A systematic review to assess the significance of the food chain in the context of antimicrobial resistance (AMR) with particular reference to pork and poultry meat, dairy products, seafood and fresh produce on retail sale in the UK.

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LAY SUMMARY

Antimicrobial resistance (AMR) in bacteria particularly that observed to antimicrobials used to treat infections in humans and animals is a major public health issue. This is due to the risk of treatment failure that could lead to an increase in duration of illness and, even death, of individuals and animals with infections caused by antimicrobial-resistant bacteria. People can become exposed to such organisms through a number of routes such as human-to-human spread, direct contact with animals, and the environment and also through the food chain. There is uncertainty around the contribution food makes to the overall AMR problem. The purpose of this systematic review was to examine published evidence between 1999 and the end of May 2016 for pork and poultry meat, dairy products, seafood and fresh produce at retail level to investigate occurrence of AMR in bacteria present in these food groups. The focus of this study was at retail level as this was perceived to be the point at which consumers are more likely to be exposed to AMR bacteria from the food chain.

This review confirmed that there is a lack of AMR prevalence data for British-produced food and, to a lesser extent in countries that export food to the UK, with a notable exception of certain major food exporting countries in northern Europe. For European exporting countries, evidence was available through both national and European Food Safety Authority (EFSA) annual integrated surveillance reports for AMR in foodborne zoonotic and indicator bacteria in animals, humans and food. Surveillance data are important for monitoring trends of resistance to critically important antimicrobials (CIAs), such as recent increases observed in ampicillin resistant in bacteria (i.e. *Salmonella* spp. and *Escherichia coli*) from Danish pork or the rise in fluoroquinolone resistance detected in *Campylobacter jejuni* from poultry in the Netherlands. There is heterogeneity in AMR prevalence levels observed across different countries that otherwise would not be detected if not for surveillance and research efforts focused on food at retail level. Such heterogeneity could be due to variations in animal production and food processing practices.

Efforts should be made to develop surveillance programmes that will identify trends in the occurrence of AMR in foods and thereby provide a framework for assessing potential risks associated with exposure to such hazards among British consumers.

The recommendations from the study include (in no particular order of importance):

- Standardisation in the selection of antimicrobials for antimicrobial susceptibility testing panels should be followed, as well use of harmonized criteria for assessment of resistance per bacteria/ drug combination for surveillance purposes of AMR in the food chain, adoption of a standardised definition for multidrug resistance (MDR) and implementation of random sampling and adequate study design for epidemiological studies focused on AMR.

- Surveillance priorities could be set using a risk-based approach, taking into account the importance of antimicrobials used for treatment in both humans and animals, and continued surveillance of the incidence and emerging resistance (including MDR) in commensal bacteria (*Enterococcus* spp. and *E. coli*) should be encouraged.

- There is a lack of information on AMR bacteria in foods of animal origin other than meat at retail level. In recent years, there have been growing numbers of outbreaks associated with milk and dairy products (cheese, butter, yogurt), seafood (fish and shellfish) and fresh produce (fruit, vegetables and salads) at national and international levels but there is scarce, scattered evidence of resistance and MDR occurrence in foodborne and commensal bacteria in these food products and its implications for public health. These gaps should be addressed also using a risk-based approach following evidence of resistance in food items as well as the extent of expected consumer exposure using consumption and import volumes.

- Data on AMR bacteria from British and imported pork meat in the UK are limited and dated. Further research and surveillance efforts are needed to ascertain AMR levels in both foodborne and commensal bacteria in pork meat in the UK.

- There is evidence of increasing levels of resistance to antimicrobials in foodborne bacteria (i.e., *Campylobacter* spp.) from poultry meat in the UK. Research and surveillance efforts should be continued to monitor AMR trends in both foodborne and commensal bacteria in British and imported chicken and poultry meats in the UK.

- Data on antimicrobial use (AMU) in food-producing animals at species level in the UK are important to explain the occurrence and dynamics of AMR, resistance genes and MDR phenotypes in a defined geographical area. More complete information should therefore be collected on the type of production systems from which food samples originate to assess the impact of animal husbandry practices as risk factors for resistance.

- There is a need for more studies to quantify the contribution of both domestic and imported foods to the occurrence of AMR in food consumed in the UK. Information on country of origin for imported products should be collected.

- Finally, further research and surveillance are needed to establish and quantify the risk of transmission of AMR for critically important antimicrobials in organisms from foods of animal and non-animal origin to humans.

EXECUTIVE SUMMARY

The aim of this study was to appraise and summarise existing evidence of prevalence and patterns of resistance to critically important antimicrobials (CIAs) as defined by the World Health Organization (WHO) in specific foodborne zoonotic pathogens and indicator commensal bacteria from both domestically-produced and imported foods at retail level that could result in the exposure of British consumers. For this purpose, a systematic review was conducted; scientific and grey literature published between 1999 and the end of May 2016 was considered. Priority was given to studies conducted on British produced food and food from the main exporting countries trading with the UK, although data was gathered and summarized for other countries and are presented in **Appendix 3**. The review investigated the prevalence of antimicrobial resistance (AMR) at phenotype level in *Campylobacter* spp. (poultry meat), *Salmonella* spp. (pork meat) and in selected commensal bacteria, namely *Enterococcus faecalis*, *Enterococcus faecium* and *Escherichia coli* in poultry and pork meat, dairy products, fish and shellfish and vegetables and fruit. Resistance to beta (β)-lactam antimicrobials (including carbapenems), fluoroquinolones, macrolides and polymyxins (i.e., colistin), and occurrence of multidrug resistance (MDR) were assessed in the bacteria of interest.

A total of 304 studies fulfilled the criteria for inclusion in this review. Eligible studies were available from 58 different countries. For the UK, 15 studies were identified; from those, eight were original articles, five were targeted surveys conducted by the Food Standards Agency (FSA) and two were surveillance reports that assessed occurrence of AMR in foodborne pathogens (*Salmonella* spp. and *Campylobacter* spp.) and commensal bacteria in pork and poultry meat.

There was a paucity of AMR data for domestically-produced food in the UK. Also the lack of surveillance data (apart from those available through the surveillance reports of the European Food Safety Authority [EFSA]) did not allow the detection of trends in AMR in food in the UK; such data would be relevant for risk assessment of exposure of British consumers. The targeted FSA retail surveys conducted since 2001 provide "snapshots" of AMR in relevant foodborne pathogens (*Salmonella* spp. and *Campylobacter* spp.) in red meat and poultry meat. There is, nevertheless, a lack of AMR data on commensal bacteria in food at retail level in the UK.

In other countries, those with most eligible studies were the USA (n= 29) and Denmark (n= 27), and to a lesser extent, China (n= 17), Brazil (n= 16), Spain (n= 14) Poland (n= 14), Turkey (n= 9), the Netherlands (n= 10) and Thailand (n= 8), which are also some of the main food exporters trading with the UK.

Although evidence from the UK and USA seem to show that prevalence of AMR is higher in bacterial isolates from conventional systems than in free range and organic systems, the data available are limited; as such it was not possible to assess this aspect in a systematic manner.

Overall, there were 189 studies that covered AMR in organisms from poultry meat and 117 in organisms from pork meat; available evidence was more scarce for AMR occurrence in dairy products (n= 33), seafood (n= 32) and fresh produce (n= 27) suggesting a paucity of data for these food groups. Furthermore, limited information was available regarding the comparison of AMR levels between different production standards (i.e. organic *versus* conventional) in all bacteria considered at retail level.

For exporting countries in Europe, evidence was available through both national and EFSA's annual integrated surveillance reports for AMR in foodborne zoonoses and indicator bacteria in animals, humans and food. AMR data were of robust quality particularly in Nordic countries and in the Netherlands. In the remaining European countries, AMR data was inconsistent up to 2011, when the harmonisation criteria for sampling and antimicrobial susceptibility testing were introduced by EFSA for mandatory surveillance conducted by European Union's (EU) Member States (MSs)[1].

There is a heterogeneity in AMR levels observed across different countries that otherwise would not be detected if not for surveillance and research efforts focused on food at retail level. This heterogeneity could be due to differences in antimicrobial usage (AMU) in animal production systems as well as differences in food processing and hygiene practices in the different countries.

It was difficult to assess patterns of MDR as authors used different criteria to identify MDR and therefore results were not often comparable across studies. Furthermore, most studies reported only MDR results selectively and due to this it was not possible to extrapolate findings to determine and compare frequency of MDR in food groups of interest within and between countries.

There was limited, dated scientific evidence available for British pork or imported pork at retail level in the UK; low prevalence levels of erythromycin resistance in *E. faecalis* and *E. faecium* isolates (8.1 and 9.6% respectively) and absence of MDR isolates (i.e., 0% vancomycin-resistant Enterococci or VRE) were noted in 2002. Between 2003 and 2007, MDR was mainly observed in *S.* Typhimurium isolates but only a reduced number of these were assessed from British pork meat. It was therefore not possible to infer on MDR trends. There was also reporting of a very low prevalence (1%) of ESBL-producers in *E. coli* isolates from British pork meat. The paucity of data from UK-produced pork is in contrast with the more extensive surveillance data available in the main pork exporting European countries that trade with the UK (i.e., Denmark, the Netherlands). Denmark has reported an increase in ampicillin resistance (up to 73% in 2013) in salmonella isolates and very low prevalence levels of fluoroquinolone resistance (up to 6%). All salmonella isolates tested in recent years were susceptible to colistin. Ampicillin resistance was also increasing in *E. coli* isolates from Danish pork meat (up to 33% in 2012). In 2013, low prevalence levels of resistance to third generation cephalosporins (3GC) (< 1.5% to cefotaxime and ceftiofur) as well as to fluoroquinolones (< 1.4% for both nalidixic acid and ciprofloxacin) were observed in *E. coli* isolates from Danish pork meat. In the Netherlands, surveillance data available (MARAN) focused on commensal bacteria; very low prevalence of resistance to ampicillin (between 0.1 and 2%) was observed in isolates from pork. Higher resistance levels to erythromycin (15% and 41.4%) in *E. faecalis* and *E. faecium* isolates respectively, were detected in Dutch pork. As in Denmark, no VRE isolates were observed. In *E. coli*, a decrease in ampicillin resistance was noted down to 12.7% in 2014 from 34% in 2006; low levels of resistance to 3GC antimicrobials were detected (1.6% cefotaxime) also in *E. coli* isolates. No resistance to meropenem was observed in 2014 also in *E. coli*. A slight increase was noted in fluoroquinolone resistance but remained low

[1] Technical specifications for the analysis and reporting of data on antimicrobial resistance (AMR) in the European Union Summary Report, EFSA Journal 2012;10(2):2587 [53 pp.] at: https://www.efsa.europa.eu/en/efsajournal/pub/2587

(< 3%); very low resistance was also reported to azithromycin (0.9%) according to MARAN data. In German pork, only limited evidence was available for the bacteria of interest. *E. coli* isolates had the highest prevalence of clavulanate-amoxicillin resistance at 13.2% and no resistance to 3GCs (cefotaxime, ceftiofur) was observed. Resistance to fluoroquinolones in *E. coli* isolates from pork was low (< 1.5% to both enrofloxacin and ciprofloxacin) but this evidence is dated (2004) and therefore it should be interpreted carefully as it may not reflect the current position in relation to exported German pork.

The USA was the main exporting country trading pork with the UK outside Europe; in the USA, ampicillin resistance has increased to 13% but a reduction has been observed in cefotaxime resistance down to 0% in salmonella isolates in 2013 since 2002 (40%); no resistance to fluoroquinolones was detected in the same year but Clinical & Laboratory Standards Institute (CLSI) guidelines for monitoring resistance are in use in that country. Low prevalence levels of ampicillin resistance (4%) in *E. faecalis* and of penicillin (8%) resistance were stated for both *E. faecalis* and *E. faecium* isolates from pork meat. Erythromycin resistance was low (8%) in *E. faecalis* isolates. In contrast to European countries, MDR prevalence levels in enterococci were observed at 8.2% for *E. faecalis* but were considerably higher for *E. faecium* isolates at 54.6%. In *E. coli* isolates, amoxicillin-clavulanic acid and ampicillin resistance prevalence levels were low (0-8% and 11.5%, respectively), in 2013. As in other countries, resistance to 3GCs and to fluoroquinolones was below 1.5%. No resistance to azithromycin was observed in *E. coli* isolates from pork meat. Up to 13.9% of *E. coli* isolates were MDR in USA pork according to NARMS surveillance data but again no information was provided regarding phenotypes.

Poultry meat (including chicken and turkey) was the food group for which there was most evidence available for the UK. There has been an upwards trend to fluoroquinolones resistance since 2001, when resistance levels were at 12.6% and 15.6% in *Campylobacter jejuni* isolates from chicken meat from conventional systems, according to FSA surveys. Resistance to ciprofloxacin and nalidixic acid was at 15% and 22%, respectively in 2005, at 21.7% and 23.1% in 2007-2008 and at a high in 2014-2015, at up to 50% and 51.5%, a sharp increase since 2005. Erythromycin resistance in *C. jejuni* from British chicken has decreased between 2005 and 2014-2015 (levels of 5% and 4.2% down to 1%). Prevalence of MDR has increased in recent years from 19.1% in 2008 to 43.4% in 2014-2015 in *C. jejuni* isolates from chicken meat at retail level in the UK; the most common phenotype was ciprofloxacin, nalidixic acid, tetracyclines and trimethoprim (n= 71). Data were scarce and dated for AMR levels in commensal bacteria isolated from British poultry meat. High levels of penicillin resistance (90% and 98%) were observed in *E. faecalis* and *E. faecium*, respectively in 2000. In 2002, resistance to erythromycin was up to 33 and 42% in *E. faecalis* and at 20 and 53% in *E. faecium* isolates from chicken and turkey meat, respectively. MDR was not investigated in commensal bacteria from British poultry meat at retail level. No data were available for AMR frequency in *E. coli* isolates from British poultry meat.

In the Netherlands, high levels of resistance to ciprofloxacin and nalidixic acid (at 63.4% in 2014, a sharp increase from 39% in 2004) were detected in *C. jejuni* isolates from poultry meat; higher levels of resistance to these fluoroquinolones (up to 100%) were observed in isolates from Polish poultry. These two countries are major exporters of poultry meat to the UK. Other exporting countries outside Europe, such as Argentina, Brazil and Chile, similarly observed high levels of fluoroquinolone resistance. In contrast, the USA reported lower resistance levels to these antimicrobials at 21.3% and at 46.2% in isolates from chicken and turkey meat, respectively. In contrast to the UK, low erythromycin resistance levels in *C. jejuni* were reported in Netherlands (0.7%) and in the USA (< 10%); no erythromycin resistance was observed in Polish poultry. Higher levels of resistance were noted in isolates from Argentinian (20%) and Brazilian (68.8%) poultry meat; Chile reported similar levels to those observed in European countries (1.8%). No MDR isolates were detected from Dutch poultry meat through surveillance up to 2013, whilst in Poland, MDR levels up to 45% were noted in *C. jejuni* isolates from poultry meat but no information was provided on common phenotypes observed.

In commensal bacteria, data were limited in exporting countries. Low levels of ampicillin resistance (1.8%) and an increase of erythromycin resistance to 51.8% were reported in *E. faecalis* isolates from Dutch poultry in 2013, according to MARAN data. In the same country, ampicillin resistance has sharply decreased in *E. faecium* isolates from poultry meat between 2002 and 2013 (i.e., 16% down to 6%). Similar levels to erythromycin resistance to those of the UK were observed in *E. faecium* from Dutch poultry meat. In the USA, a downwards trend was observed in recent years in ampicillin resistance (from 44.2% to 9.9%) in *E. faecium* and erythromycin resistance, (from 45.5% to 35.1%) in *E. faecalis* for the same period. High levels of erythromycin resistance were detected in *E. faecalis* isolates from poultry meat in Brazil at 90.2% in 2004 but it was not possible to assess if these high levels have been maintained over time. No evidence was available for MDR prevalence in enterococci isolates from Dutch poultry meats at retail level. In contrast, MDR was reported in the USA of up to 69.7% and up to 79.4% in *E. faecalis* and *E. faecium* respectively, from poultry meat. High levels of MDR were detected particularly in turkey meat. Lower levels of MDR isolates (43.9%) were noted in enterococci from Brazilian poultry meat.

In the Netherlands, ampicillin resistance was down to 40.7% in in *E. coli* isolates from chicken meat in 2014 with the highest levels of resistance (65.9%) observed in isolates from turkey meat. Cefotaxime resistance has also decreased since 2002, down to 1.9%, according to surveillance data. Colistin resistance was higher in turkey meat (4%) than in chicken meat (1.5%) in the same country but no trends were reported. In *E. coli* isolates from Polish poultry meat, higher levels of resistance were observed to ampicillin (100%), and to cefotaxime (41.7%) compared to the Netherlands and no resistance to carbapenems was detected but this was only assessed by a single study.

In the USA, high resistance to β-lactams, particularly to ampicillin (57.9%), 3GCs (up to 90.1% to cefotaxime and 90.1% to ceftriaxone) and to fluoroquinolones (97.5%) was reported in *E. coli* isolates from poultry meat from conventional systems in 2010. Ampicillin resistance was up to 55% in the same country in a recent study. These results are higher than those reported by other studies in the same country conducted years earlier, though these were based on convenience sampling[2] (i.e., non-probability sampling). No resistance was reported to erythromycin in *E. coli* isolates from USA poultry meat.

It was not possible to ascertain MDR levels in *E. coli* isolates from poultry meat in exporting countries. In a 2014 study carried out in the Netherlands on ESBL-producing *E. coli* in poultry meat colistin resistance was detected in 1.7% of isolates. In the USA, data from 2002 estimated prevalence of MDR in *E. coli* isolates to be between 10 and 26%.

A lack of AMR data was observed for milk and dairy products, seafood and fresh produce. This is particularly worrying as there is evidence of national and multi-state/multi-national outbreaks of foodborne disease with these foods as the source of human exposure. No surveillance programs, to our knowledge, assess AMR bacteria in milk, dairy products or

[2] Convenience sampling (non-probabilistic sampling)- collection of easily accessible sampling units, which are not representative of the study population. Estimates obtained in studies that follow convenience sampling are biased and therefore, should be interpreted carefully.

seafood in a systematic manner. Fresh produce is covered by some surveillance systems in European countries (i.e., the Netherlands and by EFSA) but only in recent years.

It was not possible to assess the prevalence of AMR in commensal bacteria in milk and other dairy products at retail level in the UK due to the lack of scientific evidence and surveillance data between 1999 and 2016. Lack of surveillance data was also noted for the main exporters of milk and dairy products to the UK.

Raw milk consumption could present a risk of human exposure to drug-resistant microbes if milk is not subsequently treated by pasteurisation or other forms of heat treatment which reduces the dissemination of resistant bacteria. Further research is required to quantify risk of exposure derived from milk and dairy products.

In exporting countries, limited data were available for France, Turkey and the USA; most of these studies were conducted over 10 years ago and therefore their findings may not be relevant due to changes in dairy production practices observed and in subsequent resistance trends in commensal bacteria. No resistance to amoxicillin was detected in *E. faecalis* isolates from French cheeses in 2005. Levels of ampicillin resistance of up to 36.5% and 43.8% to ciprofloxacin and to 91.7% to erythromycin were reported in *E. faecalis* isolates from milk and cheese in Turkey in 2005. Levels of resistance to ampicillin (up to 47.1%), ceftriaxone (up to 80%) and to erythromycin (96%) in *E. faecium* isolates from dairy products were detected in that year. A recent study in Turkey assessed MDR in *E. faecium* isolates from cow's milk and reported the absence of VRE. It was not possible to assess MDR across countries due to the paucity of data. In the USA, ampicillin resistance of up to 80% and to a lesser extent to ceftriaxone (30%) were observed in *E. coli* isolates from raw cow's milk; these were fully susceptible to fluoroquinolones. MDR was only observed in a small number of isolates from dairy production in both European and in the USA and it was therefore not possible to extrapolate findings. No data were available for other exporting countries such as the Republic of Ireland, Canada, New Zealand, and Israel or from the United Arab Emirates (UAE).

It was not possible to assess the frequency of AMR in commensal bacteria in seafood and fresh produce in the UK and main European exporting countries due to the lack of scientific evidence.

