. (2018)
matE*	Cadmium	Co resistance	(Zhang et al. 2018)

8.6.2 Other species and AMR after disinfection exposure

Changes in resistance to antibiotics of poultry meat isolates of *Campylobacter jejuni* and *Campylobacter coli* after *in vitro* exposure to trisodium phosphate (TSP), triclosan (TLN), QAC, cetylpyridinium chloride (CPC) and chlorhexidine diacetate (CHA) were investigated by Mavri and Smole Možina (2013). Exposure to disinfectants was stepwise with increasing concentrations over extended periods of up to 15 days. Strains were characterised to determine if any changes to AMR were adaptive or a consequence of cross resistance. The investigations determined:

- If the basis of changed resistance was active efflux, by the determination of restored sensitivity in the presence of the efflux pump inhibitors such as reserpine.
- Changes in the outer membrane protein profiles.
- Morphological changes in adapted strains were compared with the parent strains.

Repeated exposure to increasingly higher biocide concentrations for *C. jejuni* and *C. coli* resulted in modest increases in tolerance of the order of 2-4-fold in 20% of cases. In some cases, increased tolerance or resistance to other biocides and antibiotics was also observed. Resistance to erythromycin increased by 2-8-fold after a five-day exposure to all five of the biocides in three of the *C. jejuni* strains tested (56%) that were adapted to TLN, QAC and CPC. Resistance to ciprofloxacin increased by 2-4-fold after 5 or 10 passages, in some *C. jejuni* and *C. coli* strains. However, in 80% of the combinations tested, no cross-resistance was observed for antibiotics after adaptation to sanitisers. Furthermore in 30% of cases, after the stepped increased exposure to biocides, the strains were more susceptible to the tested antibiotics compared with the parent strains.

If established, the increased antibiotic resistances in the sanitiser adapted strains were stable, surviving at least ten generations of non-selective culture. Several types of active efflux pumps were identified in some of the increased tolerance strains, which also had changed outer membrane protein profiles and changes in colony morphology.

The paper authors consider that their study is seminal and established some basic information for the adaptation of campylobacters to biocides and antibiotics, that can be expanded by further work. Overall, Mavri and Smole Možina (2013) concluded that adaptation to AMR is unique to each strain of *Campylobacter* and does not result from a single species-specific mechanism. The study provides some evidence for cross-resistance in the form of efflux pumps playing some role in the increased tolerances to antibiotics in some sanitiser-adapted campylobacters.

Capita et al. (2013) undertook work in Spain to determine if meat decontaminants of the type generally regarded as safe (GRAS), and commonly used in the United

States, altered resistance to antibiotics in indigenous *Escherichia coli* populations present on chicken leg portions. The portions were immersed for 15 minutes in a solution of trisodium phosphate (TSP), acidified sodium chlorite (ASC), ascorbic acid (AA), citric acid (CA) or a mains water control and examined immediately after treatment then after five days refrigerated storage. *E. coli* isolates were qualitatively tested for resistance to twelve antibiotics using disc-diffusion. There was a significant degree of resistance observed in the tap water controls with 44/50 (88.0%) of isolates resistant to two or more antibiotics. However, the control sample isolates had a lower prevalence of resistance compared with those from the AM-treated samples. Samples treated with TSP yielded significantly more isolates that could tolerate ampicillin-sulbactam. There were increases in resistance to amoxicillin-clavulanic acid and ciprofloxacin after immersion in ASC, AA or CA; cephotaxime and nitrofurantoin after TSP immersion and trimethoprim-sulphamethoxazole after dipping in AA or CA. The CA treatment also caused an increase in tetracycline resistance in the *E. coli* population. The authors conclude their results suggest that the GRAS decontaminants tested could favour the emergence of AMR strains on poultry meat (Capita et al. 2013), but no investigation was undertaken to identify the underlying resistance mechanisms.

8.6.3 In summary:

The evidence identified supports a role for cross resistance between QAC and cadmium in *L. monocytogenes*. A combination of plasmid curing experiments and use of the efflux inhibitor reserpine provided support from two papers that the bcrABC efflux pump can remove both QAC and cadmium from bacterial cells. Zhang et al. (2018) determined that *L. monocytogenes* cured of plasmid pLMSZ08 was more sensitive to cadmium and QAC, but did not unequivocally determine if the *matE* efflux pump present on the plasmid was the source of the protection. The studies of Xu et al. (2016) and Zhang et al (2018) also provided evidence that the close proximity of genes on *L. monocytogenes* plasmids was the basis of co-resistance as the mechanism of QAC and cadmium resistance linkage in *L. monocytogenes*.

A single paper (Rakic-Martinez et al. 2011) postulated that mutation in *bcrABC* might allow increased resistance to some AM, however, the usefulness of the study was hampered by the use of an artificial selection stage and it is unknown if the study

created strains that do not exist *in vivo*. Although it is also an efflux system, there was some evidence from a single study that the *qacH* gene product in *L. monocytogenes* does not operate in an analogous manner to *bcrABC* and thereby confer increased AM tolerance at clinically relevant concentrations.

Based on a single paper, there were significant linkages between some AMR genes in *Salmonella* with QAC resistance genes being associated with copper, tetracycline and sulphonamide resistance.

There is evidence *in vitro* for cross-resistance in the form of efflux pumps playing a role in increased tolerance to antibiotics in campylobacters that were artificially adapted to some sanitisers.

Exposure to antimicrobial treatments commonly used to wash meat increased AMR in indigenous *E. coli* populations on chicken portions, although no investigation of the mechanisms operating was undertaken.

9. Study recommendations and further work

9.1 Issues related to AMR determination methods

A major barrier to meta-analyses of published studies is that different isolation and AMR determination methodologies have been used by different authors. Two protocols, namely EUCAST and CLSI dominate, although some authors devised their own testing protocols. Both of the main testing methodologies are reactive in that they periodically recalculate the breakpoints for resistance thresholds in response to reports quantifying increased resistance in bacterial populations. In addition, there can be a choice of testing methodologies that can be used e.g. resistance determination by disk diffusion or by microtitre plate. The former method reports testing results as resistant, intermediate or sensitive (R, I or S) to a given concentration of an antibiotic whereas the latter provides a defined minimal inhibitory concentration (x mg/l). In combination, these issues cause obfuscation of antibiotic resistance data and consequently greatly inhibit the ability of researchers to compare resistances between studies and to combine resistance data for metanalyses, especially if one of the studies being compared is historical.

Thus, a future area of work should be to investigate the development and adoption of more standardised testing protocols to increase the value of future research and surveillance studies by ensuring results could be analysed in combination with other, similar studies. Ideally, any standardised resistance testing methodologies should be quantitative, in terms of the phenotype, and funding bodies should be made aware of the increased long-term value of using such methodology in future project proposals, despite the increased costs. Recognising that any attempt to establish a new testing standard that would compete against the established CLSI and EUCAST quantitative protocols is a not trivial undertaking, there may be merit in investigating ways in which the usefulness of already published information could be improved. For example, looking forward, systems might be established that contained the various versions of the standards and made consideration of test organisms, quantitative information such as MICs and the assessment dates and showed how the interpretation of the results changed over time. If such systems were developed, they could also be used to simplify the determination of which antibiotics were included in different versions of the standards, which is laborious to undertake manually.

9.2 Issues arising from the use of fermentation starter cultures containing AMR

Starter cultures that are, by definition, deliberately added to fermented food products can be considered different to the natural microbiota associated with raw SPMMP because there is control by FBOs over what is deliberately added. However, there is limited information describing AMR in fermentation starter cultures. AMR genetic materials can exist in some starter bacteria and the high concentrations of starter culture cells used as an inoculum may assist plasmid-based transfer of AMR to other species, including potential human pathogens. This means that studies to determine which starter cultures pose a potential risk would be beneficial and an important first stage in preventing AMR spread through the use of starter cultures. In addition, it would be prudent to assemble guidance for FBOs describing good practices for the use of fermentation starter cultures and the importance of using strains that do not have easily mobilisable genetic elements that may transfer resistance. Following appropriate investigations, fact-based guidance on commonly used methods for curing plasmids from strains (e.g. repeated non-selective culture) could also be assembled to assist FBOs in creating strains that are safer for use in the manufacture of fermented products.

9.3 Biofilms, their community composition and their role as a physical barrier to effective sanitation

Biofilms have been shown to provide a structural barrier to disinfectants, thereby effectively lowering the sanitiser concentration to which members of the biofilm community are exposed. Many of the studies undertaken to date have been based on the use of artificially created biofilms, as a model for natural biofilms. However, the kinetics of natural biofilm formation and the interactions between species are highly complex. Some researchers have shown that some strains initially added to a mixed strain cocktail can’t compete and are absent from the final, established biofilm communities. There would be benefit in investigating the possibility of identifying standardised strains and conditions that are proven to be a good model for natural biofilms so that the relative roles of AMR and biofilms can be quantified in terms of strains, persistence abilities and effective sanitation. Given that there might be issues involved in culturing certain strains (e.g. VBNC bacteria) from biofilms, one potentially advantageous approach would be to investigate in detail natural biofilms obtained

from commercial SPMMP processing areas by examining the naturally evolved community by means of genetic methods. For example, 16S rRNA can be used to identify individual bacterial species, whilst AMR genes contained within the biofilm and individual organisms can be identified by shotgun whole genome sequencing. Undertaking such studies would also have the benefit of producing data, in the form of DNA sequences, which would be available for future study obviating many of the problems experienced in the conduct of this review.

A single paper reported that there were genes present on a plasmid that could control the expression of chromosomally located genes regulating biofilm formation in *L. monocytogenes*. However, an unravelling of the mechanism operating was not attempted. No papers were identified that explicitly reported on alternative sigma factors (which regulate the transcription of genes) altering stress responses in AMR bacteria associated with SPMMP. In general, a common regulatory mechanism enabling bacterial survival under adverse conditions occurring within the food chain involves transcriptional redirection using alternative sigma factors such as σB and modification of transcriptional activities by RNA polymerase. The mechanisms operating to preserve and spread AMR associated with SPMMP are not well understood currently. Hence investigations to determine if alternative sigma factors are important in the survival of AMR bacteria in SPMMP would improve the understanding of the preservation and spread mechanisms operating and allow the formulation of informed strategies and processing practices required to control the spread of AMR.

Overall, this study has determined that gaps in the knowledge concerning AMR associated with SPMMP exist, and many of these are due to a plethora of manners in which AMR may be reported. In the section above key gaps are noted, and the selection of which of these could be the basis of the next area of work will depend on the priorities, and budgets, of the relevant funding bodies

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11. Appendix 1 Database searching to identify literature pertinent to SPMMP

11.1 The Clarivate Analytics Web of Science search engine

An initial search was undertaken at 08:00am on the 1st January 2019, searching the 'Topic' field on the Web of Science database v5.3.1. The search string used was:

> **meat AND ((resistance OR susceptibility) AND antimicrobial? OR antibiotic?) AND (processing OR slaughter OR dress? OR chilling OR debon? OR cutting OR portioning OR modified atmosphere OR vacuum OR packing OR marinade OR fermentation OR brining OR herb?) AND (plasmid OR gene OR phage OR transposon OR integron) NOT (manure OR cheese OR retail OR wastewater OR faeces OR feces OR farm OR symbiotics OR shedding OR market? OR fish)**

which returned 399 hits.

An initial sift based on consideration of only the title and the abstract was used to identify potentially relevant papers and assemble new, relevant, search key words. The broad criteria that were applied for inclusion were that the study related to post chill meat or meat preparations, products, MSM or food contact surfaces in processing plants. In addition, the paper should have some effort focussed on antibiotic- or sanitiser-resistant bacteria. At this stage, surveillance reports were accepted into the study for further appraisal by assessment questionnaire.

The new keywords identified were:

> **Smoking, jerky, steam, pasteurisation (pasteurization), sausage, mincing, grinding, cured, brine, injection, (vacuum) massage, (processing) environment, food contact, surfaces, sanitiser, sanitizer, biocide, resistome, cooked, sliced, cold cuts**

In addition, two new parentheses for the search string were inserted to include resistance genes and terms and also specific antimicrobials (and abbreviations for the AMs). Most of the antimicrobials were taken from an EU publication on AMR in animals, humans and food (EFSA Journal 2017;15(2):4694), with additional terms

and AMs supplied from other papers. As part of the search optimisation it was noted a corresponding list of sanitisers should be prepared and included in a future round of testing. The additional search string sections were:

> **(extended-spectrum, beta?lactamase, ESBL, *amp*C, multi-drug resistance, MDR, *bla*, *mec*A, *mec*C, carbapenemase)(methicillin, vancomycin, colistin, ciprofloxacin, tetracycline?, erythromycin, amoxicillin, clavulanate, ampicillin, azithromycin, ceftazidime, chloramphenicol, clindamycin, cefotaxime, erythromycin, fusidic, fluoroquinolone, gentamicin, kanamycin, linezolid, meropenem, mupirocin, nalidixic, quinupristin, dalfopristin, rifampicin, sulfonamides, streptomycin, sulfamethoxazole, tigecycline, tiamulin, trimethoprim, AMC, AMP, AZM, CAZ, CHL, CIP, CLI, CST, CTX, ERY, FUS, GEN, KAN, LZD, MER, MUP, NAL, QD, RIF, SUL, STR, SXT, TGC, TIA, TET, TMP, amc, amp, azm, caz, chl, cip, cst, ctx, ery, fus, gen, kan, lzd, mer, mup, nal, qd, rif, sul, str, sxt, tgc, tia, tet, tmp)**

In addition, the keywords '**milk, vegetables, fruit, eggs**' were added to the exclusion section of the string.

The Boolean conditions were rewritten slightly to more tightly control the number of search results. Prior to the final revision of the second-round search string, the number of hits returned by four rounds of string adjustment were 11,471, 9,641, 5,783 and 4,211.

The revised search string was:

> **((((meat AND (resistance OR susceptibility)) AND ((antimicrobial? OR antibiotic?) OR extended-spectrum) OR beta?lactamase) OR ESBL) OR ampC) OR multi-drug resistance) OR MDR) OR bla) OR mecA) OR mech) OR carbapenemase) OR methicillin) OR vancomycin) OR colistin) OR ciprofloxacin) OR tetracycline?) OR erythromycin) OR amoxicillin) OR clavulanate) OR ampicillin) OR azithromycin) OR ceftazidime) OR chloramphenicol) OR clindamycin) OR cefotaxime) OR erythromycin) OR fusidic) OR fluoroquinolone) OR gentamicin) OR kanamycin) OR linezolid) OR meropenem) OR mupirocin) OR nalidixic) OR**

quinupristine) OR dalfopristine) OR rifampicin) OR sulfonamides) OR streptomycin) OR sulfamethoxazole) OR tigecycline) OR tiamulin) OR trimethoprim) OR AMC) OR AMP) OR AZM) OR CAZ) OR CHL) OR CIP) OR CLI) OR CST) OR CTX) OR ERY) OR FUS) OR GEN) OR KAN) OR LZD) OR MER) OR MUP) OR NAL) OR QD) OR RIF) OR SUL) OR STR) OR SXT) OR TGC) OR TIA) OR TET) OR TMP) OR amc) OR amp) OR azm) OR caz) OR chl) OR cip) OR cst) OR ctx) OR ery) OR fus) OR gen) OR kan) OR lzd) OR mer) OR mup) OR nal) OR qd) OR rif) OR sul) OR str) OR sxt) OR tgc) OR tia) OR tet) OR tmp)) AND ((((((((((((((((((((((((((((processing OR slaughter) OR dress?) OR chilling) OR debon?) OR cutting) OR portioning) OR modified atmosphere) OR vacuum) OR packing) OR marinade) OR fermentation) OR brining) OR herb?) OR smoking) OR jerky) OR steam) OR pasteurisation) OR pasteurization) OR sausage) OR mincing) OR grinding) OR cured) OR brine) OR injection) OR massage) OR environment) OR food contact) OR surfaces) OR sanitizer) OR sanitizer) OR biocide) OR cooked) OR sliced) OR cold cuts)) AND ((((((((((((((((((plasmid OR gene) OR phage) OR transposon) OR integron) OR resistome) NOT manure) NOT cheese) NOT retail) NOT wastewater) NOT faeces) NOT feces) NOT farm) NOT symbiotics) NOT shedding) NOT market?) NOT fish) NOT milk?) NOT vegetables) NOT fruit) NOT eggs))

And for the second round of testing, 522 papers were returned.

Examination of the papers revealed that a significant number of papers were surveillance not relevant to secondary processing and/or characterisation of virulence determinants in AMR bacteria found in meat and meat products and hence of limited use to this study.