AMR data were only available for *E. coli* isolates from seafood at harvest and retail levels for exporting countries outside Europe (i.e., China, Vietnam and USA). In Asian countries, the highest levels of ampicillin resistance (78.9%) were observed in *E. coli* isolates from farmed fish in China, compared to Vietnam (30% in shellfish). In China, resistance to cefotaxime was low (2.3%) and no resistance was observed to ceftiofur whilst resistance to ciprofloxacin and nalidixic acid was at 4.1% and 16%, respectively in *E. coli* isolates from farmed fish. In Vietnam, slightly higher resistance levels were observed to ciprofloxacin (10%) but higher levels of resistance were noted against nalidixic acid (25%) in *E. coli* isolates from farmed seafood (fish and shrimp) at retail level. For the USA, because the study assessed only included one isolate, this was not considered for the discussion. MDR was reported in China and Thailand; China noted 1.5% ESBL-producing *E. coli* isolates from farmed fish, whilst studies in Vietnam detected higher prevalence of ESBL-producers of 18.3% in isolates from farmed shrimp.

Amongst the main exporting European countries trading with the UK, only the Netherlands assessed AMR in vegetables and fruit as part of the MARAN surveillance program. Limited evidence was available for Spain, which is the main exporter of fresh produce to the UK. Outside Europe, limited evidence was available for the USA, Brazil and South Africa. In the Netherlands, no ampicillin resistance was reported in both *E. faecalis* or *E. faecium* isolates from fresh produce but resistance to erythromycin was at 6.3 and 25.8%, respectively. In 2012, in *E. coli* isolates from fresh produce, low prevalence levels of ampicillin, ciprofloxacin and nalidixic acid resistance (between 1.5 and 2.3%) and no resistance to either cefotaxime or colistin were detected. It was not possible to assess MDR across countries due to the paucity of data.

General recommendations

Recommendations resulting from this systematic review include (in no particular order of importance):

- Standardisation in the selection of antimicrobials for antimicrobial susceptibility testing panels as recommended by EFSA[3], the use of epidemiological cut-off values (ECOFFs) for surveillance of resistance, adoption of a standardised definition for MDR, the adoption of random sampling and adequate study design for epidemiological studies and when implementing surveillance systems for determination of AMR in the food chain as previously recommended in the Advisory Committee on the Microbiological Safety of Food (ACMSF) report published in 1999[4].

- Surveillance priorities could be set using a risk-based approach, taking into account the importance of antimicrobials used for treatment in both humans and animals, and continued surveillance of the prevalence and emerging resistance (including MDR) in commensal bacteria (*Enterococcus* spp. and *E. coli*) is also important.

- There is a lack of information on AMR bacteria in foods of animal origin other than meat at retail level. In recent years, there have been growing numbers of outbreaks associated with milk and dairy products (cheese, butter, yogurt), seafood (fish and shellfish) and fresh produce (fruit, vegetables and salads) at national and international levels but there is scarce, scattered evidence of resistance and MDR occurrence in foodborne and commensal bacteria in these food products and its implications for public health. These gaps should be addressed also using a risk-based approach following evidence of resistance in food items as well as the extent of expected consumer exposure using consumption and import volumes.

- Efforts should be made to continue to monitor resistance trends (AMR and MDR) in *Campylobacter* spp. strains and commensal bacteria from both imported and domestically-produced poultry meat in the UK; differentiation should be made for different types of poultry meat sampled (i.e., chicken and turkey meat) due to variations observed in farming management practices across species.

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[3] Technical specifications for the analysis and reporting of data on antimicrobial resistance (AMR) in the European Union Summary Report, EFSA Journal 2012;10(2):2587 [53 pp.] at: https://www.efsa.europa.eu/en/efsajournal/pub/2587

[4] https://acmsf.food.gov.uk/committee/acmsf/acmsfsubgroups/amrwg

- Research and surveillance should be developed to monitor AMR and MDR levels in foodborne pathogens (e.g., *Salmonella* spp.) and commensal bacteria from imported and domestically-produced pork meat in the UK.

- Data on antimicrobial use (AMU) in food-producing animals in the UK are important to explain the occurrence and dynamics of AMR, resistance genes and MDR phenotypes in a defined geographical area. More complete information should therefore be collected on the type of production system from which food samples originate to assess the impact of animal husbandry practices as risk factors for resistance.

- There is a need for more studies to quantify the contribution of both domestic and imported foods to the occurrence of AMR. Information on country of origin for imported products should be collected.

- Priorities should be set according to the importance of a food item in terms of exposure of consumers; for this purpose, antimicrobial consumption data by food animal species are essential for assessing the risk to British consumers via meat.

- Finally, further research and surveillance are needed to establish and quantify the risk of transmission to humans of AMR to critically important antimicrobials (CIAs) in organisms from foods of both animal and non-animal origin.

Glossary

ABC of FSM- Antibiogram Committee of French Society of Microbiology

ACMSF- Advisory Committee on the Microbiological Safety of Food

AMR- Antimicrobial resistance

AMU- Antimicrobial use

AST- Antimicrobial susceptibility testing

BSAC- British Society for Antimicrobial Chemotherapy

CDC- Centers for Disease Control and Prevention (Texas, USA)

CIAs- Critically-important antimicrobials

CIPARS- Canadian Integrated Program for Antimicrobial Resistance Surveillance

CLSI- Clinical and Laboratory Standards Institute

CRE- Carbapenem- resistant Enterobacteriaceae

DANMAP- Danish Programme for surveillance of antimicrobial consumption and resistance in bacteria from animals, food and humans

DIN- German Institute of Standards

ECDC- European Centre for Disease Prevention and Control

ECOFF(s)- Epidemiological cut-off value

EFSA- European Food Safety Authority

EMA- European Medicines Agency

ESBL(s)- Extended-spectrum β-lactamase(s)

EU- European Union

EUCAST- European Committee on Antimicrobial Susceptibility Testing

ExPEC- Extra-intestinal pathogenic *Escherichia coli*

FAO- Food and Agriculture Organisation of the United Nations

FINRES-VET- Finnish Program for Monitoring of Antimicrobial Resistance

FSA- Food Standards Agency

MAR- Multiple Antibiotic Resistance index

MARAN- Monitoring of Antimicrobial resistance and Antimicrobial Usage in Animals in the Netherlands

MDR- Multidrug resistance

MeSH- Medical Subject Headings

MIC- Minimum Inhibitory concentration

MRSA- Methicillin-resistant *Staphylococcus aureus*

MSs- Member States

NARMS- National Antimicrobial Resistance Monitoring System

NCCLS- National Committee for Clinical Laboratory Standards (renamed as CLSI after 2005)

NORM/ NORM-VET- Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway

ND- No data

N/A- Not applicable

N/S- Not stated

OIE- World Organisation for Animal Health

RoI- Republic of Ireland

spp.- Species

SVARM- Swedish Veterinary Antimicrobial Resistance Monitoring

UAE- United Arab Emirates

UK- United Kingdom

USA- United States of America

VMD- Veterinary Medicines Directorate

VRE- Vancomycin-resistant enterococci

WHO- World Health Organization

WHONET- WHO Collaborating Centre for Surveillance of Antimicrobial Resistance

3GC(s)- Third generation cephalosporins

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Background

Antimicrobial resistance (AMR) is a major public health issue. It leads to therapeutic failure and to increased morbidity and mortality of those affected with infections caused by resistant pathogens. Drug-resistant pathogens are estimated to be responsible for 25,000 deaths every year in Europe (1) and predictions are that these numbers will rise up to 390,000 by 2050 (2). Resistant infections have a negative economic impact in healthcare with a cost up to €1.5 billion annually (1). The epidemiology of AMR is complex; humans can become exposed through varied pathways such as; hospital-acquired, environmental, direct contact with pets, wildlife, food-producing animals or humans, but also through water and food. Antimicrobial use (AMU) is one of the major factors associated with the emergence and spread of AMR (3). Antimicrobials are widely used in agriculture to prevent and treat infectious diseases in livestock and plants and, in some countries outside the EU they are also used as growth promoters (AGPs) in food-producing animals (4, 5). In EU Member States, the use of AGPs has been progressively prohibited since 2006 (6). AMU is regulated in most European countries but in many countries outside the EU, antimicrobials can be purchased over the counter or are counterfeit and their use occurs often without veterinary supervision. This could pose a serious risk to consumers, as individuals could later become exposed through food to drug-resistant bacteria, resistance determinants (i.e. genes) or antimicrobial drug residues that could result in selective pressure in the gut flora. Drug-resistant foodborne pathogens such as fluoroquinolone-resistant *Campylobacter* spp. and extended-spectrum β-lactamase (ESBLs)-producing bacteria have been isolated with increasing frequency in food, food-producing animals and humans in Europe (7). Until recently, colistin resistance was thought to be only transmitted vertically but there is now evidence for horizontal transmission across bacterial strains by plasmid-borne colistin resistance determinants (8, 9). Colistin-resistant *Escherichia coli* with plasmid-mediated resistance to this antimicrobial has been recently reported in Denmark in imported frozen poultry and transmission through the food chain to humans of such organisms has been demonstrated (10). Plasmid-mediated resistance to colistin has also been identified in salmonellosis cases in humans in other European countries (8). This resistance trait has also recently been observed in pig farms in the UK (11) and in in *E. coli* isolates from broilers and turkeys in several European countries, although still at low levels (8). Furthermore, MDR in isolates of particular in salmonella serovars is a cause for public health concern (8).

There is the perception that the food chain is an important pathway for transmission of drug-resistant pathogens to humans (12); it is uncertain if this is the current trend for AMR transmission or if it is due to the selective reporting of foodborne outbreaks and target surveillance. Phylogenetic and whole genome sequence analysis of *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 in human and bovine livestock populations in Scotland has shown a greater diversity of AMR genes in isolates of DT104 from humans, by comparison to isolates found in local bovine livestock populations. This suggests that there may have been contributing animal sources other than bovine food-producing animals or foods derived from those (13, 14).

Aims and objectives

The aim of our study was to identify, appraise and summarise existing evidence of prevalence of antimicrobial resistance (AMR) observed in foodborne pathogens and commensal bacteria at retail level that could pose a risk to UK consumers. The scope of the study was restricted to AMR in pork, poultry meat, dairy, seafood and fresh produce. A systematic review was conducted through which evidence published in scientific and grey literature between 1999 and the end of May 2016 was collected and assessed. For the purpose of this review we examined resistance to specific critically important antimicrobials (CIAs), as defined by the World Health Organization (WHO) in 2012 (15): β-lactams (with emphasis in third generation cephalosporins or 3GCs and including carbapenems), fluoroquinolones, macrolides and polymyxins (colistin). This is a change from the original scope that was to assess transmission of AMR at different steps of the food chain and its impact on public health. The scope of the review was redefined after expert consultation and preliminary scope searches (please see Methods section for further information). The findings will be used to make recommendations and to identify areas where there are gaps in knowledge to guide future research efforts.

Methods

Scope search

A scope search was conducted to explore the extent and range of studies published between 1999 and 2015 using PubMed. For this purpose wide search terms covering the theme of interest were used (e.g., antimicrobial resistance, by food item and livestock species) to ascertain the volume of scientific publications available for the period of interest. Evidence from integrated surveillance reports at European level (European Food Safety Authority or EFSA) (16) was taken into consideration for identifying relevant food and bacterial combinations in the context of AMR and food safety. An assessment of the number of studies for each of the nodes ("categories") identified was conducted.

International and national experts in the AMR field from academic and governmental institutions were approached for external review of the research questions and eligibility criteria taking into account the scope of the systematic review in two separate exercises. Initially, the experts were provided with the research questions proposed in the initial tender. The initial research questions were;

a) What are the patterns, flow direction and frequency of AMR observed in foodborne pathogens and commensal bacteria transmitted via the food chain (i.e., from farm to fork) to humans that could pose a risk to UK consumers?
b) What are the patterns and frequency of AMR observed in foodborne pathogens and commensal bacteria transmitted through imported food that could pose a risk to UK consumers?
c) What is the impact of i) of infections caused by antimicrobial-resistant bacteria transmitted via the food chain in humans and ii) of bacteria carrying resistance determinants derived from food in the UK (e.g., morbidity, mortality and hospitalization rates and duration)?

The original research questions were deemed too broad for the purpose of a single systematic review. Research questions were then redefined accordingly based on the feedback provided by the experts and were further revised by the same experts during the second elicitation exercise (see below). Impact of infections caused by resistant pathogens was addressed in the background and discussion sections of this report. Furthermore, the experts were requested to provide a list of grey literature and/or scientific studies that they deemed relevant for inclusion in the review.

Research questions of this review

The final research questions used for the purpose of this review were developed taking into consideration evidence for relevant resistant foodborne pathogens and commensal bacteria observed in animals, food of animal origin and humans in European countries published by EFSA (European Food Safety Authority) (http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/4036.pdf) (16), feedback provided by experts and findings from scope searches of the literature (i.e., PubMed):

1) What is the prevalence of resistance (i.e., phenotype) observed in selected foodborne pathogens in the following meats of animal origin:

   a) *Salmonella* spp. (non-typhoidal isolates only) in pork meat to:

      i) Penicillins: Ampicillin or amoxicillin
      ii) Third generation cephalosporins: Cefotaxime or ceftriaxone or ceftiofur
      iii) Carbapenems: Imipenem, ertapenem or meropenem
      iv) Quinolones- Nalidixic acid
      v) Fluoroquinolones- Ciprofloxacin and enrofloxacin
      vi) Macrolides- Azythromycin
      vii) Polymyxins- Colistin

   b) *Campylobacter* spp. (*C. jejuni*) in poultry meat (including chicken and turkey meat) to:

      i) Fluoroquinolones: Ciprofloxacin and nalidixic acid
      ii) Macrolides: Erythromycin
      iii) Beta-lactams: Not deemed relevant
      iv) Polymyxins: Colistin

2) What is the prevalence of resistance (i.e., phenotype) observed in selected commensal bacteria in pork meat, poultry meat dairy products, seafood and fresh produce:

   a) *Enterococcus* spp. (*E. faecalis*, *E. faecium*) to:

      i) Penicillins: Ampicillin or penicillin only (cephalosporins and carbapenems are not relevant)
      ii) Macrolides: Erythromycin
      iii) Fluoroquinolones: Not deemed relevant
      iv) Polymyxins (colistin): Not deemed relevant

   b) *Escherichia coli* to:

      i) Penicillins: Ampicillin or amoxicillin
      ii) Third generation cephalosporins: Cefotaxime or ceftriaxone or ceftiofur
      iii) Carbapenems: Imipenem, ertapenem or meropenem
      iv) Quinolones- Nalidixic acid
      v) Fluoroquinolones: Ciprofloxacin and enrofloxacin
      vi) Macrolides: Azythromycin
      vii) Polymyxins: Colistin

Note$_1$: Food handlers were removed from the scope of the search due to the limited available number of studies identified and relevance during scope searches.

Note$_2$: Focus was given to assessing resistance at retail level (post-harvest stage) for pork, poultry meats, and fresh produce as it was perceived to be the point at which consumers were more likely to be exposed thereof. For dairy and seafood, consideration was given to studies focused on harvest and post-harvest stages.

Note$_3$: Livestock- associated methicillin-resistant *Staphylococcus aureus* (MRSA-398) were not assessed as such organisms have been covered under another FSA project.

Eligibility criteria

Only studies that considered fresh meat (pork and poultry only), dairy (milk and dairy products such as butter and cheese) as well as fresh produce (fruit and vegetables, including nuts) and seafood (fish and shellfish) were considered for inclusion. Only foods at retail level were considered for inclusion in this review, apart from seafood and dairy products, for which studies at harvest and post-harvest were included, due to the difficulty of segregation of studies in these two stages of the food chain. In this review, food items produced domestically (UK origin) and in the main exporting countries were assessed, due to the possibility of those foods being imported into the UK that could result in exposure to AMR by British consumers. Additional data on AMR from other countries is presented in **Appendix 3**. Original scientific articles, literature, systematic reviews, scientific opinions and surveillance reports published since 1999 until the end of September 2015 were considered for the purpose of this review. As colistin resistance emerged as a major public issue in November 2015, studies assessing colistin resistance were assessed if published up to and including May 2016. Full text manuscripts of papers that were published in English, Spanish, Portuguese and Italian were considered, as these were languages covered by researchers within the consortium.

Exclusion criteria considered included; highly processed foods (i.e., any food that has been altered from its natural state in some way, either for safety reasons or convenience**)**. Processed foods include; breakfast cereals, tinned vegetables, bread, savory snacks, ready meals, drinks (e.g., soft and carbonated drinks). Meat products (e.g., products that have been processed so that they do not look like fresh meat, for example bacon, ham or salami) and canned foods were not considered. Any type of study (or part of a study) that assessed prevalence of resistance, transmission of resistant bacteria or resistance determinants to humans in/from the following sources: companion animals (including horses) or exotic pets, direct contact with wildlife; healthcare settings (nosocomial infections) unless primary cause was a foodborne pathogen of animal origin (i.e., pork or poultry meat, seafood or dairy products) or from fresh produce (i.e., fruit or vegetables), occupational settings in veterinary practice, humans, when humans are deemed to be the source of primary infection and any studies considering horse meat were not included as in the UK horses are deemed as companion animals and horse meat consumption by British consumers is negligible.

Definitions used

Foodborne pathogens (adapted from EFSA definition) [5]

"*These are pathogenic (disease-causing) micro-organisms such as bacteria (…). Humans get foodborne infections usually through the consumption of food or drinking water contaminated by these bacteria. Infection can also occur through direct contact with food-producing animals or contaminated environment. Human-to-human transmission through faecal-oral route can also occur (e.g., secondary transmission from primary cases). They enter the body through the gastrointestinal*

[5] http://www.efsa.europa.eu/en/topics/topic/foodbornezoonoticdiseases

tract where the first symptoms often occur. Many of these micro-organisms are commonly found in the intestines of healthy food-producing animals. The risks of contamination are present from farm to fork and require prevention and control throughout the food chain".

Please note that for the purpose of this systematic review, we focused on specific foodborne pathogenic bacteria (i.e., *Salmonella* spp. in pork meat and *Campylobacter* spp. in poultry meat).

Commensal bacteria (EFSA definition) (17): *"Are those bacteria that live in or upon the (human or the animal) host without causing disease. Mostly, this co-existence is of mutual benefit. However, many commensals can cause disease if they enter body sites that are normally sterile or when the host's immune defence is impaired".*

Indicator bacteria (EFSA definition) (17): "*Those micro-organisms that are used to represent Gram-positive and Gram-negative bacteria present in the gut flora of humans and animals. EFSA recommends the use of* E. coli *(Gram-negative) and Enterococci (i.e.,* E. faecium *and* E. faecalis*) as indicators for Gram-negative and Gram-positive bacteria, respectively. The reasoning provided for the selection of these bacteria as indicators is that most resistance phenotypes present in the animal populations are usually also present in these species; these bacteria are deemed to suffer similar selective pressure and exposure to resistance determinants that other micro-organisms present in the gut flora".* According to EFSA, indicator bacteria are more suitable for the assessment of selective pressure caused by antimicrobial therapy than foodborne pathogens in livestock species due to being ubiquitous in the gut flora.

For the interpretation of AMR in the selected studies, the WHO definition was applied[6]: "*Antimicrobial resistance is resistance of a microorganism to an antimicrobial drug that was originally effective for treatment of infections caused by it. Resistant microorganisms (including bacteria, fungi, viruses and parasites) are able to withstand attack by antimicrobial drugs, such as antibacterial drugs (e.g. antibiotics), antifungals, antivirals, and antimalarials, so that standard treatments become ineffective and infections persist, increasing the risk of spread to others*".

This systematic review focused on resistance to naturally-produced, semi-synthetic and synthetic antibacterial drugs. Antivirals, antifungals and antimalarial drugs as well as biocides and heavy metals were not considered for the purpose of this systematic review. Both biocides and heavy metals are acknowledged by the authors as being capable of causing selective pressure for the occurrence of AMR in bacteria of public health interest (18).

Resistance of microorganisms was assessed at phenotype level for specified groups of antimicrobials deemed as critically important for human medicine (as defined by the WHO). Such antimicrobials include β-lactams, including carbapenems, fluoroquinolones, macrolides and polymyxins (e.g., colistin); loss of efficacy of these antimicrobials to treat severe, life-threatening bacterial infections in humans is a major public health issue (15).