After scrutiny of the second-round results, the exclusions section for the search string was further expanded to include the following terms:

NOT companion (animals) NOT vaccine NOT lipopolysaccharide? NOT neutrophil? NOT Bifidobacter? NOT Pediococc? NOT enterotoxin NOT testosterone NOT vas deferens NOT adhesion NOT invasi? NOT bison NOT rabbit? NOT duck? NOT sensory NOT actinobacillus NOT litter NOT

adjuvant? NOT thermal inactivation NOT sodium lactate NOT proto-oncogene NOT laboratory-passaged NOT ryanodine-receptor NOT carnobacterium NOT human growth hormone NOT intensive care unit NOT dose NOT optimisation NOT starch NOT school cafeterias NOT mitochondrial NOT oocytes NOT metal? NOT bloodstream infection NOT EHEC-hlyA NOT porous covalent

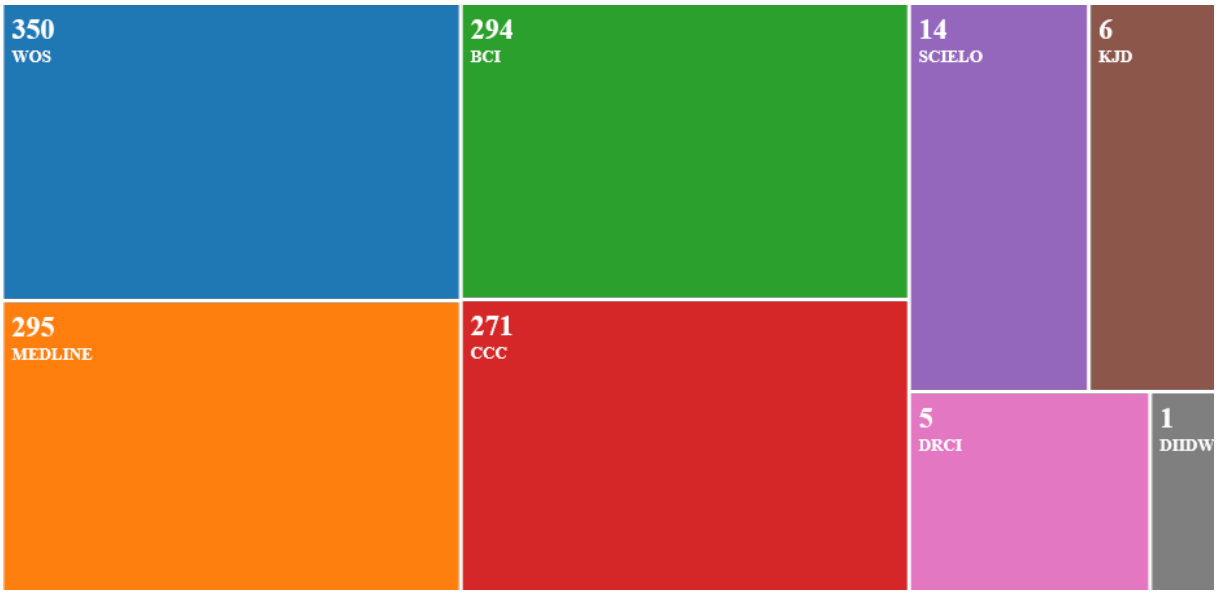
Publication language was not restricted. The range for the search was restricted to year ≥1985 for the third round of searching. The string used for the third round was:

((((meat AND (resistance OR susceptibility)) AND ((antimicrobial? OR antibiotic?) OR extended-spectrum) OR beta?lactamase) OR ESBL) OR ampC) OR multi-drug resistance) OR MDR) OR bla) OR mecA) OR mech) OR carbapenemase) OR methicillin) OR vancomycin) OR colistin) OR ciprofloxacin) OR tetracycline?) OR erythromycin) OR amoxicillin) OR clavulanate) OR ampicillin) OR azithromycin) OR ceftazidime) OR chloramphenicol) OR clindamycin) OR cefotaxime) OR erythromycin) OR fusidic) OR fluoroquinolone) OR gentamicin) OR kanamycin) OR linezolid) OR meropenem) OR mupirocin) OR nalidixic) OR quinupristin) OR dalfopristin) OR rifampicin) OR sulfonamides) OR streptomycin) OR sulfamethoxazole) OR tigecycline) OR tiamulin) OR trimethoprim) OR AMC) OR AMP) OR AZM) OR CAZ) OR CHL) OR CIP) OR CLI) OR CST) OR CTX) OR ERY) OR FUS) OR GEN) OR KAN) OR LZD) OR MER) OR MUP) OR NAL) OR QD) OR RIF) OR SUL) OR STR) OR SXT) OR TGC) OR TIA) OR TET) OR TMP) OR amc) OR amp) OR azm) OR caz) OR chl) OR cip) OR cst) OR ctx) OR ery) OR fus) OR gen) OR kan) OR lzd) OR mer) OR mup) OR nal) OR qd) OR rif) OR sul) OR str) OR sxt) OR tgc) OR tia) OR tet) OR tmp)) AND ((((((((((((((((((((((((((((((processing OR slaughter) OR dress?) OR chilling) OR debon?) OR cutting) OR portioning) OR modified atmosphere) OR vacuum) OR packing) OR marinade) OR fermentation) OR brining) OR herb?) OR smoking) OR jerky) OR steam) OR pasteurisation) OR pasteurization) OR sausage) OR mincing) OR grinding) OR cured) OR brine) OR injection,) OR massage) OR environment) OR food

contact) OR surfaces) OR sanitizer) OR sanitizer) OR biocide) OR cooked) OR sliced) OR cold cuts)) AND ((plasmid OR gene) OR phage) OR transposon) OR resistors) OR integron) NOT manure) NOT cheese) NOT retail) NOT wastewater) NOT faeces) NOT feces) NOT farm) NOT symbiotics) NOT shedding) NOT market?) NOT fish) NOT milk?) NOT vegetables) NOT fruit) NOT eggs) NOT companion) NOT vaccine) NOT lipopolysaccharide?) NOT neutrophil?) NOT Bifidobacter?) NOT Pediococc?) NOT enterotoxin) NOT testosterone) NOT vas deferens) NOT adhesion) NOT invasi?) NOT bison) NOT rabbit?) NOT duck?) NOT sensory) NOT actinobacillus) NOT litter) NOT adjuvant?) NOT thermal inactivation) NOT sodium lactate) NOT proto-oncogene) NOT laboratory-passaged) NOT ryanodine-receptor) NOT carnobacterium) NOT human growth hormone) NOT intensive care unit) NOT dose) NOT optimisation) NOT starch) NOT school cafeterias) NOT mitochondrial) NOT oocytes) NOT metal?) NOT bloodstream infection) NOT EHEC-hlyA) NOT porous covalent))

The third round of searching returned 370 papers, with a breakdown of papers grouped by matching database shown below as Figure 3:

Figure 3 The databases containing the identified peer-reviewed literature from the third round of searching. NB: Some papers appeared more than once, in different database indices.



11.1.1 The National Centre for Biotechnology Information (NCBI) Pubmed search engine

Additional databases were searched covering indices not included in the WoS platform. The same search string (with the Boolean conditions and parentheses modified as appropriate to the conventions used by each search engine). Pubmed was searched on 29/01/2019 using the string:

(((meat AND (resistance OR susceptibility)) AND (((antimicrobial? OR antibiotic?) OR extended-spectrum) OR beta?lactamase) OR esbl) OR ampc) OR multi-drug resistance) OR MDR) OR bla) OR meca) OR mech) OR carbapenemase) OR methicillin) OR vancomycin) OR colistin) OR ciprofloxacin) OR tetracycline?) OR erythromycin) OR amoxicillin) OR clavulanate) OR ampicillin) OR azithromycin) OR ceftazidime) OR chloramphenicol) OR clindamycin) OR cefotaxime) OR erythromycin) OR fusidic) OR fluoroquinolone) OR gentamicin) OR kanamycin) OR linezolid) OR meropenem) OR mupirocin) OR nalidixic) OR quinupristin) OR dalfopristin) OR rifampicin) OR sulfonamides) OR streptomycin) OR sulfamethoxazole) OR tigecycline) OR tiamulin) OR trimethoprim) OR AMC) OR AMP) OR AZM) OR CAZ) OR CHL) OR CIP) OR CLI) OR CST) OR CTX) OR ERY) OR FUS) OR GEN) OR KAN) OR LZD) OR MER) OR MUP) OR NAL) OR QD) OR RIF) OR SUL) OR STR) OR SXT) OR TGC) OR TIA) OR TET) OR TMP) OR amc) OR amp) OR azm) OR caz) OR chl) OR cip) OR cst) OR ctx) OR ery) OR fus) OR gen) OR kan) OR lzd) OR mer) OR mup) OR nal) OR qd) OR rif) OR sul) OR str) OR sxt) OR tgc) OR tia) OR tet) OR tmp)) AND (((((((((((((((((((((((((((((((((((processing OR slaughter) OR dress?) OR chilling) OR debon?) OR cutting) OR positioning) OR modified atmosphere) OR vacuum) OR packing) OR marinade) OR fermentation) OR bringing) OR herb?) OR smoking) OR jerky) OR steam) OR pasteurisation) OR pasteurization) OR sausage) OR mincing) OR grinding) OR cured) OR brine) OR injection,) OR massage) OR environment) OR food contact) OR surfaces) OR sanitizer) OR sanitizer) OR biocide) OR cooked) OR spliced) OR cold cuts)) AND (((plasmid OR gene) OR phage) OR transposon) OR resistors) OR integron) NOT manure)

NOT cheese) NOT retail) NOT wastewater) NOT faeces) NOT feces) NOT farm) NOT synbiotics) NOT shedding) NOT market?) NOT fish) NOT milk?) NOT vegetables) NOT fruit) NOT eggs) NOT companion) NOT vaccine) NOT lipopolysaccharide?) NOT neutrophil?) NOT bifidobacteria) NOT pediococcus) NOT enterotoxin) NOT testosterone) NOT vas deferens) NOT adhesion) NOT inovasi) NOT bison) NOT rabbit?) NOT duck?) NOT sensory) NOT actinobacillus) NOT litter) NOT adjuvant?) NOT thermal inactivation) NOT sodium lactate) NOT proto-oncogene) NOT laboratory-passaged) NOT ryanodine-receptor) NOT carnobacterium) NOT human growth hormone) NOT intensive care unit) NOT dose) NOT optimisation) NOT starch) NOT school cafeterias) NOT mitochondrial) NOT oocytes) NOT metal?) NOT bloodstream infection) NOT ehec-hlya) NOT porous covalent)).

and returned 183 hits. The search results were exported into Endnote using the 'Send to' option on the search results web page. The export was using the 'Citation Manager' option and the .nbib file format, which is analogous to the .RIS file exchange format used by Endnote. Endnote imported all 183 references without issue from the .nbib file when told to consider it a .RIS-format text file. Using the 'Find Duplicates' option in Endnote identified 59 duplicate papers. Thus, the Pubmed search identified 124 new papers, bringing the total number of papers in the Endnote database to 494. An initial sift based on the title and abstract determined that 28 of these papers should be taken forward for further evaluation by systematic questionnaire.

The Networked digital library of theses and dissertations (NRLTD) was also searched on 29/01/2019 using a string identical to that used for Pubmed. In addition, the constraint boxes limited the hits to theses concerned with food, resistance, meat and microbiology as their subject classifications, along with an authorship language of English, French or Portuguese as these languages could be understood by the project team. 44 hits were returned. Since NRLTD does not yet have any functionality for the export of theses, the theses were appraised for relevance to the study by reading the title and abstract inside the search results. No potentially relevant theses were identified, with the majority of works being eliminated because they were concerned with primary production or processing and unravelling the mechanisms

that conferred resistance such as the identification of point mutations in chromosomal genes conferring resistance to specific antimicrobials.

The Taylor and Frances (TandF) database was searched at https://www-tandfonline-com.libezproxy.open.ac.uk/ on 31/01/2019 using the same search string used to search NRLTD. The search took around 10 minutes to complete. Zero hits were returned. Because of the length of time before the search completed and the lack of matches, there was concern that the search engine had been unable to parse the search string. To investigate further, a simplified version lacking the exclusion conditions was used. Allowing for database-specific changes to parenthesis and Boolean conditions, the search string used was:

[All: meat] AND [[All: resistance] OR [All: susceptibility]] AND [[[[All: antimicrobial?] OR [All: antibiotic?] OR [All: extended-spectrum] OR [All: beta?lactamase] OR [All: esbl] OR [All: ampc] OR [All: multi-drug]] AND [All: resistance]] OR [All: mdr] OR [All: bla] OR [All: meca] OR [All: mech] OR [All: carbapenemase] OR [All: methicillin] OR [All: vancomycin] OR [All: colistin] OR [All: ciprofloxacin] OR [All: tetracycline?] OR [All: erythromycin] OR [All: amoxicillin] OR [All: clavulanate] OR [All: ampicillin] OR [All: azithromycin] OR [All: ceftazidime] OR [All: chloramphenicol] OR [All: clindamycin] OR [All: cefotaxime] OR [All: erythromycin] OR [All: fusidic] OR [All: fluoroquinolone] OR [All: gentamicin] OR [All: kanamycin] OR [All: linezolid] OR [All: meropenem] OR [All: mupirocin] OR [All: nalidixic] OR [All: quinupristin] OR [All: dalfopristin] OR [All: rifampicin] OR [All: sulfonamides] OR [All: streptomycin] OR [All: sulfamethoxazole] OR [All: tigecycline] OR [All: tiamulin] OR [All: trimethoprim] OR [All: amc] OR [All: amp] OR [All: azm] OR [All: caz] OR [All: chl] OR [All: cip] OR [All: cli] OR [All: cst] OR [All: ctx] OR [All: ery] OR [All: fus] OR [All: gen] OR [All: kan] OR [All: lzd] OR [All: mer] OR [All: mup] OR [All: nal] OR [All: qd] OR [All: rif] OR [All: sul] OR [All: str] OR [All: sxt] OR [All: tgc] OR [All: tia] OR [All: tet] OR [All: tmp] OR [All: amc] OR [All: amp] OR [All: azm] OR [All: caz] OR [All: chl] OR [All: cip] OR [All: cst] OR [All: ctx] OR [All: ery] OR [All: fus] OR [All: gen] OR [All: kan] OR [All: lzd] OR [All: mer] OR

[All: mup] OR [All: nal] OR [All: qd] OR [All: rif] OR [All: sul] OR [All: str] OR [All: sxt] OR [All: tgc] OR [All: tia] OR [All: tet] OR [All: tmp]] AND [[[[[[All: processing] OR [All: slaughter] OR [All: dress?] OR [All: chilling] OR [All: debon?] OR [All: cutting] OR [All: positioning] OR [All: modified]] AND [All: atmosphere]] OR [All: vacuum] OR [All: packing] OR [All: marinade] OR [All: fermentation] OR [All: bringing] OR [All: herb?] OR [All: smoking] OR [All: jerky] OR [All: steam] OR [All: pasteurisation] OR [All: pasteurization] OR [All: sausage] OR [All: mincing] OR [All: grinding] OR [All: cured] OR [All: brine] OR [All: injection,] OR [All: massage] OR [All: environment] OR [All: food]] AND [All: contact]] OR [All: surfaces] OR [All: sanitizer] OR [All: sanitizer] OR [All: biocide] OR [All: cooked] OR [All: spliced] OR [All: cold]] AND [All: cuts] AND [[All: plasmid] OR [All: gene] OR [All: phage] OR [All: transposon] OR [All: resistors] OR [All: integron]]

which returned 1,995 hits after around two minutes. Initial inspection of the publications list showed many papers related to on-farm surveillance, and foods, such as eggs, that were not the focus of the review. Thus, some exclusions were required to reduce the papers to a more manageable number. Exclusion clauses were introduced gradually as a precaution to possibly overwhelming the search engine. The search engine seemed to struggle with the three letter short codes for antimicrobials and so these were also removed, which dramatically reduced the search times to 10-15 seconds. Searching then used:

[All: meat] AND [[All: resistance] OR [All: susceptibility]] AND [[[[All: antimicrobial?] OR [All: antibiotic?] OR [All: extended-spectrum] OR [All: beta?lactamase] OR [All: esbl] OR [All: ampc] OR [All: multi-drug]] AND [All: resistance]] OR [All: mdr] OR [All: bla] OR [All: meca] OR [All: mech] OR [All: carbapenemase] OR [All: methicillin] OR [All: vancomycin] OR [All: colistin] OR [All: ciprofloxacin] OR [All: tetracycline?] OR [All: erythromycin] OR [All: amoxicillin] OR [All: clavulanate] OR [All: ampicillin] OR [All: azithromycin] OR [All: ceftazidime] OR [All: chloramphenicol] OR [All: clindamycin] OR [All: cefotaxime] OR [All: erythromycin] OR [All: fusidic] OR [All: fluoroquinolone] OR [All: gentamicin] OR [All: kanamycin]

OR [All: linezolid] OR [All: meropenem] OR [All: mupirocin] OR [All: nalidixic] OR [All: quinupristin] OR [All: dalfopristin] OR [All: rifampicin] OR [All: sulfonamides] OR [All: streptomycin] OR [All: sulfamethoxazole] OR [All: tigecycline] OR [All: tiamulin] OR [All: trimethoprim] OR [All: amc] OR [All: amp] AND [All: processing] OR [All: slaughter] OR [All: dress?] OR [All: chilling] OR [All: debon?] OR [All: cutting] OR [All: positioning]

OR [All: modified]] AND [All: atmosphere]] OR [All: vacuum] OR [All: packing] OR [All: marinade] OR [All: fermentation] OR [All: brining] OR [All: herb?] OR [All: smoking] OR [All: jerky] OR [All: steam] OR [All: pasteurisation] OR [All: pasteurization] OR [All: sausage] OR [All: mincing] OR [All: grinding] OR [All: cured] OR [All: brine] OR [All: injection,] OR [All: massage] OR [All: environment] OR [All: food]] AND [All: contact]] OR [All: surfaces] OR [All: sanitizer] OR [All: sanitiser] OR [All: biocide] OR [All: cooked] OR [All: sliced] OR [All: cold]] AND [All: cuts] AND [[All: plasmid] OR [All: gene] OR [All: phage] OR [All: transposon] OR [All: resistors] OR [All: integron]] AND NOT [All: manure] AND NOT [All: cheese] AND NOT [All: retail] AND NOT [All: wastewater] AND NOT [All: faeces] AND NOT [All: feces] AND NOT [All: fish] AND NOT [All: milk?] AND NOT [All: vegetables] AND NOT [All: fruit] AND NOT [All: eggs]]