[6] http://www.who.int/mediacentre/factsheets/fs194/en/

Multidrug resistance (MDR)

The occurrence of multidrug resistance (MDR) in commensal and pathogenic bacteria in the food chain was also assessed. For this purpose, the definition developed by Magiorakos *et al* (19) and EFSA (16) was applied; MDR bacteria are "*bacteria that have acquired non-susceptibility to at least one agent in three or more specific antimicrobial categories*"; tables for assessment of MDR for each bacteria species taking into account specific antimicrobial categories and intrinsic resistance are provided in **Appendix 1.** This definition was elaborated by a group of international experts from the European Centre for Disease Prevention and Control (ECDC) and the Centers for Disease Control and Prevention (CDC) and it is used by EFSA and ECDC.

PICO strategy

The research questions were used to define the PICO (Population, Intervention or Exposure, Comparator and Outcome) (Table 1). The PICO guided the definition of the search terms of interest for the identification of potential eligible studies for the purpose of the systematic review. The final search strategy was conducted through science database search engines, grey literature websites (e.g., national and international surveillance reports), citation tracking and experts in the domain area to identify potential relevant studies (Table 2). The search criteria were piloted by a single researcher to generate the final search terms. Any searches of the literature and criteria used were documented at all times to allow replication of the methodology used. Free text searches covered both title and abstract. Searches included MeSH (Medical Subject Headings) thesaurus headings and free text terms that cover PIO criteria (e.g. population, interventions[7] and outcomes). The free terms and MeSH headings combined with the Boolean operator "OR" and were combined with the operator "AND", at a later stage of the search process, following these two steps; (1) "population" AND "intervention" AND "outcomes" (PIO) terms. The combinations of search terms across the PIO groups were extracted separately to produce the final list of search hits from each database. Search terms for comparators were not defined, as studies with and without comparator were automatically included in the study search.

[7] Interventions were not considered for the purpose of this review as stated in Table 1. Nevertheless, authors chose to use the established PIO and PICO definitions, as those are the ones applied in systematic reviews.

Table 1- PICO strategy that was followed for the purpose of the systematic review.

PICO	Description
Population	Specified foodborne pathogens, *Salmonella* spp. were assessed in pork and *Campylobacter* spp. in poultry meat. For fresh produce (i.e., fruit and vegetables), milk and dairy products, fish and shellfish, only commensal bacteria (*Enterococcus faecium*, *Enterococcus faecalis* and *Escherichia coli*) were investigated.
(Intervention), Exposure	In this particular review, impact of intervention measures to reduce antimicrobial resistance was not investigated; the study focused on the assessment of prevalence of resistance in specific foodborne pathogens and commensal bacteria that could pose a risk for the final UK consumer: • Poultry and pork meat at retail level • Dairy at harvest and post-harvest levels • Seafood at harvest and post-harvest levels • Fresh produce at retail level Resistance to the following critically important antimicrobials groups were assessed for the bacteria of interest: • Beta-Lactams (including carbapenems) • Fluoroquinolones • Macrolides • Polymyxins (colistin) Furthermore, occurrence of MDR was also investigated for all bacteria of interest across food items.
Comparator(s)	This was not deemed as applicable for most of the eligible studies considered for the purpose of this review as this review is not focused on interventions per se. Nevertheless, comparators of interest considered for the purpose of this review included: • AMR and MDR prevalence in bacteria of interest from domestically-produced versus imported food groups; • AMR and MDR prevalence in bacteria of interest from food groups derived from conventional versus free-range and/or organic production systems
Outcome(s)	Assessment of prevalence and resistance patterns (phenotype) in bacterial populations of interest (see above "Population"). For the purpose of this systematic review, we assessed outcomes, such as the ones defined below: • Prevalence; • Counts (if number of isolates assessed was below 10); • MDR phenotypes Note: when there was lack of quantifiable outcomes, expert opinions or relevant facts were considered if available (e.g., EFSA expert opinions, literature reviews).

Study screening process

All search hits were imported automatically or manually into a reference management software (Endnote X7.3.1, Thomson Reuters, Philadelphia USA) to collate the identified literature. All identified studies and other relevant literature were screened by the authors for eligibility using a three-stage sifting approach to screen the title, abstract and full text adopting a single reviewer approach for each study. All duplicates were removed prior to the 1st stage sifting process through the reference software. The research team worked under the supervision of the Principal Investigator. A random check of excluded studies was conducted by the Principal Investigator and any discrepancies observed were discussed amongst reviewers.

Furthermore, a sample of data extracted was validated by the Principal Investigator. The number of documents identified and screened out was recorded at each stage and presented accordingly in a PRISMA diagram (please see below "Results" section).

Table 2 – Final study search strategy.

Category	Sources
Scientific databases	• Science Direct (http://www.sciencedirect.com/) • Web of Science (http://wok.mimas.ac.uk/) • PubMed (http://www.ncbi.nlm.nih.gov/pubmed/)
Reference tracking	Reference lists of all studies selected for inclusion were searched to identify further relevant studies
Grey literature	• http://www.globalhealthlibrary.net/php/index.php (World Health Organization) • www.cdc.gov (Centre for Diseases Control and Prevention, USA) • www.ecdc.europa.eu (European Centre for Diseases Prevention and Control) • www.phe.gov.uk (Public Health England, UK) • www.efsa.europa.eu (European Food Safety Authority) • www.ema.europa.eu (European Medicines Agency) • www.fao.org (Food and Agriculture Organisation of the United Nations) • www.food.gov.uk (Food Standards Agency) • http://www.vmd.defra.gov.uk (Veterinary Medicines Directorate, UK) • http://www.danmap.org/ (DANMAP, DTU, Denmark) • http://www.vetinst.no/ (NORM-VET, Norwegian Veterinary Institute, Norway) • http://www.rivm.nl/ (MARAN, RIVM, The Netherlands) • http://www.sva.se/ (SVARM, SVA, Sweden) • http://www.fda.gov/ (NARMS, FDA/CDC, USDA, USA) • http://www.phac-aspc.gc.ca/ (CIPARS, Public Health Agency of Canada, Canada) • Consultation with AMR experts

Data extraction process

A template for data extraction was prepared by the research team based on the PIO (Population, Intervention and Outcome(s)) presented in Table 1 as an Excel document (Microsoft Office 2013, Microsoft UK, Berkshire UK). This template was tested prior to implementation. Once implemented, the template was used by reviewers to collect the data from eligible studies. Study characteristics (e.g., study design, sample size, sampling methods amongst others) and outcome(s) of interest were described and summarised accordingly. To synthesise the data extracted and evaluate its quality a narrative approach was used according to the framework described by the Economic and Social Research Council and recommended by the University of York Centre for Reviews and Dissemination (http://www.york.ac.uk/inst/crd/) (20). This was used to; a) develop a preliminary synthesis of findings of the integrated studies, b) investigate relationships within and between studies (e.g., prevalence of resistance in the UK and other countries that are exporters to the UK of food products of interest) and c), evaluate the degree of robustness of the synthesis. The findings of this study were used to inform the Food Standards Agency (FSA). Gaps in knowledge identified through this review will help to guide research in the domain of interest.

Risk of bias assessment

The risk of bias assessment was conducted only for studies where random sampling (e.g., randomised clinical control trials, longitudinal cohort, case-control studies or cross-sectional) was reported by the authors. For studies where convenience sampling was applied an assumption was made of low quality and of high risk of bias, as results were not deemed to be representative for the food item of interest. Expert opinions and literature reviews were not assessed for bias; nevertheless, findings of these studies were still considered for inclusion in the systematic review, if deemed relevant by the research team. Risk of bias assessment was conducted after the data extraction process. Templates were created in Word document for the assessment of risk of bias according to study design (**Appendix 2**). Bias was assessed following the criteria stipulated by the PRISMA statement (21). For this effect, bias in individual studies was assessed at a) study (i.e. large reporting of small against large scale studies), and b) outcome (i.e. selective reporting). The Newcastle-Ottawa Scale (NOS) tool was adapted and used to assess risk of bias at study and outcome levels in observational studies (22).

Results

For the purpose of this review, 8,520 scientific studies were screened for AMR occurrence in pork and poultry meat, dairy products, seafood and fresh produce at retail level.

A total of 304 studies fulfilled the criteria for inclusion in this review. Eligible studies were available from 58 different countries; for the UK, eight original articles, five FSA surveys and two surveillance reports were eligible for inclusion. In other countries, the country with most eligible studies were the USA (n= 29) and Denmark (n= 27), and to a lesser extent, China (n= 17), Brazil (n= 16), Spain (n= 14) Poland (n= 14), Turkey (n= 9), Netherlands (n= 10) and Thailand (n= 8), which are also some of the main food exporters trading with the UK.

Overall, there were 189 studies that covered AMR in poultry meat and 117 in pork meat; there was less evidence available for AMR occurrence in dairy products (n= 33), seafood (n= 32) and vegetables and fruit (n= 27), that indicate a paucity of data for these food groups. Furthermore, there was scarce information regarding the comparison of AMR prevalence levels between different production standards (i.e. organic *versus* conventional) in all bacteria considered at retail level. It was therefore not possible to assess this aspect in a systematic manner. Overall, there were 82 eligible studies that assessed occurrence of AMR and MDR in *Campylobacter jejuni* in poultry meats, 142 for *Enterococcus* spp. (*E. faecalis* and *E. faecium*) and 149 for *Escherichia coli*, respectively in poultry, and pork meats, dairy, fresh produce and seafood and 59 studies for *Salmonella* spp. (non-typhoidal) in pork meat (Fig. 4).

A word of caution: Most studies included in this review were deemed at a high risk of bias due to the lack of representativeness of data (i.e. findings were based on convenience sampling) and lack of comparability of studies (i.e., diversity of methods used to assess susceptibility to antimicrobials).

From the studies assessed, only 32 (9.5%) conducted random sampling and were mostly studies based on surveillance data - either directly through surveillance reports (EFSA, DANMAP, MARAN, SVARM, FSA surveys) or indirectly through scientific articles (UK *Campylobacter* spp. surveys and Chinese National Surveillance Program for Foodborne Pathogens). These studies presented a low to moderate risk of bias (see **Appendix 2**). Summary tables of AMR data per bacteria and food item of interest are presented in **Appendix 3**; a full list of the eligible studies is available in **Appendix 4**. All raw data extracted can be assessed in **Appendix 5**.

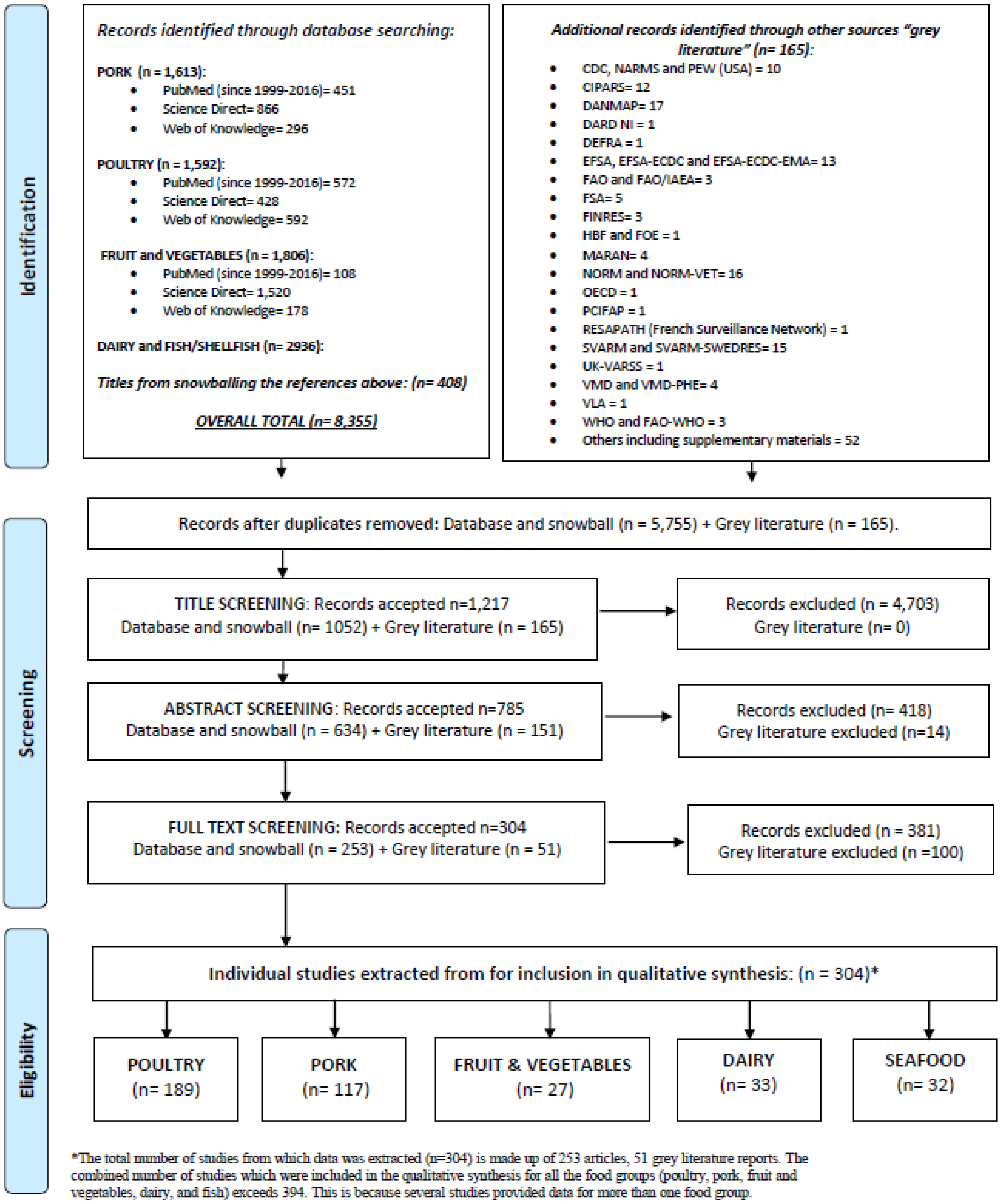

Figure 1- PRISMA diagram of the three-stage screening process for poultry and pork meat, dairy products, seafood and fresh produce (n= 304) (23).

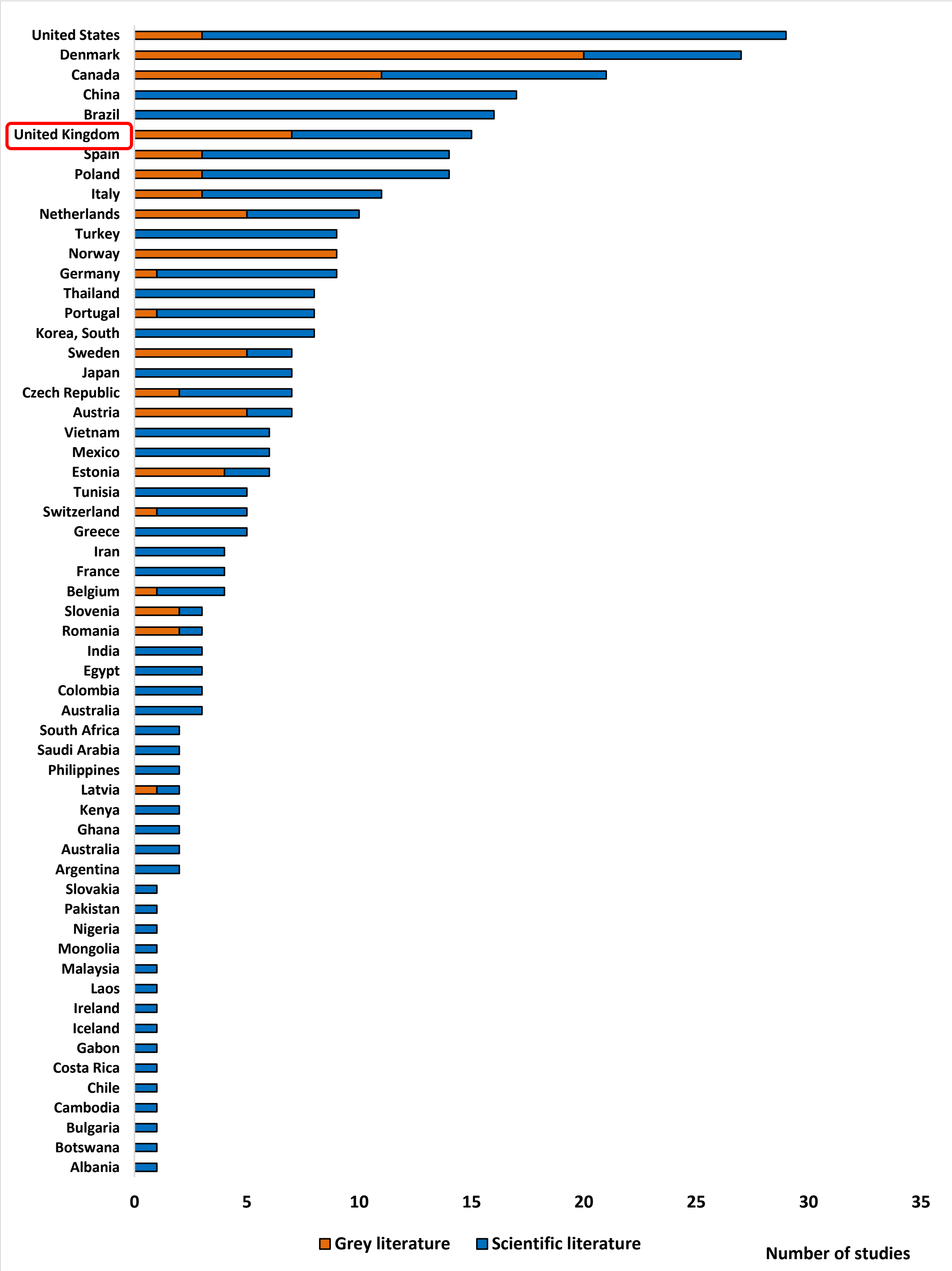

Figure 2- Number of scientific studies and grey literature identified per country deemed eligible for inclusion in this review.

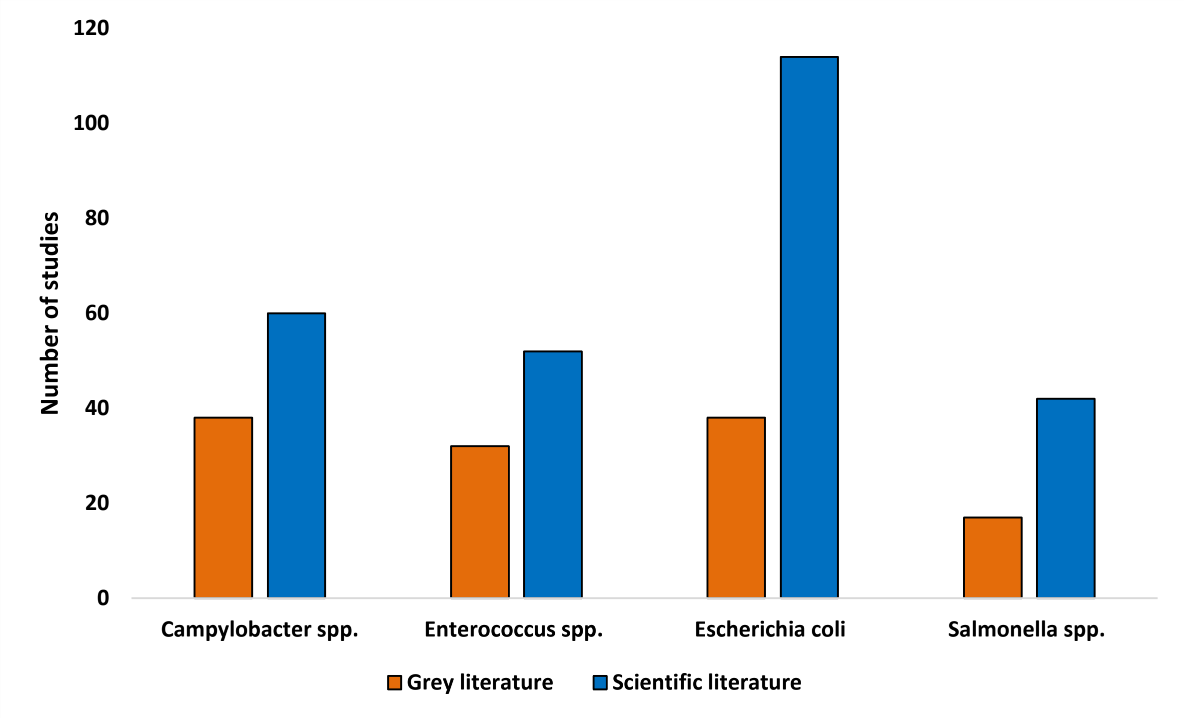

Figure 3- Number of eligible studies per bacteria of interest.