Additional care was required when searching the TandF database, because it had a tendency to change terms which were detected as spelling mistakes and thereby alter the search terms. For example, ‘santiser’ was changed to the US spelling whilst ‘brining’ became ‘bringing’, ‘slicing’ became ‘splicing’ etc. Furthermore, as the numbers of conditions increased, there was evidence that there were alterations made by the search engine that were likely to exclude papers. As an example, sections of the search string that were altered by the database before use and thus different to what was entered included: (underlined for emphasis)

… AND [All: all: gene] <u>AND [[All:]] OR [All: []] AND</u> [All: all: phage] <u>AND [[All:]] OR [All: []] AND</u> [All: all: transposon] AND <u>[[All:]] OR [All: []]</u> AND [All: all: resistors] <u>AND [[All:]] OR [All: []] AND</u> [All: all: integron]] …

The issue was partly solved by removing spaces preceding the search terms and deleting wildcards such as '?'. A near final string of:

> **[All:antimicrobial] OR [All:antibiotic] OR [All:extended-spectrum] OR [All:lactamase] OR [All:chilling] OR [All:debon] OR [All:cutting] OR [All:positioning] OR [All:modified]] OR [All:atmosphere]] OR [All:vacuum] OR [All:packing] OR [All:marinade] OR [All:fermentation] OR [All:brining] OR [All:smoking] OR [All:jerky] OR [All:steam] OR [All:pasteurisation] OR [All:pasteurization] OR [All:sausage] OR [All:grinding] OR [All:cured] OR [All:brine] OR [All:injection] OR [All:sanitizer] OR [All:sanitiser] OR [All:biocide] OR [All:cooked] OR [All:sliced] OR [All:plasmid] OR [All:gene] OR [All:phage] OR [All:transposon] OR [All:resistors] OR [All:integron]] AND NOT [All:fish] AND NOT [All:milk] AND NOT [All:vegetables] AND NOT [All:fruit] AND NOT [All:eggs]**

was then used. However, the string was still subject to automatic 'correction' and so the TandF database was searched using the highly simplified string:

> **antimicrobial meat resistance NOT slaughter NOT animals NOT eggs NOT milk**

The search completed in around 15 seconds and returned 50 papers. As was found when using the NRLTD database, there was no provision for exporting the search results *en masse* and so review of titles and abstracts was undertaken from inside the search results listing. Possibly because of the limited scope of journals from a single publisher, the majority of the papers related to either livestock on farms, or their feed. A number of papers were also concerned with AMR detection methodologies. A single paper that was concerned with reformed lamb meat was potentially relevant and exported into the Endnote database using .RIS as the intermediary file format. The number of papers in the Endnote database after the TandF database search was 495.

The American Society for Microbiology (ASM) journals were searched. The journals that are indexed are Antimicrobial Agents and Chemotherapy, Applied and Environmental Microbiology, Clinical and Vaccine Immunology, Clinical Microbiology Reviews, EcoSal Plus, Eukaryotic Cell, Infection and Immunity, Journal of

Bacteriology mBio, Microbiology and Molecular Biology Reviews, Microbiology Resource Announcements, Microbiology spectrum and Molecular and Cellular Biology. An index of journal titles searched by WoS is available online. All of the ASM journals are indexed by the WoS platform and so no searching of the ASM journals database using the ASM engine was undertaken. ASM books were searched on 02/02/2019 using the string:

> **antimicrobial meat resistance.**

From 12 hits, nine potentially relevant book chapters were identified from the titles. Export from the ASM website is problematic and so the references were manually entered into Endnote. As part of that process, abstracts were read. After abstract screening, the number of potentially relevant chapters was reduced to five.

Articles published by SpringerLink were searched on 05/02/2019. The standard search string yielded zero hits. The string was simplified by removing the antibiotic names and short codes and some of the gene names and acronyms. In addition, the wild cards were removed. The simplified search string used was:

> **meat AND (resistance OR susceptibility) AND (antimicrobial OR antibiotic OR extended-spectrum OR lactamase OR multi-drug resistance OR carbapenemase OR methicillin OR vancomycin OR colistin OR ciprofloxacin OR tetracycline OR erythromycin OR amoxicillin OR clavulanate OR ampicillin OR azithromycin OR ceftazidime OR chloramphenicol OR clindamycin OR cefotaxime OR erythromycin OR fusidic OR fluoroquinolone OR gentamicin OR kanamycin OR linezolid OR meropenem OR mupirocin OR nalidixic OR rifampicin OR sulfonamides OR streptomycin OR sulfamethoxazole OR tigecycline OR tiamulin OR trimethoprim) AND (processing OR slaughter OR dress OR chilling OR cutting OR positioning OR modified atmosphere OR vacuum OR packing OR marinade OR fermentation OR bringing OR herb OR smoking OR jerky OR steam OR pasteurisation OR pasteurization OR sausage OR mincing OR grinding OR cured OR brine OR injection OR massage OR environment OR food contact surfaces OR sanitizer OR sanitizer OR biocide OR cooked**

OR spliced OR cold cuts) AND (plasmid OR gene OR phage OR transposon OR resistors OR integron)

which generated 475 hits when the search field was limited to the subgroups of ‘bacteriology’, ‘public health’, ‘epidemiology’, infectious diseases’, ‘medical microbiology’ and ‘health promotion and disease prevention’. The search results were saved as comma separated values (.csv) and imported into Endnote using the application-provided Springer import file. A high proportion of the hits (n=114) were conference abstracts, with no additional information. SpringerLink indexes 3536 journals. It is probable there is a high degree of overlap between WoS and SpringerLink because there were 135 duplicate references compared with the previously identified literature. No additional new papers were identified when the remaining 226 papers were scrutinised for possible inclusion into the review. Overall, the 226 remaining papers were not considered to be informative to the review.

The Oxford University Press (OUP) database was searched on 05/02/2019. The standard search string retrieved 1,671 papers, which was considered too many to comfortably manage. Thus, some more exclusions were added to the search string to remove wild animal surveillance, and generic resistance mechanisms papers. When the OUP database was searched using:

((meat AND (resistance OR susceptibility)) AND (antimicrobial? OR antibiotic) AND (((((((((((((((((((((((((((((((((((processing OR slaughter) OR dress?) OR chilling) OR debon?) OR cutting) OR portioning) OR modified atmosphere) OR vacuum) OR packing) OR marinade) OR fermentation) OR brining) OR herb?) OR smoking) OR jerky) OR steam) OR pasteurisation) OR pasteurization) OR sausage) OR mincing) OR grinding) OR cured) OR brine) OR injection,) OR massage) OR environment) OR food contact) OR surfaces) OR sanitizer) OR sanitizer) OR biocide) OR cooked) OR sliced) OR cold cuts)) AND ((plasmid OR gene) OR phage) OR transposon) OR resistors) OR integron) NOT manure) NOT cheese) NOT retail) NOT wastewater) NOT faeces) NOT feces) NOT farm) NOT symbiotics) NOT shedding) NOT market?) NOT fish) NOT milk?) NOT vegetables) NOT fruit) NOT eggs) NOT companion) NOT vaccine) NOT lipopolysaccharide?) NOT neutrophil?) NOT Bifidobacter?) NOT

Pediococc?) NOT enterotoxin) NOT testosterone) NOT vas deferens) NOT adhesion) NOT invasi?) NOT bison) NOT rabbit?) NOT duck?) NOT sensory) NOT actinobacillus) NOT litter) NOT adjuvant?) NOT thermal inactivation) NOT sodium lactate) NOT proto-oncogene) NOT laboratory-passaged) NOT ryanodine-receptor) NOT carnobacterium) NOT human growth hormone) NOT intensive care unit) NOT dose) NOT optimisation) NOT starch) NOT school cafeterias) NOT mitochondrial) NOT oocytes) NOT metal?) NOT bloodstream infection) NOT EHEC-hlyA) NOT porous covalent) NOT wild))

845 papers were returned, which was considered more manageable. There is no facility to download search results *en masse* at OUP and so screening for relevance was undertaken on the OUP website, with potentially useful papers being compared manually to the existing publications database for the identification of duplicates. Relevant papers were imported one-at-a-time using the website-provided 'cite' option and using a native Endnote file format. The OUP website also had difficulty parsing the search string, many of the exclusion keywords (e.g. farm, eggs, vegetables) appeared in the titles of the papers returned by the search. Consequently, many papers were excluded and only three additional papers were identified.