Please note that some studies were included more than once as they covered more than one eligible bacterial species or strain. "*Campylobacter* spp." included *C. jejuni*, *C. jejuni* and *C. lari*; "*Enterococcus* spp." included only *E. faecalis* and *E. faecium* and "*Salmonella* spp." covered a wide range of Salmonella serovars including *S.* Derby, *S.* Typhimurium, *S.* Kentucky, *S*. Heidelberg, *S.* Agona amongst others.

It was estimated that 68.1% of studies applied clinical MIC (Minimum Inhibitory Concentration) breakpoint criteria to assess susceptibility of isolates to antimicrobials of interest; for this estimation, the reviewers assumed that CLSI guidelines (if not stated otherwise by authors) were related to clinical breakpoints. Only a small proportion of studies (n= 37) followed epidemiological cut-offs (ECOFFs) to assess AMR in foodborne pathogens and commensal bacteria occurring in the food groups of interest. For approximately a fifth of the studies (n= 64) it was not possible to assess characteristics of breakpoints applied by authors (even when specific standards for antimicrobial susceptibility testing or AST were reported) (Fig. 4).

A large number of eligible studies (70.4%) included in this review followed CLSI and NCCLS[8] guidelines for the purpose of determining occurrence of AMR; one of the studies reported using both sets of standards (Fig. 5). Only 14.5% of studies used EUCAST standards to support their interpretation of AST. One study applied both EUCAST and CLSI guidelines. Four percent of studies followed other guidelines (e.g. National guidelines). Nine percent of studies did not provide any indication of the guidelines followed.

[8] Renamed as CLSI after 01/01/2005.

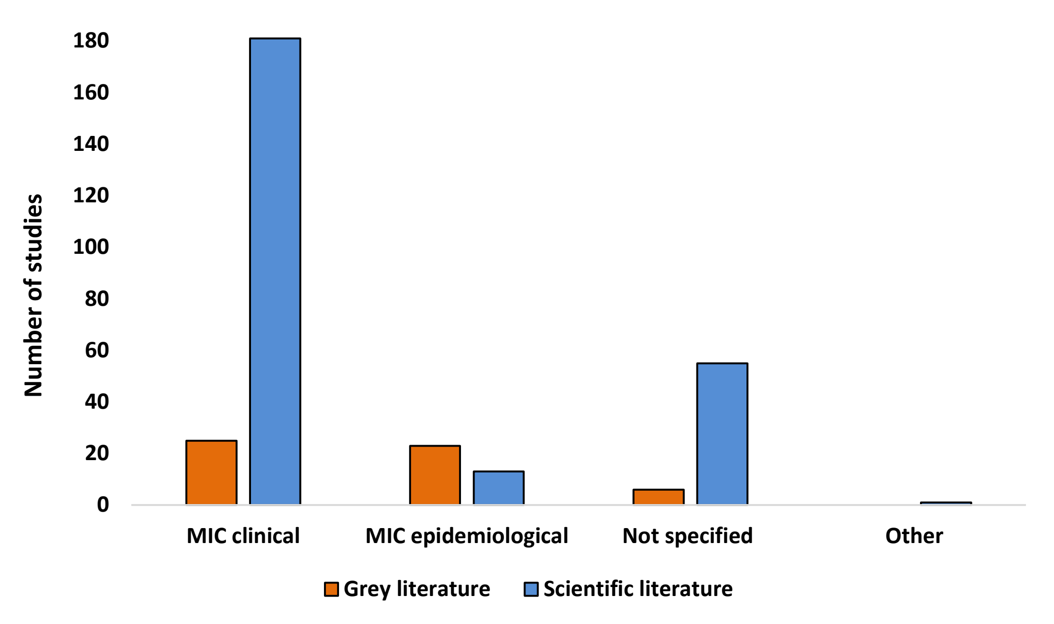

Figure 4- MIC breakpoints applied by eligible studies in this review.

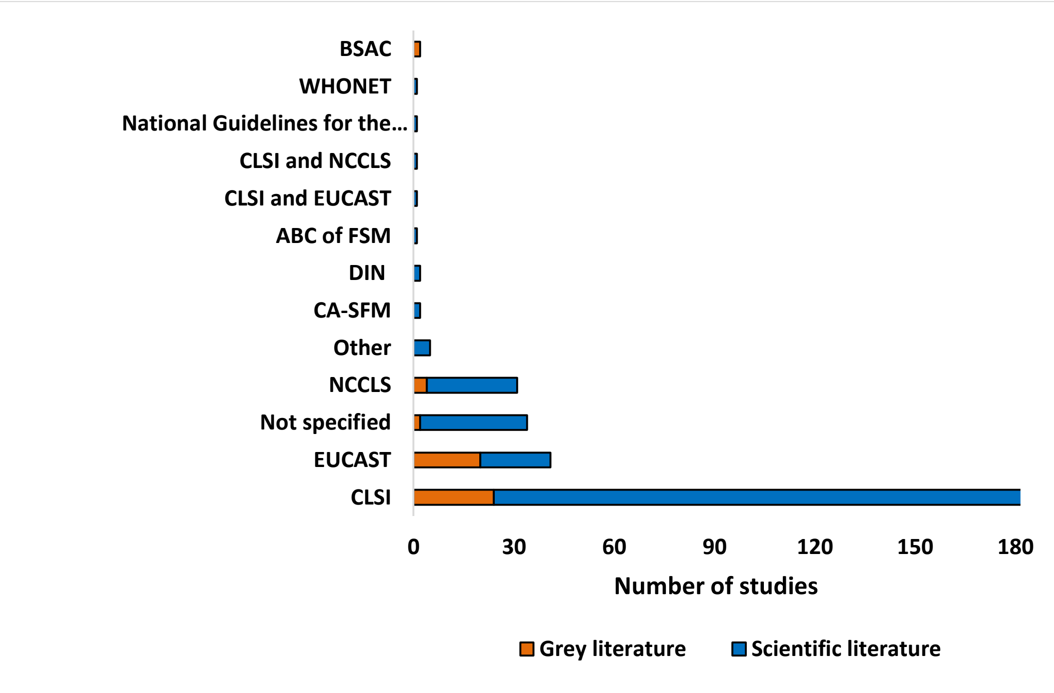

Figure 5- Standard for MIC interpretation criteria adopted by eligible studies in this review.

1. Pork meat

A total of 117 studies examined AMR in pork meat, from which 78 were scientific studies (e.g. original articles and reviews published in peer-reviewed journals) and

39 surveillance reports (grey literature). Four studies assessed AMR and MDR in pork meat at retail level in the UK; two original articles and two FSA surveys. All but one of the eligible studies (n= 115) utilised a cross-sectional study design; the remaining study was a review (Figure 6). Ninety studies (77.6%) conducted convenience sampling, whilst 20.7% applied random sampling. Two studies did not provide information about the type of sampling.

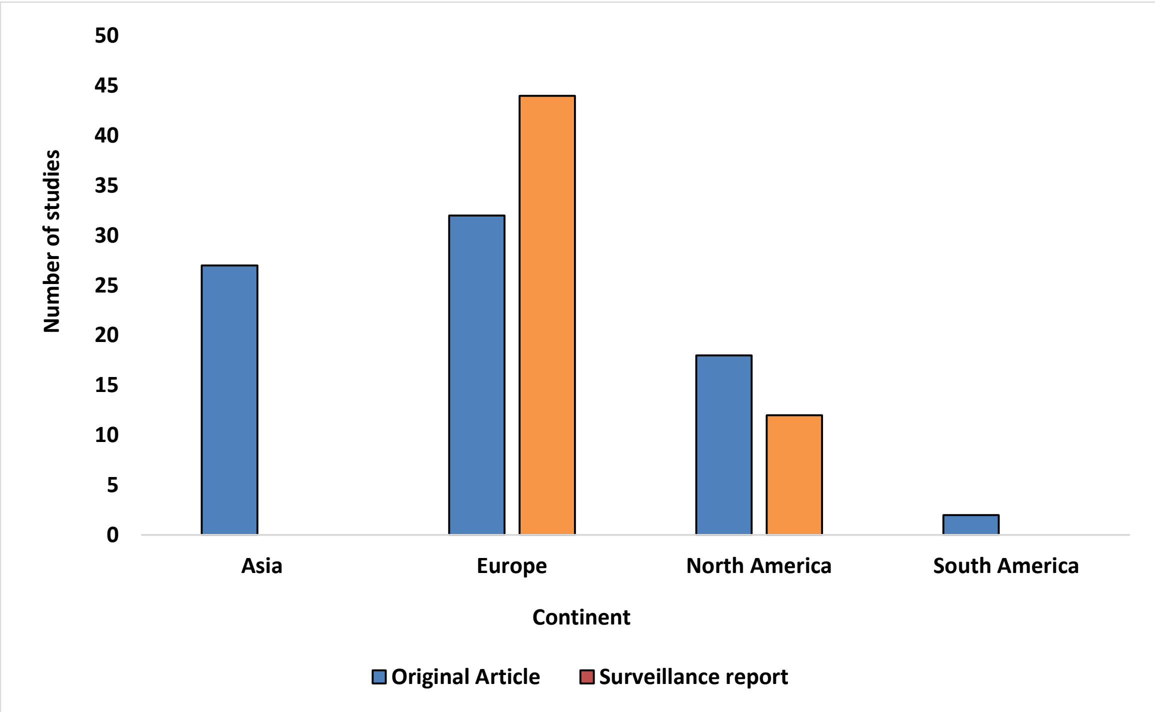

Figure 6- Distribution of eligible scientific and grey literature for antimicrobial resistance in pork meat per continent.

The methods used to assess AMR within pork meat varied, as described in Table 20. Eighty-seven (75%) of eligible studies applied CLSI standards (or NCCLS, as CLSI was previously known prior to 2005), and to a lesser extent, European Committee on Antimicrobial Susceptibility Testing (EUCAST) standards (14.7%); 5.1% followed other standards (e.g. national guidelines) and in 5.2% of studies, authors did not state the type of standards followed to assess AMR (Table 3).

Table 3- Standards applied in eligible studies assessing AMR in bacteria of interest in pork meat.

AMR Standards	Studies (non-grey literature)	Grey literature	Total
Antibiogram Committee of French Society of Microbiology (ABC of FSM)	1	0	**1**
Clinical and Laboratory Standards Institute (CLSI)	53	22	**75**
National Committee for Clinical Laboratory Standards (NCCLS)	7	4	**11**
The European Committee on Antimicrobial Susceptibility Testing (EUCAST)	6	13	**19**
National Guidelines for the Laboratory Detection of Extended Spectrum β-Lactamase- producing bacteria (ESBLs)	1	0	**1**
British Society of Antimicrobial Chemotherapy	0	1	**1**
Not specified	10	0	**10**
Total	**78**	**37**	**117**

[1]NB: NCCLS was rebranded as CLSI as of 01/01/05.

For the purpose of this review, we will focus on the frequency of AMR in British pork and in pork from main exporting countries trading with the UK. In Europe, these countries were Denmark, the Netherlands, Germany and Belgium (Figure 6). The USA is the sole exporting country of pork in the American continent into the UK. The UK does not import pork meat from African or Asian countries. For further AMR data on pork meat at retail level from other countries, please refer to **Appendix 3**.

The largest volume of both study and grey literature references was from European countries (54.3%); followed by American (24.8%), and Asian countries (20.9%); in the latter there were no surveillance reports available in English for the purpose of this review. There were no eligible studies for food items produced or imported from African countries (Figure 7).

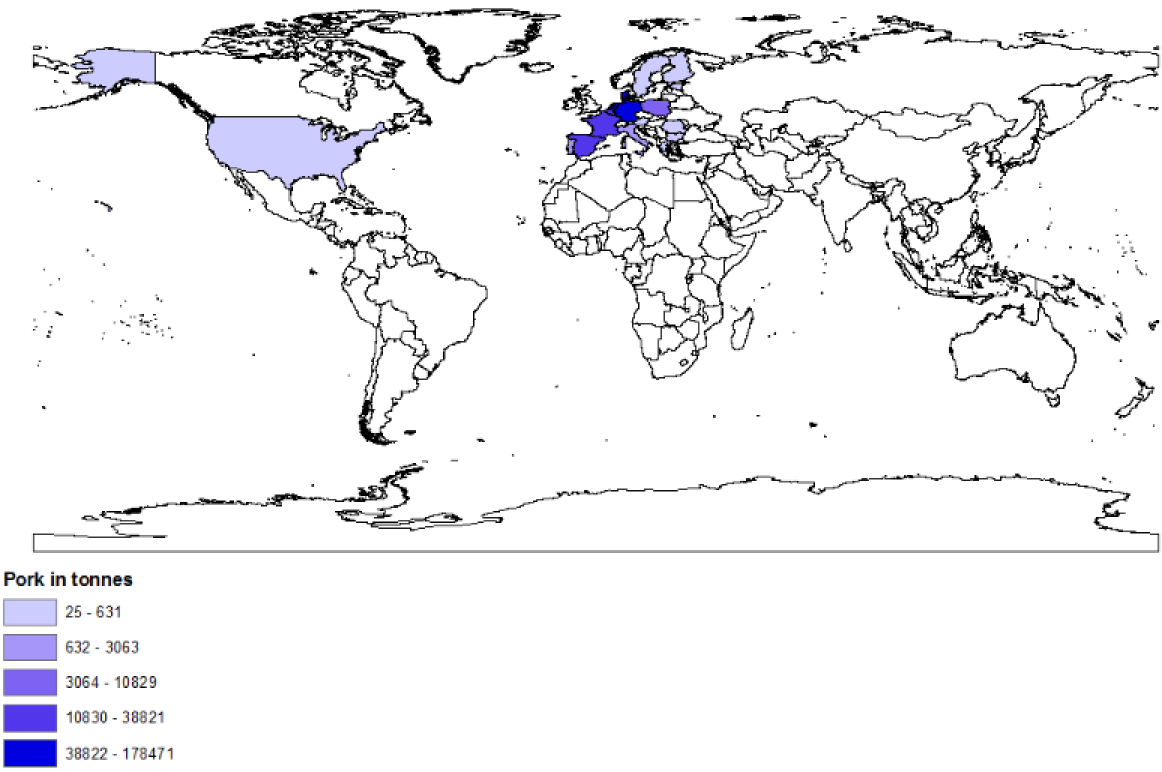

Figure 7- Exported pork meat (in tonnes) per country into the UK in 2015 (Source: HM Revenue and Costumes, UK).

Salmonella spp.

There were 20 studies from which data pertaining to AMR *Salmonella* spp. in isolates from pork meat were extracted; ten surveillance reports and ten scientific original studies. From those, two studies assessed AMR in salmonella isolates from pork meat in the UK at retail level (24). In the main pork exporting countries; ten in Denmark (25-34), three in Germany (35-37) and one for both Belgium and the Netherlands (38). In addition, four studies providing AMR data related to pork produced in the USA were also included in this review (39-42). (**Appendix 3** summarises the main findings per continent and studies of *Salmonella* spp. isolates in other countries).

Beta-Lactams

UK

A large study in the UK assessed of 1,440 samples of fresh pork meat and pork offal at retail and food premises level, between 2003 and 2005 (43). The samples for this study were collected using a convenience sampling strategy and therefore its findings cannot be generalised to all pork meat produced in the UK. This study reported resistance to ampicillin in all of the nine *S.* Typhimurium definitive phage type (DT) 104 isolates tested; in contrast, *S.* Derby (n= 8), *S.* Typhimurium DT 208 (n= 3) and *S.* Newport (n=3) isolates were fully susceptible to this antimicrobial. A survey conducted by FSA between 2006 and 2007 randomly sampled 1,693 samples of pork meat at retail level; this study only reported a small number of salmonella isolates (1-3 per strain) tested (*S.* Typhimurium DT 120, *S.* Typhimurium phage type (PT) U311, *S.* Typhimurium DT 109, *S.* Cerro, *S.* Derby and *S.* Virchow PT 26); from these, 2/2 *S.* Typhimurium U311, 1/1 *S.* Typhimurium DT 109 and 1/1 *S.* Typhimurium DT 120 isolates from British pork were resistant to ampicillin but were also MDR (see MDR section below) (44).

Europe

Denmark, the main exporter of pork meat into the UK has observed an increase in ampicillin resistance of *S.* Typhimurium since 1999 from 20% to 73%, mainly in isolates from imported pork meat (country of origin unknown); in domestically produced pork, resistance to ampicillin has increased up to 4% in the same time period. In contrast, resistance to amoxicillin-clavulanic acid has remained low (i.e., up to 2%) in both domestically-produced and imported pork (27-32). All salmonella isolates from pork meat tested as part of the Danish Programme for surveillance of antimicrobial consumption and resistance in bacteria from animals, food and humans (DANMAP) were susceptible to ceftiofur.

The Netherlands reported to EFSA as part of the European annual surveillance program that no resistance to ampicillin had been observed in *Salmonella* spp. isolates in 2006 (38). Under the same surveillance system, Belgium noted ampicillin resistance up to 27% in *Salmonella* spp. in domestically-produced pork in the same year (38). Germany, in contrast, reported lower levels of ampicillin resistance than those observed in other exporting countries of only 8.3% in *S*. Derby in a study conducted in 2007 (36); this estimate was based in a small convenience sample size (i.e., non-probabilistic sampling), whilst in the other countries, a representative sample calculation (i.e., random sampling) was applied to estimate resistance levels. No resistance to imipenem or meropenem was detected in the same study (36).

North, Central and South America

In *Salmonella* spp. from the USA, resistance to ampicillin increased from 0% to 13% between 2002 and 2013; none of the *S.* Typhimurium isolates were found to be resistant to this antimicrobial during this period, though only a small number of isolates were tested as part of the National Antimicrobial Resistance Monitoring System (NARMS) (39). NARMS surveillance detected a peak on cefotaxime resistance at 7.1% in isolates from pork meat in 2011 but a reduction to 0% was observed in 2013 (39). Two studies assessed AMR in *Salmonella* spp. isolates from pork meat in the USA, but these only assessed a small number of isolates (41, 42); in both studies, no resistance was detected to amoxicillin-clavulanic acid. From these studies, one study (42) reported four *S.* Typhimurium DT 104 isolates from ground pork meat to be resistant to ampicillin, but these isolates were MDR, presenting additional resistance to chloramphenicol, florfenicol, streptomycin, sulfamethoxazole and tetracyclines.

Fluoroquinolones

UK

In the UK, the study conducted by Little *et al* between 2003 and 2005 tested only a small number of different salmonella isolates for resistance to ciprofloxacin and nalidixic acid; the highest level of resistance observed was to nalidixic acid, in which three out of nine isolates of *S.* Typhimurium DT 104 were resistant to this antimicrobial (43).

Europe

In Denmark, levels of ciprofloxacin (0-6%) and nalidixic acid (0-3%) resistance in *S.* Typhimurium isolates from domestically-produced pork remained low between 2001 and 2005 (26-30). Slightly higher levels of resistance to the same antimicrobials were observed in *S.* Typhimurium isolated from imported pork (country of origin unknown), with up to 8% and up to 11% resistance to ciprofloxacin and nalidixic acid, respectively (26, 27, 29, 32, 33); it was not possible due to the small number of samples to assess if there was a significant difference in resistance levels to these antimicrobials between Danish and imported pork. Two smaller studies in Denmark that applied convenience sampling, did not detect resistance to these antimicrobials in *S*. Schwarzengrund (45) or *S*. Rissen (46); in the latter, samples of imported pork from Thailand were assessed.

North, Central and South America

In the USA, no resistance to ciprofloxacin or nalidixic acid was detected between 2001 and 2013 in salmonella isolates of relevance to public health through the NARMS (41, 42, 47).

Macrolides

UK

There were no data available regarding the resistance to macrolides of *Salmonella* spp. isolates from pork meat in the UK.

Europe

There were no data available regarding the resistance to macrolides of *Salmonella* spp. isolates from pork meat in the main pork exporting countries in Europe.