The Cambridge Core database of Cambridge University Press (CUP) journals and books was searched using the same search string as was used for the OUP database. The search was undertaken on 06/02/2019. A total of 741,890 hits were returned, which was clearly too many to sift. Inspection of the first few returned papers showed the same issues as those experienced for OUP, with many of the exclusion terms having been ignored.

Cambridge Core has a search instruction guide, however it is an overview and does not cover Boolean operations for searching. The approach taken to resolve the issue of no exclusions and an excessive number of hits was to attempt to rebuild the search string using the guide. A basic search of **((meat AND (resistance OR susceptibility))** was informative. It returned 48,205 hits. Inspection of the returned results revealed the Boolean operators were apparently ignored, even for such a simple string, and any of the included search terms was matched as a result.

Further investigations revealed that **meat antibiotic** returned 7,959 hits and **meat antibiotic processing** returned 156,470 hits. **meat antibiotic NOT processing** also returned 156,470. The same number of hits (156,470) was returned for **NOT meat NOT antibiotic NOT processing**. The Cambridge Core customer services unit was emailed and asked whether the search engine supported Boolean searching on 06/02/2019. A response was received from CUP from Kitty Franco using the email address academictechsupport@cambridge.org and correspondence was commenced regarding the search engine capabilities on 13/02/2019. On 26/02/2019, the Cambridge Core technical support confirmed that their search engine does not currently support Boolean operators and will search only for the keywords included. Further, Boolean operators are treated simply as search keywords, which was why large numbers of hits were generated. Therefore, the search string was modified to remove exclusions and all Boolean operations. The search string:

> **meat resistance susceptibility antimicrobial? antibiotic processing slaughter dress? chilling debon? cutting portioning modified atmosphere vacuum packing marinade fermentation brining herb? smoking jerky steam pasteurisation pasteurization sausage mincing grinding cured brine injection, massage environment food contact surfaces sanitiser sanitizer biocide cooked sliced cold cuts plasmid gene phage transposon resists integron**

was used to search the Cambridge Core on 04/03/2019. The search returned 408,855 hits. The Cambridge Core does allow searches to be saved and exported, albeit with limitations. The results files are paged with 20 hits displayed per page. Export is only possible on a per-page basis. The more than 20,000 pages of hits were considered impractical to download on a page-by-page basis and so the search string was modified in a similar fashion to the TandF string, but without the Boolean exclusions. When:

> **antimicrobial meat resistance**

was used to search the Cambridge Core, 41,897 hits (2,095 pages) were returned. A search using only '**meat**' returned 4,730 results (237 pages). Journals relating to nutrition, parasitology, animal health, the environment and helminths were excluded

from the search results. The subject areas of mathematics and statistics and probability were also excluded, and consequently there were 250 remaining hits. These references were downloaded on a page-by-page basis. Running a 'find duplicates' search on the 250 hits revealed six duplicates, one copy of each of which was deleted, leaving 247 hits. These papers were appraised for inclusion by two reviewers. The search term '**meat**' was very general hence a large proportion of papers concerned livestock surveillance preslaughter, foodborne outbreaks, case control investigations of outbreaks and comparisons between food and human isolates. Many of the papers were historical (<1985) and very few made any consideration of any antimicrobial resistance of isolates. Consequently, only 7 papers were considered relevant enough to be further appraised.

The Elsevier database, Science direct was searched on 07/02/2019 using the URL (universal resource locator). The search index supports Boolean operations, but with a limit of eight conditions per query. Publication types used were review articles, research articles, book chapters and conference abstracts. A search string was constructed by using general terms, inspecting the resultant hits and limiting the searches by the inclusion of terms that excluded papers that were not relevant. The search string used was

> **(meat AND resistance AND antibiotic AND processing AND bacteria) NOT clinical NOT animal NOT feed NOT wastewater**

which returned 475 hits. The relevance of the papers was assessed by inspection of the title and abstract in the search results listing and potentially relevant papers individually marked and downloaded as .RIS files directly into Endnote. Large numbers of the papers were excluded because their focus was bacteria whose AMR status was not determined as part of the study. After the removal of duplicates (n=6), two new papers were added to the literature list to be further appraised.

The Wiley online library, including books, has been indexed as part of the WoS platform since 2002 and was therefore included in the WoS searches.

11.1.2 Summary of database searches

A summary of the numbers of papers identified and positively appraised for further evaluation is shown as Table 19. Papers were subject to initial exclusion if they did

not relate to an aspect of SPMMP, AMR genetic materials or AMR bacteria assessed by reading the title and abstract of the publication. Only a single instance of publications that were identified more than once by the searches were used.

Table 19 A summary of the numbers of papers identified by the database searches. WoS is web of science, NTCLTD is the networked digital library of theses and dissertations, TandF is Taylor and Francis, ASM is the American society for microbiology, OUP is Oxford university press, CUP is Cambridge University Press.

Database	Number of hits identified by the search (after removal of duplicates)	Number of relevant papers taken forward for further appraisal*
WoS	370	97
PubMed	124	28
NRCLTD	44	0
TandF	50	1
ASM	12	5
SpringerLink	266	0
OUP	845	3
CUP	247	7
Elsevier	475	2
Total	2433	143

*Papers were discarded if they were considered to be outside the scope of the review by both reviewers after reading the paper title and abstract.

11.2 Search effectiveness determinations

A check to determine the effectiveness of the keyword searching was undertaken. Twelve papers from each of the WoS and Pubmed search datasets were selected at

random and ten were similarly selected from the Springer results. The cited reference lists were examined to see if they contained additional papers not identified by the searches. The references from a mixture of papers considered possibly useful and those that were discarded during the initial sift were examined for overlooked papers. The justification for the approach was that exclusion was based on suitability for the current review, but a high proportion of papers were in closely related areas such as AMR in primary processing. Because of the different approaches to publications, export by different search engines, all of the literature cited by the WoS-identified papers was selected at random and searched; the PubMed-identified papers, with the previously-identified duplicates removed were randomly chosen and examined and all of the SpringerLink-identified papers were used for selection. Table 20 shows a summary of the cited literature examined.

Table 20 A summary of the randomly selected literature examined, and any new papers identified in the cited literature from Web of Science

Random reference no.	Paper	No. new papers
11	Escolar et al. (2017)	2
50	Alizadeh et al. (2016)	0
64	Bengtsson and Wierup (2006)	0
125	Frasao et al. (2015)	0
180*	Campos Calero et al. (2018)	0
196	Lavilla Lerma et al. (2014)	0
218	Manageiro et al. (2017)	0
241	Oppliger et al. (2012)	0
249	Paludi et al. (2011)	0
266	Schlegelova et al. (2004)	0
335	Skowron et al. (2016)	0
354	Wang et al. (2008)	0

Table 21 A summary of the randomly selected literature examined, and any new papers identified in the cited literature from PubMed

Random reference no.	Paper	No. new papers
13	Jiang et al. (2017)	2
15	Li et al. (2017)	0
21	Ortiz et al. (2016)	0
22	Ryu et al. (2012)	1
73	Hong et al. (2012)	0
88	Maravic et al. (2018)	0
89	Maravic et al. (2013)	0
90	Marshall and Levy (2011)	0
94	Montoya et al. (1992)	0
98	Nuesch-Inderbinen et al. (2013)	0
105	Poulsen et al. (2012)	0
113	Taviani et al. (2008)	

Table 22 A summary of the randomly selected literature examined, and any new papers identified in the cited literature from SpringerLink

Reference no.	Paper	No. new papers
6	Doherty (2011)	0 (no refs)
75	Nand et al. (2015)	0 (no refs)
101	Kurt-sukur et al. (2017)	0 (no refs)
113	Bellamkonda et al. (2007)	0 (no refs)
115	Levis (2017)	0
130	Rees et al. (1988)	0
209	Evans (1991)	0
234	Grimes et al. (2013)	0
283	Vahrson et al. (1997)	0
349	Mahler (1974)	0

*WoS article 180 was supplementary data, which was not peer reviewed and had no citations. The main article (WoS number 8; Campos Calero et al. (2018)) was randomly selected as a substitute. Only the first citation of a newly identified potentially relevant paper from the randomly selected paper citations is shown.

Two additional papers were identified from the WoS papers using title only (Fallah et al. 2012; Allen et al. 2016). The Fallah et al paper (2012) was discarded after the abstract text was read because it fell outside the inclusion criteria for the study. The Allen review was retained for general background purposes and because the abstract mentioned sanitiser resistance and association with food contact surfaces. Papers discussing the acquisition of quaternary ammonium resistance (Buffet-Bataillon et al. 2012), tetracycline resistance in meat products (Koo and Woo 2011), and the transfer of resistance in bacteria isolated from meat products (Sunde and Norstrom 2006), were newly-identified. potentially relevant papers from the reference lists of the PubMed random selections.