North, Central and South America

No resistance to azithromycin was detected in *Salmonella* spp. isolates from pork meat in the USA between 2011 and 2013 (47).

Colistin

UK

No studies assessed colistin resistance in salmonella isolates from pork meat at retail level in the UK.

Europe

In Denmark, all salmonella isolates from domestically-produced pork were fully susceptible to colistin (27-32).

In Germany, no resistance to colistin was observed in *S.* Derby isolates from pork in 2007 but only 12 isolates were assessed (36). No data on colistin resistance were available in eligible studies for the Netherlands and Belgium, countries which are also substantive exporters of pork into the UK.

North, Central and South America

No data were available in eligible studies regarding occurrence of colistin resistance in salmonella isolates from pork meat at retail level in the USA.

Multidrug resistance (MDR) in *Salmonella* isolates

UK

In the UK, a cross-sectional study conducted between 2003 and 2005 detected MDR isolates mainly in *S.* Typhimurium isolates (*S.* Typhimurium DT 104, *S.* Typhimurium DT 10b, *S*. Typhimurium PT U310, *S*. Typhimurium U302, *S*. Typhimurium DT 193) but also in *S*. Derby. Only a small number of MDR isolates were reported in this study. MDR phenotypes often involved resistance to ampicillin, streptomycin, tetracyclines and nalidixic acid (43). In a survey by the FSA in 2007, a reduced number of isolates from British pork were MDR; these were *S.* Typhimurium DT 120 (1/1), *S.* Typhimurium U311 (2/2) and *S.* Typhimurium DT 109 (1/1) (44). These isolates were all resistant to streptomycin, sulphonamides and tetracyclines. It was not possible to assess MDR trends due to the small number of isolates tested (44).

Europe

No eligible studies reported MDR isolates in Danish or Dutch pork at retail level. In a study in Germany in 2004, Schwaiger *et al* (37) noted that both of the two isolates of *S.* Typhimurium DT 104 detected within a sample of 250 pieces of pork meat at processing and retail level in 2004 were MDR, with a resistance profile including ampicillin, clavulanate-amoxicillin, piperacillin, streptomycin, doxycycline, florfenicol and chloramphenicol (37). A further study in Germany, conducted between 2006 and 2007 observed one MDR isolate in a convenience sample of S. Dublin (n= 8) in pork meat at retail; this isolate was resistant to sulfamethoxazole, spectinomycin, streptomycin and tetracyclines (36). A study conducted in the same year, also reported MDR isolates in 63% of *S.* enterica serotype 4,5,12,i: (n= 20) resistant to ampicillin, sulfamethoxazole, streptomycin and tetracyclines (35).

North, Central and South America

In the USA, MDR resistance was observed in a small number of *S.* Typhimurium isolates, in both NARMS surveillance data (39) and research studies (40, 42) between 1998 and 2004; from those, four *S.* Typhimurium DT 104 isolates were resistant to ampicillin, chloramphenicol, florfenicol, streptomycin, sulfamethoxazole and tetracyclines (42). In another study (40), two *S*. Typhimurium isolates detected in ground pork meat presented the same MDR phenotype as in the study by White *et al* (2001) but one of the isolates was additionally resistant to florfenicol. Although the NARMS reported MDR in *S*. Typhimurium, *S.* Newport and *S.* Heidelberg isolates, it did not report their specific phenotypes (39). In the study conducted by White *et al* (2001), one isolate of S. Agona was resistant to tetracyclines, streptomycin and sulfamethoxazole and one isolate of *S.* Heidelberg was resistant to those antimicrobials and additionally to kanamycin (42).

Enterococcus faecalis

Twenty-one studies focused on prevalence of AMR and MDR in *E. faecalis* isolates from pork in the UK (n= 1) and the main exporting countries trading with this country (i.e., Denmark, the Netherlands, Germany, Belgium and the USA). From these, 17 were surveillance reports, three were original studies and one was a review. All studies but one were conducted at retail level; the single exception study was conducted at processing (e.g. packaging prior to wholesale) (48). Thirteen of the studies conducted random sampling; the remainder followed a convenience sampling approach.

Beta-Lactams

UK

A study in the UK conducted between 2001 and 2002 detected 161 *E. faecalis* isolates from 255 samples of pork meat, but these were not assessed for resistance to antimicrobials in this group (24).

Europe

In Denmark, no resistance to either penicillin or ampicillin was detected in *E. faecalis* isolates from both domestically-produced and imported pork meat (country of origin unknown) between 1999 and 2012 (27, 29, 31, 49-56). Stable low levels of resistance to ampicillin in isolates from fresh pork at retail level were detected by surveillance in the Netherlands; only 0.1% from 2003-2014 (57-59). In Germany, the only eligible study reported a single *E. faecalis* isolate from pork meat at processing level that was fully susceptible to ampicillin; this finding is not generalisable to German pork due to the small sample size (48). No recent evidence was available for *E. faecalis* isolates from pork meat produced in Belgium.

North, Central and South America

In the USA, a study by McGowan-Spicer *et al* between 2000 and 2001 reported 8.3% resistance to penicillins in *E. faecalis* isolates from pork meat but used a convenience-based sampling (60). More recently, the NARMS reported very low to low prevalence levels of penicillin resistance (between 0 and 4%) in *E. faecalis* isolates from pork meat at retail between 2002 and 2013 (47).

Fluoroquinolones

Not deemed relevant in *Enterococcus* spp. Resistance to fluoroquinolones for *E. faecalis* from pork meat at retail level is not presented in this report but can be accessed in **Appendix 3**.

Macrolides

UK

In the UK, low prevalence levels of resistance to erythromycin at 8.1% were observed in *E. faecalis* isolates from pork meat at retail level in a study conducted between 2001 and 2002; there is a lack of more recent data on levels of resistance for this country (24).

Europe

In Denmark, surveillance data showed that erythromycin resistance in *E. faecalis* isolates from domestic and imported fresh pork meat collected at retail level between 1999 and 2013 varied between 0 and 12% (with the peak occurring in 2009), but there was no visible trend across the years (27, 31, 49-56). In the Netherlands, similar findings were observed of resistance up to 15% to erythromycin in 2015 in *E. faecalis* isolates but with only 2% being reported back in 2012 (57-59). No recent evidence was available for *E. faecalis* isolates from pork meat produced in Germany or Belgium.

North, Central and South America

In the USA, a study by McGowan-Spicer *et al.* conducted between 2000 and 2001 reported 8.3% erythromycin resistance in *E. faecalis* isolates from pork meat cuts (60). More recently, the NAMRS' surveillance system reported prevalence levels of erythromycin resistance in *E. faecalis* isolates from pork meat of between 4.5% and 9.1% in 2007; in 2013, resistance to this antimicrobial was 7% but there were no detectable trends across the years (47).

Colistin

Not deemed relevant in *Enterococcus* spp.

Multidrug resistance (MDR) in *Enterococcus faecalis* isolates

UK

In the sole study conducted in the UK (24), no VRE isolates were observed in British pork meat between 2001 and 2002; no recent data were available to determine if this is still the case. No other MDR phenotypes were assessed.

Europe

No MDR isolates were reported between 1999 and 2013, according to DANMAP (27-31, 49-54, 61, 62) and MARAN data (57-59). No data on MDR prevalence in *E. faecalis* isolates from German pork were available (48). In the review conducted in Belgium, although occurrence of VRE was discussed, no information was provided the prevalence of VRE in pork meat (63).

North, Central and South America

Of the two studies eligible in the USA, only the NARMS reported prevalence of MDR bacteria in pork meat between 2002 and 2013 (39). The NARMS reported MDR levels in *E. faecalis* isolates from pork meat between 15.7% (2002) and 8.2% (2013), with a downwards trend during the surveillance period, though these estimates were based on convenience sampling. No information was provided on the phenotypes in these isolates.

Enterococcus faecium

Twenty-six publications assessed AMR in *E. faecium* isolates from pork meat. One eligible study was conducted in the UK and used a random sampling methodology to collect 255 samples of fresh pork meat at retail level between 2001 and 2002 (24). Studies in the main pork exporting European countries were: Denmark (n= 14), and the Netherlands (n= 2). No eligible studies were available for Germany or Belgium, the two other major exporting European countries for pork meat into the UK. For the

USA, two eligible studies were assessed. **Appendix 3** presents further AMR data for *E. faecium* isolates from pork meat at retail level for these and other countries.

Beta-Lactams

UK

The only study conducted in the UK did not assess resistance to β-lactam antimicrobials in *E. faecium* isolates from pork meat (24).

Europe

Denmark, the main exporter of pork meat into the UK reported relatively low prevalence of resistance to penicillin, between 0 to 6.3%, with no evident trend across the years between 2003 and 2014 in *E. faecium* isolates from domestic pork meat at retail; similar prevalence levels were also reported for ampicillin between 2008 and 2014 (27, 49, 51-55). Comparable prevalence levels of resistance to penicillin and ampicillin were observed in isolates from imported pork (country of origin unknown) for the same time period, apart from 2009, for which resistance to both antimicrobials was reported at 9% according to DANMAP data (53). Slightly higher resistance prevalence to ampicillin were noted in Dutch pork of 8% in 2010, though MARAN data indicated resistance to ampicillin in the order of only 2% in *E. faecium* isolates from pork in 2011 (57-59).

North, Central and South America

The NARMS reported penicillin resistance of up to 8% between 2002 and 2013 in the USA, with no clearly definable trend during this period of study. These estimates were based on convenience sampling and therefore are not generalisable (47). Resistance to ampicillin was not assessed in the same study.

Fluoroquinolones

Not relevant in Enterococci. Data on resistance to fluoroquinolones in *E. faecium* isolates from pork meat at retail level per continent can be accessed in **Appendix 3**.

Macrolides

UK

In the UK between 2001 and 2002, 9.6% of *E. faecium* isolates (n= 114) from 255 British pork samples were resistant to erythromycin (24); however, this finding is dated and may not represent current resistance levels.

Europe

In Denmark, erythromycin resistance in domestically-produced pork meat at retail ranged from 3 to 35% in 2009. At the latest assessment in 2013, erythromycin resistance was 14.8%, though no evident temporal trend between 1999 and 2013 was detected (27, 31, 49-56, 61). In imported pork (country of origin unknown), erythromycin resistance was lower than in Danish pork at 3% but only 32 *E. faecium* isolates were assessed (51). In the Netherlands, resistance to erythromycin in *E. faecium* isolates from domestic pork meat was very high (41.4%) as per 2014, although this estimate was based on a convenience sample (58, 64).

North, Central and South America

No eligible studies assessed erythromycin resistance in *E. faecium* from pork meat at retail level in the USA.

Colistin

Not deemed relevant in *Enterococcus* spp.

Multidrug resistance (MDR) in *Enterococcus faecium* isolates

UK & Europe

No data on MDR was available on *E. faecium* isolated from British (24), Danish (27-31, 49, 50, 52-54, 61, 62) and Dutch (57-59) pork meat at retail level in eligible studies.

North, Central and South America

MDR isolates were more common in *E. faecium* (54.6% in 2003) than in *E. faecalis* isolates from pork meat at retail level in the USA, according to NARMS, though no data were provided on the phenotypes observed (39).

Escherichia coli

No eligible studies pertaining to AMR in *E. coli* from pork meat were available for the UK.

For the main pork exporting countries several eligible studies were identified for; Denmark (n= 15), the Netherlands (n= 3) and Germany (n= 1). No studies assessed AMR in *E. coli* in retail pork meat in Belgium. Two studies assessed AMR in *E. coli* from pork meat in the USA. Data on AMR in *E. coli* isolates detected in pork meat at retail level in other countries can be accessed in **Appendix 3**.

Beta-lactams

Europe

In Denmark, low prevalence levels of amoxicillin-clavulanic acid resistance (1%) were reported between 2003 and 2004 in *E. coli* isolates from domestically-produced pork (27, 55). In contrast, ampicillin resistance had risen from 8% in 1999 to 33% in 2012 (53). Cefotaxime and ceftiofur resistance were low (0-1.4% and 0-1%, respectively) in *E. coli* isolates from Danish pork between 1999 and 2013 (16, 27-31, 50, 52-54, 61, 62). DANMAP did not assess the occurrence of resistance to carbapenems in *E. coli* isolates. In the Netherlands, the second major exporter of pork into the UK, ampicillin resistance in *E. coli* isolates from pork meat was estimated at 34% between 2006 and 2007 but it has decreased to 12.7% in 2014 (57-59). In contrast, the levels of cefotaxime resistance in Dutch pork have remained low (up to 1.6%) between 2002 and 2014 (57-59). No resistance to meropenem was observed in *E coli* isolates in 2014; it was not possible to assess trends for resistance to this antimicrobial due to the lack of susceptibility data for previous years (58). Germany presented the highest prevalence of amoxicillin-clavulanic acid resistance (13.2%) in *E. coli* isolates form pork, amongst the main exporters of pork meat to the UK. Nevertheless, these observations were collected in 2004 and may not be representative of current AMR levels in German pork (37). No resistance to cefotaxime or to ceftiofur was detected in German pork and only very low levels to resistance to imipenem (0.5%) were observed, in line with the findings with other pork exporting countries (37).

North, Central and South America

In the USA, a study conducted in 2006 reported resistance to amoxicillin-clavulanic acid in 8% in *E. coli* isolates from pork chops but applied convenience sampling (65). According to NARMS, resistance to amoxicillin-clavulanic acid was at 6.8% in 2009 but it has decreased to 0.9% in 2014 in *E. coli* isolates from pork chops at retail level (39). Resistance to ampicillin was 16.1% in 2005 but decreased to 11.5% in 2013 (NARMS data) (39). Levels of ceftiofur and ceftriaxone resistance were low (below 1.5%) between 2002 and 2013, apart from 2009, when resistance was up to 6.8% for both antimicrobials in *E. coli* isolates from pork meat (39).

Fluoroquinolones

UK

No eligible studies assessed resistance in *E. coli* isolates from pork meat in the UK.

Europe

In Denmark, the major pork exporter to the UK, DANMAP detected a decrease in nalidixic acid resistance from 4% in 2001 to 1% in 2013, together with low ciprofloxacin resistance (1.4%) in *E. coli* isolates from domestically-produced pork meat, whilst in imported pork (unknown country of origin), levels of resistance to nalidixic acid and to ciprofloxacin increased from 0% to 10% between 1999 and 2013 (31, 49-55, 61). In Dutch pork, a slight increase of resistance to ciprofloxacin (0 to 3%) and nalidixic acid (0 to 2.7%) was observed between 2002 and 2014 (57-59). In Germany, resistance to both enrofloxacin and ciprofloxacin in *E. coli* isolates from pork meat was low (up to 1.5%) in 2004 (37) but no recent data were available on present resistance levels.

North, Central and South America

Resistance to nalidixic acid was reported by a study in the USA in 1999 in *E. coli* isolates from minced pork at 10% and from pork chops at 12% (66), whilst NARMS described prevalence levels between 2002 and 2013, of between 0.46-1.46% for the same antimicrobial (47). Findings from both the NARMS and the scientific study should be interpreted carefully as in both cases convenience sampling was conducted.

Macrolides

UK

No data were available in eligible studies for pork meat in the UK.

Europe

No data were available for azithromycin resistance in *E. coli* isolates from Danish or from German pork meat. The prevalence of azithromycin resistance was very low (0.9%) in isolates from Dutch pork meat in 2014; it was not possible to assess trends due to the lack of surveillance data for previous years (58).

North, Central and South America

In the USA, NARMS did not detect resistance to azithromycin in *E. coli* isolates from pork at retail level between 2011 and 2013 (47).

Colistin

UK

No data were available in eligible studies for pork meat in the UK.

Europe

No resistance to colistin was reported in *E. coli* isolates from domestically-produced pork in Denmark whilst in imported pork (country of origin unknown), prevalence of colistin resistance was detected at 2% between 1999 and 2013 (16, 29-31, 49, 51-54, 62). *E. coli* isolates from Dutch pork between 2008 and 2010 and also 2014 were fully susceptible to colistin, apart from 2010 when colistin resistance was detected in 0.5% of isolates (58). Resistance to colistin was low (1.5%) in isolates from German pork but these findings date from 2004 (37) and may not reflect current trends.

North, Central and South America

For the USA between 2011 and 2013 the NARMS did not report on colistin resistance in *E. coli* isolates from pork meat at retail level.

Multidrug resistance (MDR) in *Escherichia coli* isolates

UK

One single study was identified for the UK; this study reported that ESBL and AmpC-producers were detected in 1% of *E. coli* isolates from British pork meat (67); none of the six isolates detected were resistance to carbapenems or to polymyxins (i.e., colistin) (67). This study used selective media for fluoroquinolone-resistant ESBL-producers and therefore may have underestimated the true prevalence of ESBL-producers (67).

Europe

In Denmark, DANMAP reports noted ESBL-producing bacteria in more than 20% of *E. coli* isolates from domestically-produced pork in 2013. Similar findings were observed also in imported pork (country of origin unknown) in the same country (50). In the Netherlands, a study conducted by Overdevest *et al* (2011) (68) detected ESBL-producing bacteria in 1.8% of *E. coli* isolates from pork meat in 2008, whilst MARAN reported prevalence of ESBL-producing *E. coli* of 4% from pork meat in 2014, with a decrease in the prevalence levels of ESBL-producing bacteria observed in recent years (58). No data were available for German pork.

North, Central and South America

In the USA, up to 21.1% of *E. coli* isolated from pork chops were MDR in 2004; in 2013, prevalence of MDR *E. coli* was down to 13.9% but no information on AMR phenotypes was provided in the NARMS reports (39).

2. Poultry meat

Overall, 189 studies eligible studies assessed AMR in poultry meat at retail level, including 144 'scientific studies' (e.g. original articles and reviews published in peer-reviewed journals) and 45 surveillance reports (grey literature) (Figure 8).

The majority of eligible studies (98.9%; n= 187) were cross-sectional studies; two were reviews (63, 69), and two did not mention type of study design applied (70, 71). Regarding sampling methodologies, 149 (78.8%) eligible studies used convenience sampling and only a small proportion of studies applied random sampling (17.5%, n=33). The remaining seven studies (3.7%) did not mention what sampling methodology was applied.

The standards used to assess AMR in poultry meat varied, as described within Table 4. A large number of eligible studies (61.7%) applied MIC clinical breakpoints whilst only 16.1% used ECOFFs. The remaining studies did not provide information on the breakpoints applied to assess resistance (22.3%).

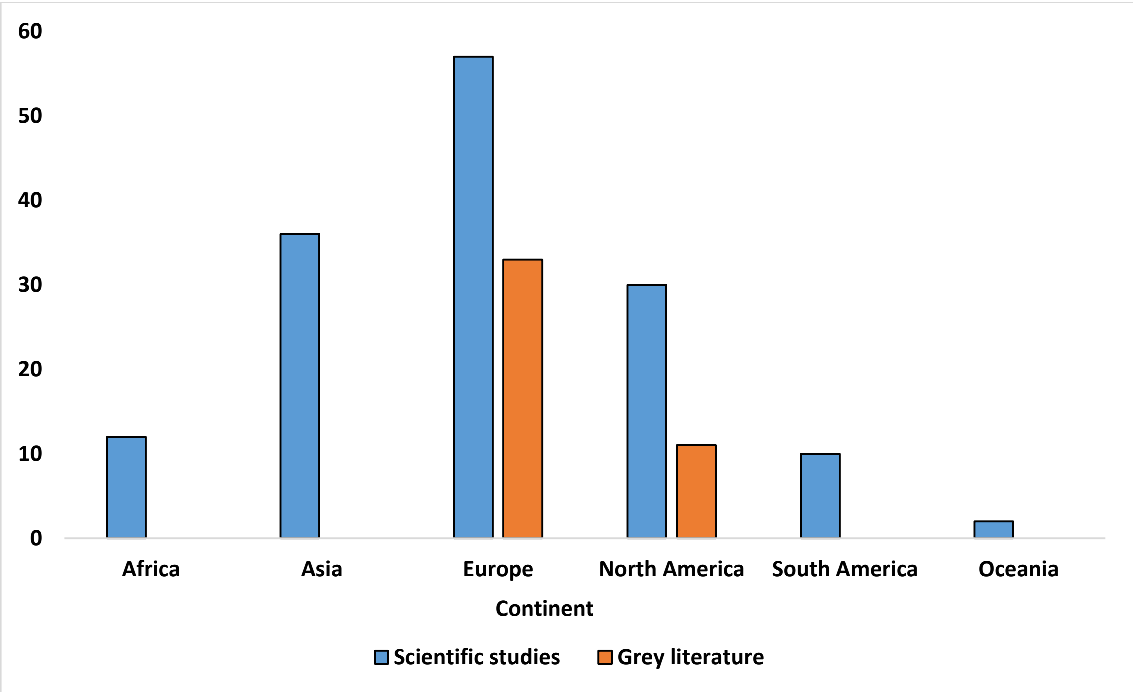

Figure 8- Distribution of eligible scientific and grey literature for antimicrobial resistance in poultry meat per continent.

Table 4- Standards applied in eligible studies assessing AMR in bacteria of interest in poultry meat.

AMR Standards	Studies (non-grey literature)	Grey literature	Total
Clinical and Laboratory Standards Institute (CLSI)	82	23	**105**
The European Committee on Antimicrobial Susceptibility Testing (EUCAST)	15	17	**31**
CLSI and EUCAST	1	0	**1**
National Committee for Clinical Laboratory Standards (NCCLS)	17	4	**21**
CLSI and NCCLS	1	0	**1**
WHONET	1	0	**1**
Other	4	0	**4**
Not specified	22	0	**22**
National Guidelines for the Laboratory Detection of ESBL	1	0	**1**
Total	**144**	**45**	**189**

*NB: NCCLS is a previous version of CLSI, as per 01/01/05.

**The authors referenced other studies rather than a specific accreditation system.

For the purpose of this report, only eligible studies focused on AMR findings in British poultry and on poultry produced in the main exporting countries trading with the UK have been used. The main poultry exporters considered were; Europe: the Netherlands, Poland and the Irish Republic; Elsewhere: the USA, Brazil, Argentina and Chile (North and South America), South Africa (Africa), Thailand (Asia) and Australia (Oceania) (Figure 9). AMR and MDR phenotypes observed in bacteria of interest in poultry meat in other countries can be accessed in **Appendix 3**.

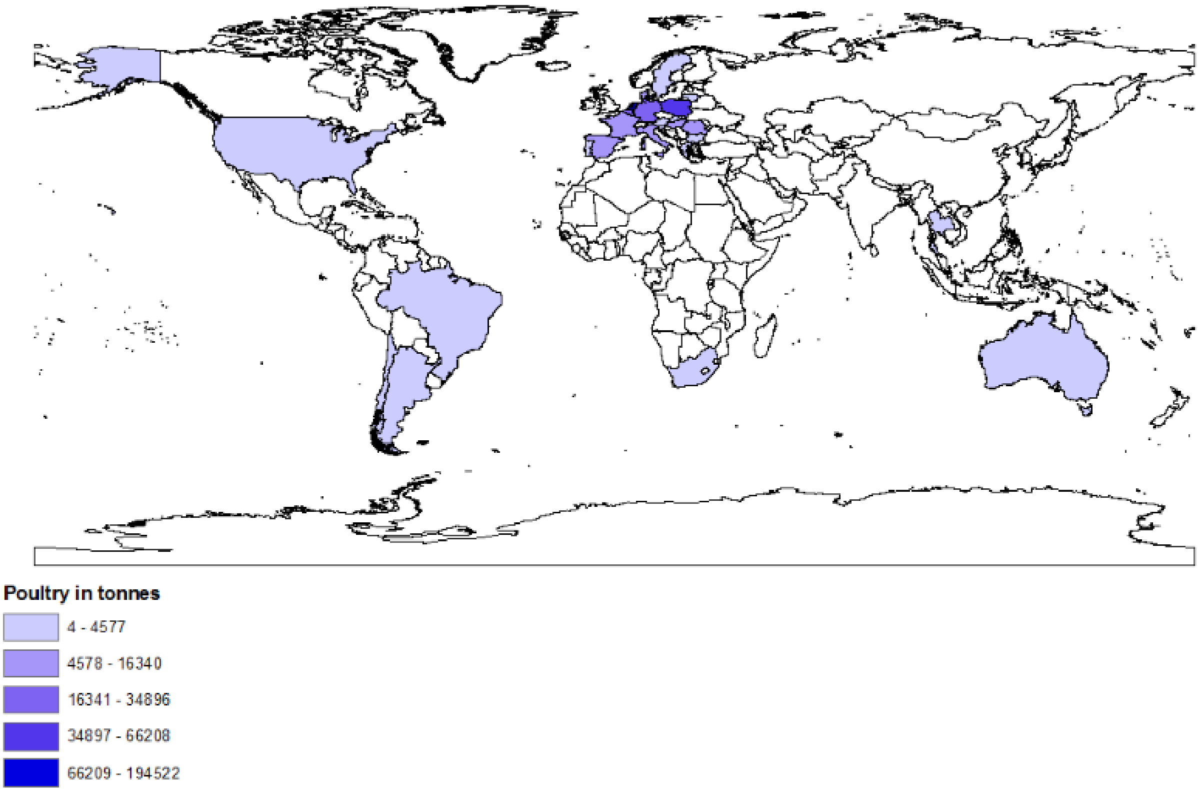

Figure 9- Exported poultry meat (in tonnes) per country into the UK in 2015. (Source: HM Revenue and Costumes, UK).

Campylobacter jejuni

Five studies assessed AMR in *C. jejuni* isolates from poultry meat produced in the UK, three of which were original scientific articles (72-74) and two were national survey reports conducted by FSA (75, 76). Two studies applied convenience sampling and three random sampling (73, 75, 76). All studies focused on domestically-produced poultry apart from that of Wilson *et al* (2003) and the FSA surveys (75, 76) that also assessed AMR in imported fresh and frozen chicken meat from European (Republic of Ireland or RoI and the Netherlands) and non-European countries (Thailand).

In exporting countries, for the Netherlands there were three eligible studies, all of which were surveillance reports (57-59), and eight studies for Poland, from which three were included in EFSA surveillance reports (49, 61, 62); the remaining were scientific original articles (77-81). Only data reported by EFSA were obtained through random sampling (62); all other studies followed a convenience sampling strategy. Data from European countries can also be found under the “UK” section as imported meat assessed as part of FSA surveys (75, 76).

Five studies assessed AMR in the USA; four were original scientific studies (82-85) and one a surveillance report (39). Only one of the scientific studies adopted a random sampling approach (83). Two studies assessed AMR in *C. jejuni* isolated from Brazilian poultry, both of which were original articles of studies using convenience sampling (71, 86). The same was observed for the only eligible study conducted in Argentina (87) and in Chile (88).

No studies were available for Thailand, the main poultry meat exporter to the UK located in Asia; data for this country were assessed in the "Europe section" as AMR in isolates from Thai chicken meat were assessed as part of a survey conducted by FSA in the UK at retail level in 2001 (75). No data were available for poultry meat from Australia or South Africa.

Beta-Lactams

Resistance to β-lactam antimicrobials was not deemed relevant for the purpose of this report. Data on resistance levels to β-lactam antimicrobials in *C. jejuni* and other campylobacter isolates in poultry meat at retail level per continent can be accessed in **Appendix 3**.

Fluoroquinolones

UK

Three studies and two survey reports assessed fluoroquinolone resistance in *C. jejuni* isolates from poultry meat in the UK (72-75, 89). Wilson *et al* (2003) estimated fluoroquinolone resistance in *C. jejuni* isolates from a convenience sample of domestically-produced chicken meat (n= 412) between 1995 and 2000 and noted nalidixic acid and ciprofloxacin resistance at 6.4% and 8.5%, respectively, whilst in imported chicken meat (country of origin unknown) resistance to these antimicrobials was higher, at 18.2% (72). In 2001, a FSA survey reported higher prevalence of ciprofloxacin and nalidixic acid (12.6% and 15.6%, respectively) resistance in *C. jejuni* isolates from conventional systems, compared to those observed in isolates from free-range (3.1% and 9.4%) and organic production systems (both at 2%) (75). This survey also assessed AMR in *C. jejuni* isolates from imported chicken meat from the Netherlands and from Thailand (75). Prevalence of resistance to fluoroquinolones (ciprofloxacin and nalidixic acid) was at 12% in *C. jejuni* isolates from chicken meat imported from the Netherlands (75). Only one isolate was assessed from Thai chicken meat and was MDR and therefore it is discussed in the relevant MDR section (75). A study by Wimalarathna *et al* (2013) which considered findings from two cross-sectional surveys conducted in 2001 (FSA survey reported above) and between 2004 and 2005 reported a slight increase of prevalence from 15% to 22% for both ciprofloxacin and nalidixic acid resistance in *C. jejuni* isolates from poultry meat (73). A follow-up FSA survey in 2007-2008 demonstrated prevalence of ciprofloxacin resistance at 21.7% and of nalidixic acid resistance at 23.1% in *C. jejuni* isolates from British chicken meat from conventional systems (76), which is slightly higher than those observed in 2001 (see above) (75). It was not possible to infer on the trends of fluoroquinolones resistance in *C. jejuni* isolates from chicken meat from free-range (n= 3) and organic (n= 7) production systems due to the small number of isolates tested (76). In the same survey, resistance to ciprofloxacin and nalidixic acid was demonstrated in three and four out of seven *C. jejuni* isolates from imported chicken meat from Poland (76). In a subsequent survey by FSA in 2014-2015, prevalence levels of ciprofloxacin and nalidixic resistance were reported at 50% and 51.5%, respectively in isolates from conventionally-produced British chicken meat; slightly lower prevalence levels of resistance to these antimicrobials (40.7% and 48.1%, respectively) were observed in isolates from British chicken meat of free-range production systems (89). In the same survey, isolates were also taken from organic chicken meat samples; due to the reduced number of isolates tested (n= 5), it was not possible to make inferences on AMR levels in this specific production system (89). There was a upwards trend observed in prevalence levels of fluoroquinolone resistance between 2001 and 2015, with a major increase observed between 2008 and 2015 in British chicken meat from conventional production systems and, to a lesser extent, also in chicken meat from free-range systems (89). It was not possible to assess resistance levels to fluoroquinolones across years in British chicken meat from organic systems due to the small sample size (89).

<u>Europe</u>

In the Netherlands, the major exporter of poultry meat into the UK, high prevalence of resistance were reported for both ciprofloxacin and nalidixic acid with an increase observed between 2004 (39%) and 2014 (63.4%) in *C. jejuni* isolates at retail level (57-59). In contrast, in Poland, the second largest exporter of poultry meat into the UK from Europe, resistance in *C. jejuni* to both ciprofloxacin and nalidixic acid were very high at 100% and 95.7%, respectively in 2012 as reported by this country to EFSA (62). High resistance levels to these antimicrobials have also been reported in other studies in the same country (49, 61, 77, 78, 80, 90), apart from a study conducted between 2003 and 2005 that reported a 5% prevalence of ciprofloxacin-resistant isolates but applied a convenience sampling strategy (81). Although the UK receives poultry from the RoI, there were no data related to fluoroquinolone resistance in poultry meat from the RoI in eligible studies.

<u>North, Central and South America</u>

The only study conducted in the USA (the largest exporter of poultry meat to the UK outside Europe), between 1999 and 2000 that followed random sampling estimated ciprofloxacin and nalidixic acid resistance in poultry meat at 25% and 32%, respectively (83). In a study conducted between 2001 and 2002, all of four *C. jejuni* isolates from turkey mincemeat were susceptible to ciprofloxacin (82). In the USA, prevalence of ciprofloxacin and nalidixic acid resistant isolates varied between 2002 (17.2%) and 2013 (11.2%), with a peak observed in 2009, when resistance was 21.3% in *C. jejuni* isolates from chicken meat at retail level, according to NARMS data (39). Similar findings were reported in another study in the USA conducted by Thakur *et al* (2009) between 2001 and 2002, where ciprofloxacin resistance was 19%, whilst in a study in Michigan state by Fitch *et al* (2005), 13 isolates of *C. jejuni* from 113 samples of chicken meat were fully susceptible to ciprofloxacin but the year the study was conducted was not provided by authors. Only one study in 2007 compared ciprofloxacin resistance of *C. jejuni* isolates from chicken meat from conventional, intensive systems with those from organic production systems; this study reported 69% in intensive systems versus a prevalence of 41% resistance in organic systems (85). Ciprofloxacin and nalidixic acid resistance in isolates from turkey meat at retail level in the USA was up to 60% in 2010 and was at 46.2% in

2011, though the number of *C. jejuni* isolates tested from turkey meat was very small (39). Results from the studies quoted above were based on convenience sampling.

Three studies reported on fluoroquinolone resistance in *C. jejuni* isolates in the main poultry exporting countries to the UK in South America: Argentina (87), Brazil (86) and Chile (91). These countries had very high prevalence of fluoroquinolone resistance in *C. jejuni* isolates compared to European countries and the USA. Studies in Brazil demonstrated resistance to nalidixic acid at 93.8% and to ciprofloxacin at 100% in 2009 (86). Similarly in Argentinian studies, 86.7% and 100% resistance was detected in *C. jejuni* to nalidixic acid and to ciprofloxacin, respectively (87). Studies in both Brazil and Argentina were cross-sectional and applied convenience sampling (86, 87). In Chile, lower prevalence of ciprofloxacin resistance at 58.2% in *C. jejuni* isolates from domestically-produced poultry was observed in a survey conducted between 2006 and 2010, but again a convenience sampling strategy was applied (88).

Macrolides

UK

In the UK, in a survey with convenience sampling conducted between 1995 and 2000, erythromycin resistance was estimated at 5% in *C. jejuni* isolates from domestically-produced chicken meat (72). In imported poultry meat (country of origin unknown) prevalence of erythromycin resistant isolates was similar (3%) between 1995 and 2000 (72). Surveys with random sampling conducted in the UK in 2001 (75) and between 2004 and 2005 noted lower levels of erythromycin resistance at 0% and 2.5% from chicken meat at retail level, respectively (73). The survey conducted in 2001 by FSA reported very low prevalence of erythromycin resistance (0-0.2%) in *C. jejuni* isolates from chicken meat from conventional, free-range and organic systems (75). No erythromycin resistance was observed in *C. jejuni* isolates from chicken meat imported from the Netherlands produced in conventional systems in the same survey (75). In domestically-produced poultry meat in the UK, erythromycin resistance was low at 1.5% in a survey conducted between 2003-2005 (74). Similar prevalence levels for erythromycin resistance were observed in a later survey conducted by FSA between 2007 and 2008 at 1.8% in isolates from British chicken meat from conventional systems; it was not possible to assess trends for AMR in meat from free-range and organic systems in the UK (76). In the same FSA survey, two out of seven *C. jejuni* isolates from imported chicken meat from Poland were resistant to erythromycin; the number of isolates was too small for inferences on AMR prevalence (76). In the latest survey by FSA (2014-2015), levels of erythromycin resistance varied between 0% (free-range systems) and 1% (conventionally-produced systems) in *C. jejuni* isolates from British chicken meat (89).

Europe

In Poland, all *C. jejuni* isolates from poultry meat were susceptible to erythromycin (49, 61, 62, 78-81), apart from a study conducted between 2008 and 2009 that assessed *C. jejuni* from poultry meat and in poultry offal, with prevalence of erythromycin resistant isolates at 11.4% (77). In the Netherlands, similar prevalence of erythromycin resistant isolates to those estimated for the UK were observed of up to 6% in 2008 and of only 0.7% in poultry meat in 2014, according to MARAN data (57-59). No eligible studies assessed AMR in *C. jejuni* isolates from poultry produced in the RoI.

North, Central and South America

In the USA, a survey conducted between 1999 and 2000 that applied random sampling estimated prevalence of erythromycin resistant isolates at 42% in poultry meat (83). NARMS reported erythromycin resistance as low (e.g., < 10%) between 2002 and 2013 in *C. jejuni* isolates from chicken meat at retail level (39). Similar findings were observed in a study in the Louisiana state by Han *et al* (2009) in 2006 comparing prevalence of resistance in *C. jejuni* isolates from chicken meat produced in conventional systems versus those produced in organic systems, where erythromycin resistance was higher, with levels up to 30% in conventional systems against 12% in organic poultry. In contrast, in turkey meat, levels of erythromycin resistance ranged between 10% in 2008 and 0% in 2013; the NARMS applies a convenience sampling approach, therefore, findings should be interpreted carefully (39).

In Brazil, the largest exporter of poultry meat to the UK in South America, erythromycin resistance was reported at 25.5% (year of study unknown) (86) and at 68.8% (71) in *C. jejuni* isolates from fresh poultry meat in 2009. Both studies applied convenience sampling. In Argentina, lower resistance levels to the same antimicrobial (20%) were reported but no information was provided on the year the survey was conducted and only a small number of *C. jejuni* isolates were tested (87). Chile, in comparison, reported very low prevalence of erythromycin resistant isolates in poultry meat (1.8%) in a survey conducted between 2006-2010 in the Metropolitan region but again this estimate was also based on convenience sampling (88).

Colistin

None of the eligible studies assessed colistin resistance in *C. jejuni* isolates from poultry meat in the UK or in any of the major poultry exporting countries.

Multidrug resistance (MDR) in *Campylobacter jejuni* isolates

Due to the reduced number of isolates assessed across studies and to the non-representativeness of findings due to convenience sampling, it was not possible to assess trends or estimate prevalence of common MDR phenotypes.

UK

One study and two surveys reported MDR isolates in *C. jejuni* isolates from British poultry meat (74, 75, 89). The FSA survey in 2001 (75) reported a 12% prevalence (126/1046) of MDR in *C. jejuni* isolates from British chicken meat from conventional systems, whilst lower resistance levels (9.4%, 3/32) were detected in isolates from free-range systems; it was not possible to determine the prevalence of MDR in isolates from organic systems due to the small number of isolates tested. The most common phenotypes observed in the FSA survey in 2001 were; ampicillin, kanamycin, tetracyclines and neomycin (n= 39), and ampicillin, tetracyclines, nalidixic acid and ciprofloxacin (n= 62) in isolates from British chicken meat from conventional production systems (75). In the same survey, one single *C. jejuni* isolate from imported Thai chicken meat was MDR with the phenotype: ampicillin, ciprofloxacin, nalidixic acid and tetracyclines; no MDR isolates were detected in *C. jejuni* (n= 25) from imported Dutch chicken meat (75). The study by Little *et al* (2009) noted low prevalence of MDR in *C. jejuni* isolates, with the highest prevalence being observed in isolates from turkey meat (16.7%), followed by duck meat (11%); no MDR isolates were observed in chicken meat between 2003 and 2005. In a later FSA survey conducted between 2007 and 2008, no MDR was detected in isolates from organic chicken meat but only a small number of isolates were tested (76); a prevalence of 19.8% (101/510) MDR was observed in *C. jejuni* isolates from British chicken meat from conventional production, which represents a slight increase in MDR since 2001; the most common phenotype was: ampicillin, ciprofloxacin, nalidixic acid and tetracyclines (n= 86) (75, 76). In the same survey, MDR was observed in *C. jejuni* isolates from imported poultry meat from Poland (4/7), with resistance to ampicillin, ciprofloxacin, nalidixic acid and tetracyclines; two isolates were also resistant to tetracyclines. Due to the small number of isolates assessed, it was not possible to infer on MDR trends from imported Polish meat (76). The latest FSA survey conducted between 2014 and 2015 reported a prevalence of 43.4% of MDR in *C. jejuni* isolates from conventionally-produced chicken meat at retail level; the most common phenotype observed was: ciprofloxacin, nalidixic acid, tetracyclines and trimethoprim (82.6%, 71/86) (89). In the same survey, a lower prevalence of MDR was observed in *C. jejuni* isolates from free-range chicken meat; one MDR isolate was detected among five *C. jejuni* isolates from organic poultry but no inferences could be made due to the reduced number of isolates tested from this particular production system (89).

<u>Europe</u>

No MDR isolates were reported in eligible studies in the Netherlands. In contrast, in Poland, three studies identified MDR *C. jejuni* isolates from poultry (78, 79, 81). The AMR phenotypes identified in the study by Wieczorek *et al* (2012) included; ciprofloxacin, streptomycin and tetracyclines (n= 2, 1.6%) and ciprofloxacin, nalidixic acid, streptomycin and tetracyclines (n= 8, 6.5%) and in the study by Rozynek *et al* (2008), ampicillin, ciprofloxacin and tetracyclines (n= 3) (81). The remaining study (78) identified 45% (18/40) MDR isolates but did not report phenotypes.