As part of the citations checking process, another duplication with an author surname mis-spelling was noted (Nuesch-Inderbinen et al. 2013); an alternate random choice was made. In addition, another potentially-relevant paper (Casagrande Proietti et al. 2018) was identified during the process of obtaining PDF copies of the papers. The additional paper appeared as a recommendation from the journal publisher.

In total, 148 papers were flagged to be taken forward for further appraisal by systematic questionnaire.

Wherever possible, electronic or PDF copies of the papers were sourced. For the papers and book chapters that were not available electronically, a paper photocopy was obtained. As part of downloading PDF copies of the papers, seven further publication duplicates (Jayaratne et al. 1990b; Heir et al. 1995; Hufner et al. 2007; Gomez et al. 2014; Fernandes et al. 2017; Ferreira et al. 2017; Gowda et al. 2017) identified by both WoS and Pubmed were noted. The primary issue for software detection of duplicates appeared to be use of author Christian names as opposed to initials by Pubmed. One paper (Reuter and Sassepatzer 1979) was unable to be sourced. However, the paper was in a German language journal and had only one citation from the senior author in 40 years hence it was considered of little relevance. Overall, 142 papers were therefore determined suitable for review.

The papers were systematically appraised for provenance, relevance, objectivity, methodology, presentation and timeliness (PROMPT) using a standardised questionnaire (Appendix 2). Two papers were selected at random (papers 64 and 86) for discussion between reviewers prior to the commencement of appraisals. Reviewers were asked to use paper copies of the form shown in Appendix 2 and mark their question response scores using a horizontal line through a vertical linear sliding scale. The questionnaires were designed to be general, so they were suitable for use over a range of publication topics. However, questions could be excluded from being answered by

checking a ‘not applicable’ box, if they were deemed to be not appropriate for the paper. The form responses were digitised using a page feed scanner (DS-1630, Epson, Hemel Hempstead, Herts, UK) and the scores read using software (Fizz v2.50B, Biosystèmes, Burgundy, France). A response scale of between 0 and 5, with a precision of two decimal places; was used for each of the questions asked. The appraisal results were manually inspected to confirm that the number of the paper, first author and reviewer had been captured accurately before the Fizz application stored the scanned appraisal information into a relational database (SQL-server 2017; Microsoft, Redmond, WA, USA). The stored appraisal results were retrieved using Access 2016 (Microsoft) as a front-end to the SQL-server database using a link connection (rather than downloading the information into Access).

The purpose of the appraisal was to determine the relevance of the paper content for inclusion into the review in the context of the review questions asked. The authors wish to make clear that any exclusion of a paper from the review was not a comment on the quality of the work reported.

11.2.1 Second-round searching

On 9th July 2019, the searches were repeated using the original search strings with the intention of including papers published in 2019. Whenever possible, a date restriction of ≥01/06/2018 was included into the search to reduce the number of previously identified being papers returned. The date restriction was chosen to ensure that papers published towards the end of 2018, but only added to the databases after a delay, were identified by the review.

Both Google Scholar and also Google were included in the July 2019 searches to identify any missed relevant peer-reviewed materials and gray literature. Both Google and Google Scholar limit searches to 32 keywords and both engines attempt to match all the terms searched for as a default. Consequently, a simple search string was used for both engines that was:

(meat AND resistance AND antibiotic AND processing AND bacteria) -clinical -animal -feed -wastewater

The Google Scholar search returned ‘around 7,170’ hits. A standard Google search returned ‘about 147,000’ hits. It was not practical to review all the hits from these searches. Google ranks hits on a number of factors, including a favourable listing for payment. However, relevance to individual and multiple search terms and academic content are also favourably treated by Google and so the first 12 pages of results (120 hits) from each search were scrutinised, justified by an assumption they would be the most relevant. Most hits from the standard Google search engine were to peer reviewed scientific papers, or to sources such as Pubmed showing an AMR paper. Most hits had been previously identified by the original database searches. Technical reports,

largely from government-sponsored research, and bodies such as the WHO, were also returned in the search results.

A summary of the additional searches is shown as Table 23.

Table 23 A summary of the additional papers identified by re-running the searches in July 2019 The Web of Science version available in July 2019 was v5.3.2, which was different to the v5.3.1 used for the initial searches.

Database	Number of additional items identified with duplicates excluded	Number of relevant papers remaining after initial sift and systematic appraisal
WoS	38	3
PubMed	0	0
NRCLTD	2	0
TandF	25	0
ASM	0	0
SpringerLink	2	0
OUP	14	0
CUP	13	0
Elsevier	9	0
Google Scholar	7,170	0
Google	147,000	7

11.2.2 Third-round searching

Helpful discussions with Dr Derek Brown (NHS Scotland) regarding an early draft of this review lead to a recommendation that the search strategy, which had included the three letter short codes used to denote specific antibiotics (Section 11 above), should be expanded to include the single and two letter codes used by some journals. The search string was edited to that shown below

(additional keywords in **bold**) and the WoS database searched again. The revised searching was undertaken on 4th December 2019 using version 5.3.4 of the database.

((((meat AND (resistance OR susceptibility)) AND ((antimicrobial? OR antibiotic?) OR extended-spectrum) OR beta?lactamase) OR ESBL) OR ampC) OR multi-drug resistance) OR MDR) OR bla) OR mecA) OR mech) OR carbapenemase) OR methicillin) OR vancomycin) OR colistin) OR ciprofloxacin) OR tetracycline?) OR erythromycin) OR amoxicillin) OR clavulanate) OR ampicillin) OR azithromycin) OR ceftazidime) OR chloramphenicol) OR clindamycin) OR cefotaxime) OR erythromycin) OR fusidic) OR fluoroquinolone) OR gentamicin) OR kanamycin) OR linezolid) OR meropenem) OR mupirocin) OR nalidixic) OR quinupristin) OR dalfopristin) OR rifampicin) OR sulfonamides) OR streptomycin) OR sulfamethoxazole) OR tigecycline) OR tiamulin) OR trimethoprim) OR AMC) OR AMP) OR AZM) OR CAZ) OR CHL) OR CIP) OR CLI) OR CST) OR CTX) OR ERY) OR FUS) OR GEN) OR KAN) OR LZD) OR MER) OR MUP) OR NAL) OR QD) OR RIF) OR SUL) OR STR) OR SXT) OR TGC) OR TIA) OR TET) OR TMP) OR amc) OR amp) OR azm) OR caz) OR chl) OR cip) OR cst) OR ctx) OR ery) OR fus) OR gen) OR kan) OR lzd) OR mer) OR mup) OR nal) OR qd) OR rif) OR sul) OR str) OR sxt) OR tgc) OR tia) OR tet) OR tmp **OR Ap) OR A) OR Cf) OR Cm) OR C) OR Cp) OR Gm) OR G) OR Km) OR K) OR Na) OR St) OR S) OR Tc) OR Te) OR T) OR Su) OR Sx) OR Tm)**)) AND ((((((((((((((((((((((((((((((((((processing OR slaughter) OR dress?) OR chilling) OR debon?) OR cutting) OR portioning) OR modified atmosphere) OR vacuum) OR packing) OR marinade) OR fermentation) OR brining) OR herb?) OR smoking) OR jerky) OR steam) OR pasteurisation) OR pasteurization) OR sausage) OR mincing) OR grinding) OR cured) OR brine) OR injection,) OR massage) OR environment) OR food contact) OR surfaces) OR sanitizer) OR sanitizer) OR biocide) OR cooked) OR sliced) OR cold cuts)) AND ((plasmid OR gene) OR phage) OR transposon) OR resistors) OR integron) NOT manure) NOT cheese) NOT retail) NOT wastewater) NOT faeces) NOT feces) NOT farm) NOT symbiotics) NOT shedding) NOT market?) NOT fish) NOT milk?) NOT vegetables) NOT fruit) NOT eggs) NOT companion) NOT vaccine) NOT lipopolysaccharide?) NOT neutrophil?) NOT Bifidobacter?) NOT Pediococc?) NOT enterotoxin) NOT testosterone) NOT vas deferens) NOT adhesion) NOT invasi?) NOT bison) NOT rabbit?) NOT duck?) NOT sensory) NOT actinobacillus) NOT litter) NOT adjuvant?) NOT thermal inactivation) NOT sodium lactate) NOT proto-oncogene) NOT laboratory-passaged) NOT ryanodine-receptor) NOT carnobacterium) NOT human growth hormone) NOT intensive care unit) NOT dose) NOT optimisation) NOT starch) NOT school cafeterias) NOT

mitochondrial) NOT oocytes) NOT metal?) NOT bloodstream infection) NOT EHEC-hlyA) NOT porous covalent))

The search returned 1201 hits, which reduced to 1180 when the date range was restricted to ≥1985. We noted that this was significantly more papers than the 370 that the original search had identified. Investigations revealed the use of the '**OR A**' search term was identifying 'a' as an indefinite article and removal of the term reduced the number of papers by more than half, all of which were imported into the Endnote database. The 'remove duplicates' function of the software was used to remove previously identified papers, leaving 132 new papers. The papers were sifted for relevance by reading the title and abstract as described previously (Section 1.13). Twelve new papers were identified (Cadena et al. 2019; de Souza et al. 2019; Hachemi et al. 2019; Hao et al. 2019; Maertens et al. 2019; Manso et al. 2019; Matle et al. 2019; Perez-Rodriguez and Taban 2019; Perin et al. 2019; Sadek et al. 2019; Silva et al. 2019; Tasmin et al. 2019), with seven being assessed by two reviewers (Section 1.14) for inclusion into the review. It should be noted that all of these were published in the latter half of 2019, indicating that the original searches had been thorough, with no historical section of the literature failing to be identified by the absence of the shorter antibiotic codes. The review was subsequently edited to include the newly identified papers, which related mostly to Table 5.