<u>North, Central and South America</u>

The study by Thakur *et al* (2009) conducted in the USA between 2001 and 2002 in chicken meat reported MDR at 7.2%. The definition of MDR applied in this study was resistance to two or more antimicrobials and according to the MDR definition used in this systematic review, these isolates (resistant to doxycycline and ciprofloxacin) would not be classified as MDR as such. This study did not find MDR in *C. jejuni* isolates from ground turkey meat examined during the same time period (82). MDR was identified in *C. jejuni* isolates from poultry meat in the USA at increasing prevalence levels (0-11.1%) between 2002 and 2009, according to NARMS; these estimates were based on convenience sampling and therefore might not be accurate (39). No MDR phenotypes were provided.

MDR was not addressed in the studies of *C. jejuni* from Brazilian poultry meat (71) (86). In Argentina (year of study unknown), a high prevalence of MDR resistance - defined by authors as resistance to three or more antimicrobials, independently of class - was identified to ciprofloxacin and erythromycin amongst *C. jejuni* isolates (n= 15) (87). Therefore these isolates could not be identified as MDR as defined in this review. No MDR data were provided for Chilean poultry.

Enterococcus faecalis

Only one eligible scientific article assessed AMR in *E. faecalis* isolates in British poultry meat and applied random sampling (24).

In exporting European countries, only two surveillance reports in the Netherlands with convenience sampling (57, 58) were identified.

In North and South American countries there was a scientific study and a surveillance report (NARMS) for USA poultry meat (39, 60) and one scientific study for Brazil (92). All of these studies applied convenience sampling. No studies were available for Argentina, Australia, Chile, South Africa or Thailand.

Beta-lactams

UK

In the UK, a survey conducted by Hayes *et al* (2003) between 2001 and 2002 estimated 84% and 90% prevalence of penicillin resistance in *E. faecalis* isolates from turkey and chicken meat at retail level, respectively based on random sampling (24). No data were available in this study on ampicillin resistance in British poultry meat.

Europe

In the Netherlands, ampicillin resistance was very low (1.8%) in poultry meat at retail level in 2013, but this estimate was based on a convenience sample (58).

North, Central and South America

In the USA, according to NARMS, resistance to penicillins was very low ranging between 0.5% and 0% in *E. faecalis* isolates from chicken meat at retail level according to an earlier survey between 2000 and 2001 (60) and between 2002 and 2013 (39). In the same country, slightly higher prevalence levels of penicillin resistance (up to 1.5% in 2005) were observed in turkey meats according to NARMS but all *E. faecalis* isolates from turkey meat in 2013 were susceptible to this antimicrobial (39). No data regarding resistance to β-lactam antimicrobials in *E. faecalis* was available for isolates from Brazilian, Argentinian or Chilean poultry meat.

Fluoroquinolones

Not deemed relevant in Enterococci. Data on resistance to fluoroquinolones in *E. faecalis* isolates from poultry meat at retail level per continent can be accessed in **Appendix 3**.

Macrolides

UK

In a survey in the UK between 2001 and 2002, resistance to erythromycin was observed in 33% and 42% of *E. faecalis* isolates from fresh chicken and turkey meats sampled at retail level (24).

Europe

In the Netherlands, MARAN surveillance detected similar prevalence levels of erythromycin resistance to those observed in British poultry, at 32% for the same year; an increase up to 51.8% was, however, reported in 2013 (57-59).

North, Central and South America

In Brazil, 90.2% of *E. faecalis* isolates from poultry meat between 2002 and 2004 in the state of the Rio de Janeiro were found to be resistant to erythromycin (92).

In the USA, resistance to erythromycin in *E. faecalis* isolates from chicken meat at retail level showed a downwards trend between 2002 (45.5%) and 2013 (35.1%), though these estimates were based on convenience sampling (39).

No data regarding resistance to macrolides in *E. faecalis* isolates were available from Argentinian or Chilean poultry meat.

Colistin

Not deemed relevant in Enterococci.

Multidrug resistance (MDR) in *Enterococcus faecalis* isolates

UK

MDR was not assessed in the study that investigated AMR in *E. faecalis* isolates from British poultry meat (24).

Europe

MDR was not assessed in neither of the studies investigating AMR in *E. faecalis* isolates from Dutch poultry meat (57, 58).

North, Central and South America

In Brazil, prevalence of MDR was high (43.9%) in *E. faecalis* isolates from poultry meat between 2002-2004 with the following phenotypes reported; tetracyclines, erythromycin and streptomycin (n= 11), chloramphenicol, tetracyclines, streptomycin and erythromycin (n= 3), chloramphenicol, tetracyclines and erythromycin (n= 3), chloramphenicol, tetracyclines and streptomycin (n= 1) (92).

In the USA, prevalence of MDR isolates was 69.7% in *E. faecalis* isolates from poultry meat between 2002 and 2011, according to NARMS; this was based on convenience sampling and no information was provided on the phenotypes observed (39). The MDR definition used by the NARMS was "resistance to three or more antimicrobial classes" but list of relevant antimicrobial classes were not provided. This was the only study that provided MDR data in the USA.

Enterococcus faecium

Only one study investigated AMR prevalence in *E. faecium* isolates from British poultry meat (24). In exporting countries, two surveillance reports provided data for Dutch poultry (57, 58); no studies were available for this commensal bacteria from poultry meat in Poland and in the RoI.

In the USA, only NARMS reported on AMR prevalence in *E. faecium* isolates from poultry meat (39). In Brazil, a single study was identified (92). Both studies conducted convenience sampling. No studies were available for poultry meat produced in Argentina, Australia, Chile, South Africa or Thailand.

Beta-lactams

UK

In the UK between 2001 and 2002, high prevalence (98%) of penicillin resistant isolates were observed for *E. faecium* compared to *E. faecalis* (above) isolates from both chicken and turkey meat at retail level (24).

Europe

In Dutch poultry meat, no data were available for penicillin resistance; nevertheless, MARAN studies detected a decrease of ampicillin resistance in *E. faecium* isolates between 2003 and 2009 (16% down to 6%), though these estimates were based in convenience sampling (57).

North, Central and South America

In the USA, penicillin resistance in *E. faecium* isolates from chicken meat at retail level has decreased between 2002 and 2013 from 44.2% down to 9.9%, as indicated by recent NARMS data (39). A downwards trend was also observed for isolates recovered from turkey meat for the same time period, though resistance levels remained high at 39.6% in 2013 from 50.6% back in 2002 (39).

There is a paucity of data on β-lactam antimicrobial resistance for *E. faecium* in Brazilian poultry at retail level; the only eligible study conducted in the Rio de Janeiro state assessed a sample size that was too small as only two isolates were tested (92). No data were available for β-lactam antimicrobial resistance in Argentinian and Chilean poultry meat at retail level in eligible studies.

Fluoroquinolones

Not deemed relevant for Enterococci. Data on resistance to fluoroquinolones in *E. faecium* isolates from poultry meat at retail level per continent can be accessed in **Appendix 3**.

Macrolides

UK

In the UK, erythromycin resistance in *E. faecium* varied greatly between isolates from chicken (20%) and turkey (53%) meats, according to a survey conducted between 2001 and 2002 (24); there is a paucity of data regarding resistance trends to macrolides in poultry meat in the UK.

Europe

In the Netherlands, the MARAN reports stated that resistance to erythromycin in *E. faecium* isolates from poultry meat at retail level have varied between 19 and 57% for 2003-2009 and 2013 (57, 58); in 2009, erythromycin resistance was at 40% in poultry meat (57) and similar resistance levels (43.1%) were also observed in 2013 but only turkey meat was tested (58).

North, Central and South America

In the USA, erythromycin resistance in *E. faecium* isolates from chicken meat has ranged between 9.5% in 2006 to up to 29.6% in 2013, as indicated by NARMS data (39). In turkey meat, higher prevalence rates were reported for erythromycin for the same period, though there seems to be a downwards trend, as resistance has decreased from 50.6% (2002) down to 39.6% in 2013 (39). There are scarce data on macrolide resistance in *E. faecium* from Brazilian poultry at retail level; the only eligible study in the state of Rio of Janeiro assessed a sample size that was too small as only two isolates were assessed (92). No data were available for macrolide resistance in Argentinian and Chilean poultry meat at retail level in eligible studies.

Colistin

Not deemed relevant in Enterococci.

Multidrug resistance (MDR) in *Enterococcus faecium* isolates

UK

In the only eligible study conducted in the UK, no data were provided on MDR occurrence in *E. faecium* isolates from poultry meat (24).

Europe

The same applied to Dutch poultry across surveillance data (57, 58).

North, Central and South America

In the USA, in a study conducted in 2007 no VRE isolates were observed in chicken meat but only a convenience sample of meat was collected and the number of *E. faecium* isolates assessed was not provided (93). According to NARMS, high prevalence of MDR (between 48.7% and 79.4%) was detected in *E. faecium* isolates from chicken meat between 2002 and 2013, with the peak observed in 2003 (39). It was not possible to assess trends in these data, as convenience sampling was conducted. NARMS reported that prevalence of MDR isolates was higher in turkey meat for the same time period, ranging between 75.6% and 93.5%, with the peak in 2006 (39).

No MDR data were available for Argentinian, Brazilian or Chilean poultry meat in eligible studies.

Escherichia coli

No studies assessed AMR in *E. coli* isolates from British poultry. In European exporting countries, two surveillance reports and one scientific article were identified for the Netherlands, which all applied convenience sampling (57, 59, 94). No studies were identified for Poland or the RoI.

In contrast, six scientific studies and one surveillance report provided information on AMR in *E. coli* isolates from poultry meat in the USA (39, 65, 66, 95-98), while only one study provided data for Brazilian poultry for the period 2006-2007 (99). All of these studies conducted convenience sampling.

Beta-Lactams

UK

No data was available for the UK in eligible studies.

Europe

In the Netherlands in 2010, 100% resistance to cefotaxime was reported in *E. coli* isolates from fresh chicken meat, but a convenience sampling was adopted and therefore findings should be interpreted carefully (68). MARAN data in 2014 demonstrated lower prevalence (40.7%) of ampicillin resistant isolates in *E. coli* isolates from poultry meat compared to 68% observed back in 2008 (57-59). Nevertheless, the highest levels of ampicillin resistance were detected in turkey meat, with 65.9% in 2014 compared to 2010-2011 when these were up to 76.1% (57-59). In the Netherlands, cefotaxime resistance in poultry meat has decreased down to 1.9% in 2014 since 2002; the highest prevalence of cefotaxime resistance in *E. coli* was observed in 2011 at 22% (57-59). In Dutch turkey meat, cefotaxime resistance in *E. coli* isolates was low at 2.3% in 2014 according to surveillance data (57-59).

In Poland, the single eligible study that assessed β-lactam antimicrobial resistance in *E. coli* from fresh chicken meat at retail only tested a small number of isolates (n= 12); from those, 100% were resistant to ampicillin, 58.3% to ceftriaxone and 41.7% to cefotaxime, and all isolates were susceptible to carbapenems (i.e., imipenem and meropenem) (100). No data were available for Irish poultry meat at retail level.

North, Central and South America

In the USA between 1999 and 2006, lower levels of resistance to β-lactam antimicrobials were reported by different studies in *E. coli* isolates from turkey meat; with high levels of resistance observed to ampicillin (22- 47.1%), and to a lesser extent, to clavulanate-amoxicillin (4- 13.6%) (66, 96, 97).

In the USA, the study conducted by Xia *et al* in 2006 (2011), investigated prevalence of AMR in extra-intestinal *E. coli* (ExPEC) isolates from a convenience sample of 415 fresh chicken breasts; this study detected low levels of resistance to ceftriaxone (20%), ampicillin (18%), clavulanate-amoxicillin (12%) and to ceftiofur (8%) but was conducted in 2006 (65). The study by Xia *et al* (2011) also noted lower prevalence rates of resistance to clavulanate-amoxicillin (8%), ampicillin (25%), cefotaxime and ceftriaxone (2% for both) in ExPEC isolates in ground turkey meat (65). Of note is that ExPEC strains can cause opportunistic infections in humans (68).

In the USA in 2010, high prevalence rates of resistance to β-lactam antimicrobials were reported in *E. coli* isolates from broiler meat produced in conventional systems, particularly to 3GCs; cefotaxime (90.1%), ceftriaxone (88.4%) and to a lesser extent to penicillins: clavulanate-amoxicillin (76.9%) and ampicillin (57.9%) (101). This was in contrast with a study conducted in 1999 by Schroeder *et al* that reported lower prevalence of resistance to ampicillin (32%), ceftiofur (6%) and ceftriaxone (2%) in fresh meat but noted higher ampicillin resistance levels (55%) in ground poultry meat; this applied also a convenience sampling strategy (66).

Fluoroquinolones

UK

No studies assessed resistance to fluoroquinolones in *E. coli* isolates from British poultry.

Europe

In the Netherlands, a cross-sectional study conducted in 2010 estimated 14% ciprofloxacin resistance in *E. coli* isolates from fresh chicken meat (68). In Polish poultry, a recent study reported 25% resistance to ciprofloxacin but only 12 *E. coli* isolates were tested (100). None of the eligible studies assessed fluoroquinolone resistance in isolates from poultry from RoI.

North, Central and South America

In the USA, no resistance to ciprofloxacin was reported in both ground and fresh chicken meat in earlier studies conducted in 1999, though levels of nalidixic resistance varied between 0 and 8% in fresh chicken meat and was 16% in ground chicken meat (66, 97). In a survey conducted between 2001 and 2003, low prevalence of resistance to ciprofloxacin (1.5%) and to nalidixic acid (range: 4-36%) in *E. coli* isolates from poultry meat was observed; the authors of this study did not distinguish between commensal *E. coli* and opportunistic ExPEC (97). In turkey meat resistance to ciprofloxacin (0-1%) was lower between 1999 and 2006. For nalidixic acid it would appear to have been a decrease in resistance from 25% down to 8.7% during the same time period (66, 97); this was across studies based on convenience sampling with different study designs and the variation observed may have been spurious in this case. In a study by Xia *et al* in 2006, ExPEC isolates from fresh chicken meat were fully susceptible to ciprofloxacin and only 11% of these were resistant to nalidixic acid (65). In the same study, in ExPEC isolates detected in turkey ground meat, lower levels of resistance were observed than for poultry meat, with 1% and 10% resistance levels to ciprofloxacin and nalidixic acid, respectively (65). In the USA, high prevalence rates of resistance to ciprofloxacin and nalidixic acid (both at 97.5%) were observed in *E. coli* isolates from broiler meat from conventional farms in 2012 (101).

Macrolides

UK

No eligible studies assessed macrolide resistance in *E. coli* isolates from British poultry.

Europe

No studies assessed macrolide resistance in *E. coli* isolates from Dutch, Polish or Irish poultry meat.

North, Central and South America

In the USA, no resistance to erythromycin was observed in *E. coli* isolates from both chicken and turkey meats at retail level by NARMS between 2011 and 2013 (39).

Colistin

UK

No data were available for British poultry meat in eligible studies.

Europe

In the Netherlands, colistin resistance was higher in turkey than in poultry meat, at 4.5% and 1.5%, respectively in 2014, according to MARAN (58). A recent study in the Netherlands also reported colistin resistance at similar levels (1.7%) in poultry meat samples collected in 2009 and 2014 (94).

North, Central and South America

In the USA, resistance to colistin was not reported for *E. coli* isolates from poultry meat at retail level in eligible studies.

Multidrug resistance (MDR) phenotypes in *Escherichia coli* isolates

UK

In the UK, a single study conducted in 2006 provided limited information on the occurrence of ESBL-producing *E. coli* isolates from domestically-produced and imported poultry meat (102); in British poultry, prevalence of ESBL-producers was low (1.6%, 1/62), whilst in imported poultry meat (country unknown), prevalence of ESBL producers was up to 17.5%. This study only assessed quinolone-resistant ESBL-producers and therefore may have excluded other MDR phenotypes occurring in *E. coli* isolates from poultry meat.

Europe

In the Netherlands, a study conducted by Kluytmans *et al* (2016) assessed the occurrence of colistin resistance retrospectively in ESBL-producing *E. coli* isolated in 2009 and 2014 from poultry meat; low prevalence levels of resistance of 1.5% and 1.7% were reported, respectively (94).

North, Central and South America

In the USA, a survey conducted between 2001 and 2002 identified MDR prevalence levels of between 10 and 26% in *E. coli* isolates (n= 175) from poultry meat; it was not possible to ascertain from this study the phenotypes observed and estimates were based in a convenience sample (95). In a study in 2006 only 0.7% of 415 ExPEC isolates from poultry meat were found to be MDR isolates, with resistance to amoxicillin-clavulanate, ampicillin, cefoxitin, ceftiofur, ceftriaxone, kanamycin, streptomycin, sulphonamides and tetracyclines (68). In 2007, a study by Chan *et al* (2008) in the same country did not detect ESBL-producing *E. coli* in poultry meat but only 12 food samples were taken and the number of isolates tested was not provided so the relevance of these findings is limited (93).

3. Dairy products

Thirty-three studies assessed occurrence of AMR in commensal bacteria in dairy products (Fig. 10); from those, 32 (96.9%) were original scientific articles and one (3.1%) was a literature review. No surveillance reports were included amongst the eligible studies for these food items. All but two of the original studies conducted convenience sampling; the remaining studies conducted random sampling (103) (104). Of these studies, 31 applied clinical breakpoints to assess AMR and two did not state breakpoints applied. The majority of studies (72.7%) followed CLSI standards (Table 5).

There was no evidence available for dairy products produced in the UK. Therefore, the focus of this section was on the main exporting countries that trade with the UK; France (n= 2), Turkey (n= 4) (Europe), and the USA (n= 2) (America). No data were available for the RoI, Canada, New Zealand, Israel or from United Arab Emirates (UAE) that are the main exporters of dairy products to the UK, together with France (Figure 11).

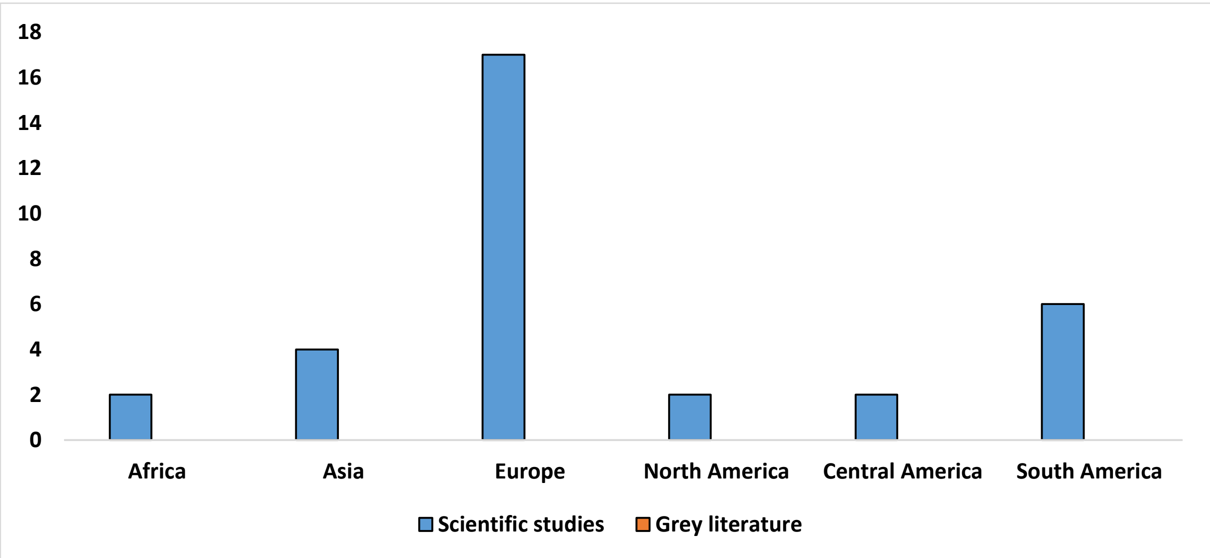

Figure 10- Distribution of eligible scientific and grey literature for antimicrobial resistance in dairy products per continent.