11.3 Statistical analyses of paper inclusions to assess broadly equivalent appraisal scoring between reviewers

Four reviewers (R1-R4) appraised the identified papers for acceptance into, or rejection from, the review. In order to determine whether the reviewers were appraising the papers in a broadly similar manner, statistical analyses were undertaken to compare the totals of accepted and rejected papers. Each paper was initially appraised by two reviewers and a pairwise comparison matrix was set up whereby each reviewer was compared, overall, to the three other reviewers. Individual author comparisons were generated randomly and overall each initial comparison set contained between 24 and 26 papers for each reviewer. The statistical test used to assess the degree of agreement between appraisers was Cohen's kappa (Cohen 1960; Smeeton 1985). Cohen's kappa (κ) tests interrater reliability for the assignment of objects into non-overlapping categories and the calculated value maps to a range of between -1 and +1. For the purposes of this review, κ was used to determine how reliably each reviewer assigned each paper into the category of accepted or rejected compared with the other three reviewers.

11.3.1 Appraisal divergence

An arbitration process was established for those papers where one reviewer scored a paper as unsuitable, but the other scored it suitable for inclusion. In brief, a third reviewer also assessed the

contested paper and the majority decision from all three appraisals was used to decide the suitability of the paper (i.e. 2/3 appraisals were used to accept or reject a paper). The subjective opinion of the project team was that the inclusion of a third review would provide a more balanced assessment compared with arbitration decided by a mean score calculated from just two reviewer appraisals. The approach is well established because it is used by several journals to arbitrate papers submitted for publication with a split review decision. The third reviewer was decided mostly by reviewer availability, consideration of reviewers' workload and the likelihood that papers could be reassessed in a timely manner of a few days.

12. Appendix 2 The literature appraisal questionnaire

Appraiser ☐ John ☐ Bob ☐ Janet ☐ Mike Systematic Appraisal Number [ ] Paper first author_____

Provenance section	**---→ increasing score**	**Not applicable**
Q1. What is the provenance of the senior author or all authors as assessed by numbers of general peer reviewed publications?	0 _____ >100 papers	☐
Q2. What is the provenance of the senior author or all authors as assessed by numbers of AMR-related peer-reviewed publications?	0 _____ >100 papers	☐
Relevance section		
Q1. Is the study surveillance or a comparative experiment before and after a secondary process?	Surv _____ Comp Exp	☐
Q2. Did the study make specific mention of the use of controls that were appropriate to what was measured?	No _____ Yes	☐
Q3. Were bacteria with any credible potential (e.g. *L. monocytogenes*) for causing human illness studied?	Commensal _____ Pathogen	☐
Q4. Did the study focus on a compound(s) relevant to those used in the treatment of human illness or a food contact surface sanitiser?	No _____ Antibiotic/Sanitiser	☐
Objectivity section		
Q1. Was there potential bias or no bias declared by the authors such as:		
A. Research funding from a third party that might have a vested interest?	No _____ Yes	☐
B. Employment from a commercial entity that might benefit from the report?	No _____ Yes	☐
C. An author's declaration of a potential conflict of interest?	No _____ Yes	☐
Methods section		
Q1. Did the study use natural (N) or cultured (C) cells?	C _____ N	☐
Q2. If the work included investigating effect of 2ndary food processing on AMR, was processing done in lab or plant??	Lab _____ Plant	☐
Q3. If under commercial conditions, were multiple plants studied?	No _____ Yes	☐
Q4. Were there determinations undertaken on different days and on different batches of product?	No _____ Yes	☐

Q5. Were enough samples tested overall for the study to be considered robust?	0 _____ 5	☐
Q6. Were enough samples used for each data point such that they can be considered typical?	0 _____ 5	☐
Q7. Were the number of replicates per data point calculated using the variance determined from pilot work?	0 _____ 5	☐
Q8 Was any AMR resistance determined by phenotyping (e.g. disk diffusion/breakpoints) or genotyping, i.e. detection of resistance genes	Genetics _____ Culture	☐
Presentation: Q1. Were the results presented in a clear manner suitable for extraction and inclusion into the review?	No _____ Yes	☐
Timeliness: Q1. Is the paper recent or might it be of lesser relevance because it is historical (H) and what it reports may have moved on?	H _____ Recent	☐

13. Appendix 3 Groups for AMR classified using their base chemistry

Table 24 Antibiotics classified by activity type and base chemistry

Antibiotic	Drug class
Amikacin	Aminoglycoside
Amoxicillin	Penicillin
Amoxicillin/clavulanic acid	Penicillin/β-lactamase inhibitor
Ampicillin	Penicillin
Ampicillin-sulbactam	Penicillin/β-lactamase inhibitor
Azithromycin	Macrolide
Bacitracin	Cyclic polypeptide
Benzylpenicillin	Penicillin
Cefepime	Cephalosporin (4th generation)
Cefoperazone	Cephalosporin (3rd generation)
Cefotaxime	Cephalosporin (3rd generation)
Cefoxitin	Cephalosporin (2nd generation)
Ceftazidime	Cephalosporin (3rd generation)
Ceftiofur	Cephalosporin (3rd generation)
Ceftriaxone	Cephalosporin (3rd generation)
Cefuroxime	Cephalosporin (2nd generation)
Cefalothin	Cephalosporin (1st generation)
Chloramphenicol	Nitrobenzine

Antibiotic	Drug class
Ciprofloxacin	Fluoroquinolone (2nd generation)
Ciprofloxacin-Gentamicin	Fluoroquinolone/aminoglycoside
Clindamycin	Aminoglycoside
Colistin aka polymyxin E	Cyclic polypeptide
Co-trimoxazole aka Trimethoprim/sulfamethoxazole	Amino oxymethyl benzyl pyrimidine/sulfonamide
Doxycycline	Tetracycline
Enrofloxacin	Fluoroquinolone
Ertapenem	Carbapenem
Erythromycin	Macrolide
Florfenicol	Nitrobenzine (derivative)
Fusidic acid	Fusidane
Gentamicin	Aminoglycoside
Imipenem	Carbapenem
Kanamycin	Aminoglycoside
Levofloxacin	Fluoroquinolone
Lincomycin	Aminoglycoside
Linezolid	Oxazolidinone
Meropenem	Carbapenem
Methicillin	Penicillin
Moxifloxacin	Fluoroquinolone

Antibiotic	Drug class
Mupirocin	Polar fatty acid
Nalidixic acid	Fluoroquinolone
Neomycin	Aminoglycoside
Nitrofurantoin	Nitrofuran
Norfloxacin	Fluoroquinolone
Oxacillin	Penicillin
Penicillin	Penicillin
Piperacillin/tazobactam/cefalexin	Penicillin/β-lactamase inhibitor/Cephalosporin (1st generation)
Piperidimic acid	Heterocyclic amine carboxylic acid (pyridopyrimidine)
Quinupristin/dalfopristin	Streptogramin/Streptogramin
Rifampicin	Cyclic polypeptide
Streptomycin	Aminoglycoside
Sulfamethoxazole	Sulfonamide
Sulfisoxazole	Sulfonamide
Sulfonamide	Sulfonamide
Sulfadimethoxine	Sulfonamide
Teicoplanin	Glycopeptide
Tetracycline	Tetracycline
Tiamulin	Pleuromutilin
Tigecyclin	Tetracycline

Antibiotic	Drug class
Tilmicosin	Macrolide
Trimethoprim	Amino oxymethyl benzyl pyrimidine
Vancomycin	Glycopeptide

Table 25 Sanitiser classified by active agent chemistry

Sanitiser class
Iodophore
Acid-anionic
Halogen
Peracetic acid
Hypochlorite
Cationic detergent

14. References

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