Table 5- Standards applied in eligible studies assessing AMR in bacteria of interest in dairy products

AMR Standards	Scientific studies	Grey literature	Total
Clinical and Laboratory Standards Institute (CLSI)	24	0	24
National Committee for Clinical Laboratory Standards (NCCLS)	3	0	3
Antibiogram Committee of French Society of Microbiology (ABC of FSM)	2		2
The European Committee on Antimicrobial Susceptibility Testing (EUCAST)	2	0	2
German Institute for Standards (DIN)	0	0	0
Not specified	2	0	2
Total	**33**	**0**	**33**

[1] NB: NCCLS is a previous version of CLSI, as per 01/01/05;

[2]Antibiogram Committee of the French Society for Microbiology

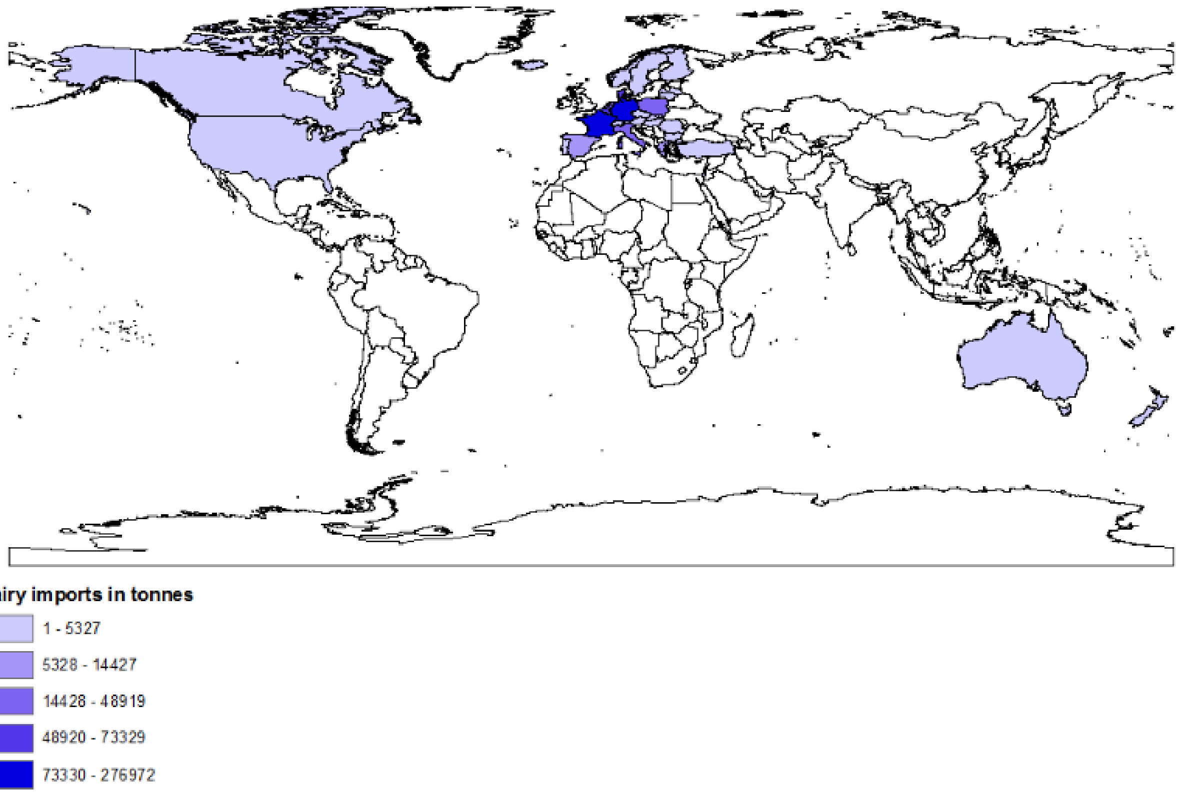

Figure 11- Exported dairy products (in tonnes) per country into the UK in 2015. (Source: HM Revenue and Costumes, UK).

Enterococcus faecalis

Only four studies investigated the occurrence of AMR in *E. faecalis* isolates from milk and dairy products; these were conducted in France (105) and Turkey (103, 106, 107).

Beta-Lactams

UK

No data were available for resistance patterns in non-MDR isolates of *E. faecalis* from dairy products from UK and France.

Europe

In Turkey, resistance to ampicillin was reported in *E. faecalis* isolates from both milk and cheese at 36.5% and 30.6%, respectively in 2000 in two separate studies (103, 106). In another study conducted by Yuksel *et al* (2015), lower levels of resistance to ampicillin were detected (20%) but a convenience sample was used and no information was provided on the year the study was performed (107).

North, Central and South America

No eligible studies assessed resistance in relevant exporting countries.

Asia

No eligible studies assessed resistance in relevant exporting countries.

Fluoroquinolones

Not deemed relevant in Enterococci. Data on resistance to fluoroquinolones in *E. faecalis* from dairy products at retail level per continent can be accessed in **Appendix 3**.

Macrolides

UK

No eligible studies assessed resistance in *E. faecalis* from dairy products in the UK.

Europe

In France, resistance to erythromycin was detected at 67.1% of *E. faecalis* isolates from cow cheese production in 2005 (105).

In Turkey, high levels of erythromycin resistance were observed in *E. faecalis* isolates from milk (91.7%) and cheese (90.3%) (103, 106). In another study conducted by Yuksel *et al* (2015) with a convenience sample of traditional Turkish cheese, lower prevalence levels of resistance to erythromycin were noted (66.7%) but only 15 isolates were tested.

Colistin

Not deemed relevant in Enterococci.

Multidrug resistance (MDR) in *E. faecalis* isolates

UK

No eligible studies assessed MDR in *E. faecalis* isolates from dairy products in the UK.

Europe

In France in 2005, 60.7% of *E. faecalis* isolates (n= 79) from raw and pasteurised milk intended for cheese production were MDR. In the MDR isolates, the most common resistance phenotype was; chloramphenicol, erythromycin, kanamycin, minocycline and tetracyclines (105). The authors used a different definition for MDR (i.e. resistant to two or more antimicrobials) and therefore the final estimates of MDR by the authors of this study were different than those presented in this report. No data were available for dairy products from Turkey.

Enterococcus faecium

There were no evidence for occurrence of resistance in *E. faecium* isolates from dairy products in the UK for the period between 1999 and 2016.

In Europe, data regarding AMR prevalence in *E. faecium* isolates from dairy products were only available in four studies conducted in Turkey (103, 106-108). No eligible studies were available for main exporting countries located in North America, or in the Asian or the Oceania continents.

Beta-Lactams

UK

No data were available in eligible studies for dairy products in the UK.

Europe

In Turkey in 2000, ampicillin resistance in *E. faecium* isolates from raw milk was estimated at 47.1%, according to a study using convenience sampling by Citak *et al* (2005); in cheese samples in the same year, resistance was estimated at 32% in a further study by Citak *et al* (2004) where random sampling was adopted (106). In another study by Yuksel *et al* (2015), ampicillin resistance was lower at 11.4% in cheese but there was no information either on the size of food sample by convenience sampling or the year in which the study was conducted (107).

Fluoroquinolones

Not deemed relevant for Enterococci. Data on resistance to fluoroquinolones in *E. faecium* isolates from dairy products at retail level per continent can be accessed in **Appendix 3**.

Macrolides

UK

No data were available in eligible studies for dairy products in the UK.

Europe

For the main European exporting countries, data on AMR in *E. faecium* isolates from dairy products were only available for Turkey. A study conducted in 2000 in raw milk samples at processing level detected high prevalence levels of erythromycin resistance (92.3%) in *E. faecium* isolates but these estimates were based on convenience sampling (103); resistance levels up to 96% were detected in Turkish white cheese in the same year at retail level. This study applied a random sampling approach (106). In another study (year unknown) published in 2015 by Yuksel *et al*, erythromycin resistance was reported at lower levels in cheese at 57.1%; a convenience sampling strategy was adopted but there was no information on the number of food samples tested (107).

Colistin

Not deemed relevant for Enterococci.

Multidrug resistance (MDR) in *Enterococcus faecium* isolates

UK

No data were available in eligible studies for dairy products in the UK.

Europe

In Turkey, three studies assessed the occurrence of VRE in milk (103, 108) and cheese (106). In the study by Citak *et al* (2005) conducted in 2000, 48% of *E. faecalis* isolates recovered were resistant to vancomycin and also resistant to erythromycin, rifampicin, gentamycin, ampicillin and ceftriaxone (103). VRE isolates were observed in 76.3% of *E. faecium* isolates in Turkish traditional white cheese in 2000; no information was provided on their phenotypes (106). In milk, in the study by Ḉetinkaya *et al* (2013), no VRE isolates were observed in raw milk (108).

Escherichia coli

No studies assessed AMR occurrence in isolates from dairy products in the UK.

Only three studies assessed the occurrence of resistance in *E. coli* isolates from dairy products in eligible studies in France (109) and the USA (110, 111).

Beta-Lactams

UK

No data were available for dairy products in the UK.

Europe

A single study assessed the occurrence of resistance in *E. coli* isolates from a variety of French dairy products (milk and cheese) and reported MDR isolates. These results are described below in the relevant section (109).

North, Central and South America

A study conducted in the USA reported high prevalence of resistance to ampicillin (80%) and to ceftriaxone (30%) in *E. coli* isolates from raw cow milk (54 samples) but no indication of the year of the study was provided, and only a convenience sample was taken. From these, 10 isolates were tested of which seven (70%) were MDR (111) (see relevant section below for further information on MDR phenotypes observed).

Fluoroquinolones

UK

No data were available in eligible studies for dairy products in the UK.

Europe

A single study assessed the occurrence of resistance in *E. coli* isolates from a variety of French dairy products (milk and cheese) and reported MDR isolates (109). These findings are described below in the relevant section.

North, Central and South America

In the USA, no resistance to enrofloxacin was observed in *E. coli* isolates (including those that were MDR) from bulk milk tank samples in a study by Straley *et al* (2006).

Macrolides

UK

No data were available for *E. coli* from dairy products from the UK.

Europe

A single study assessed the occurrence of resistance in *E. coli* isolates from a variety of French dairy products (milk and cheese) and reported MDR isolates. These findings are described below in the relevant section (109).

North, Central and South America

None of the eligible studies assessed macrolide resistance *in E. coli* isolates from dairy products in main exporting countries.

Colistin

UK

No data were available for *E. coli* from dairy products from the UK.

Europe

Resistance to colistin was reported in MDR *E. coli* isolates from French dairy products (please see section below for details on observed phenotypes) (109).

North, Central and South America

None of the eligible studies assessed colistin resistance in *E. coli* isolates from dairy products in the USA.

Multidrug resistance (MDR) in *Escherichia coli* isolates

UK

No data were available in eligible studies for *E. coli* isolates from dairy products in the UK.

Europe

A single study assessed the occurrence of AMR in *E. coli* isolates from a variety of French dairy products (milk and cheese) through convenience sampling (109); this study assessed only two isolates, both of which were MDR with the following phenotypes; ticarcillin, rifampicin, imipenem, sulphonamides, tetracyclines, piperacillin/tazobactam, chloramphenicol, amoxicillin-clavulanic acid, ceftazidime, amoxicillin, aztreonam, cefalotin, colistin, ceftazidime, mecillinam and ampicillin (n= 1) and ceftazidime, aztreonam, cefalotin, colistin, and mecillinam (n= 1). Due to the limited number of isolates, these resistance levels and patterns cannot be extrapolated to the overall French dairy production.

North, Central and South America

In the study by Berge *et al* (2007), 32% of *E coli* isolates (out of 1,577 isolates) tested in bulk cow milk during processing were MDR (110); due to the reporting in clusters by authors, it was not possible to ascertain occurring phenotypes.

The review by Straley *et al* (2006), noted that three out of ten *E. coli* isolates were MDR in bulk dairy milk samples. The resistance phenotypes observed were: ampicillin, chloramphenicol, florfenicol and ceftiofur (n= 2); ampicillin, chloramphenicol, tetracyclines, gentamicin, ticarcillin, ticarcillin/clavulanic acid and ceftiofur (n= 1) (111).

4. Seafood

Most studies dealing with AMR in fish and shellfish have the limitation of very small sample sizes, in addition to the previously mentioned issues that compromise representativeness and comparability of many studies identified in this review.

Thirty-two of the eligible studies of this systematic review assessed AMR in fish and shellfish between 1999 and May 2016 (Figure 12). None of these focused on seafood produced in the UK. All the studies were original scientific articles, cross-sectional in nature and followed a convenience sampling approach. No surveillance reports included in this systematic review monitored the occurrence of AMR in this food group. From the studies included, 22 (68.8%) were conducted at retail level and 10 (31.2 %) at capture level. All but six studies applied clinical breakpoints to assess AMR in isolates of interest; the remaining studies did not indicate breakpoints used. Twenty-six studies followed CLSI standards and one EUCAST standards; three studies did not state the standards applied (Table 6).

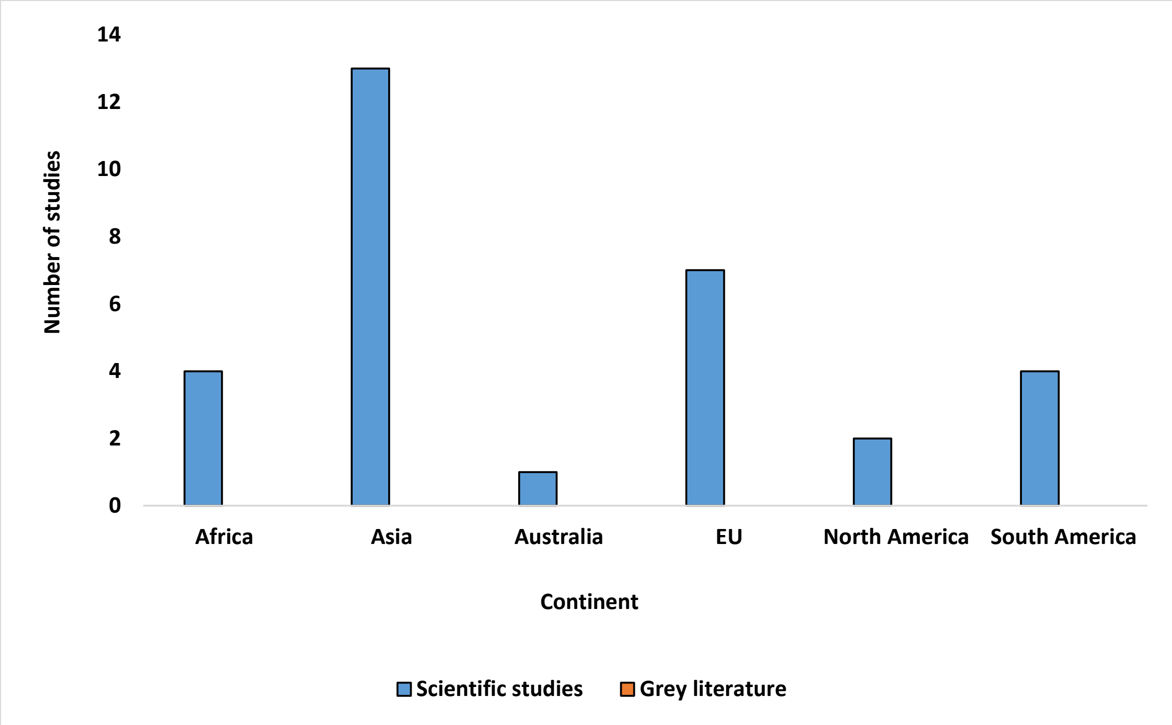

Figure 12- Distribution of eligible scientific and grey literature for antimicrobial resistance in seafood per continent.

For the purpose of this report, AMR in commensal bacteria of interest were assessed in the main seafood exporting countries trading with the UK: USA (America), China and Vietnam (Asia) (Fig. 13). There were no AMR data in the main European exporters of seafood to UK; Iceland, Sweden and Denmark, Faroe Islands and Norway, and Turkey. There were no data available for AMR in Burma, one of the main Asian exporting countries trading with the UK.

Table 6- Standards applied in eligible studies assessing AMR in bacteria of interest in seafood.

AMR Standards	Studies (non-grey literature)	Grey literature	Total
Clinical and Laboratory Standards Institute (CLSI)	24	0	24
National Committee for Clinical Laboratory Standards (NCCLS)	3	0	3
The European Committee on Antimicrobial Susceptibility Testing (EUCAST)	1	0	1
Not specified	4	0	3
Total	**32**	**0**	**32**

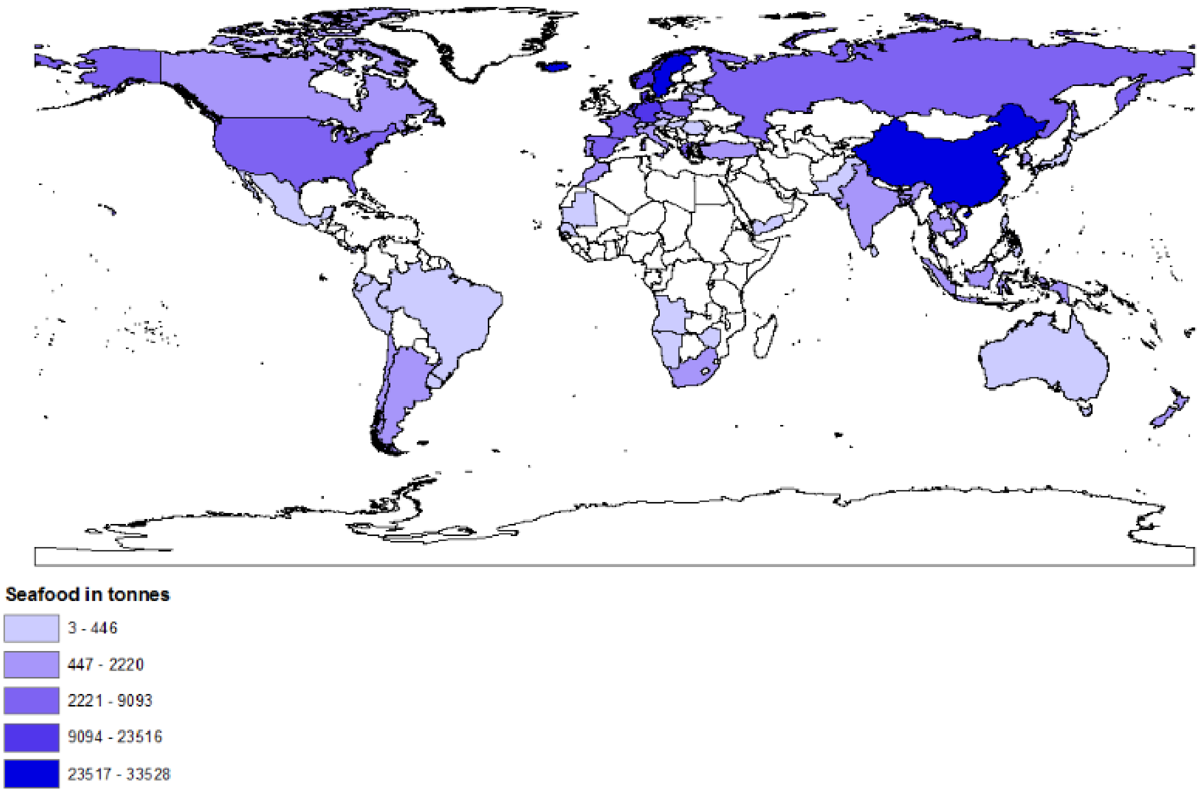

Figure 13- Exported seafood (in tonnes) per country into the UK in 2015 (Source: HM Revenue and Costumes, UK).

Enterococcus faecalis

No eligible studies were available for *E. faecalis* isolates from seafood from the UK or main exporting countries that trade these food items with the UK. Therefore, AMR and MDR information will not be provided in this report. AMR and MDR profiles for *E. faecalis* isolated from seafood in other countries can be accessed in **Appendix 3**.

Enterococcus faecium

No eligible studies were available for *E. faecium* isolates from seafood farmed or captured in the UK or in the main exporting countries that trade these food items with the UK. Therefore AMR and MDR information will not be provided in this report. AMR and MDR profiles for *E. faecium* isolates from seafood in other countries are provided in **Appendix 3**.

Escherichia coli

No eligible studies were available for *E. coli* isolates from seafood in the UK.

No eligible studies assessed AMR in *E. coli* from seafood for the main exporting countries apart from one study in China (the main exporting country to the UK outside Europe) (112), one study in the USA (113) and four studies in Vietnam (114-

117), which is the 10th main country trading seafood with the UK. All but one of these studies were conducted at retail level; the remaining study was performed in Vietnam at harvest level in aquaculture sites (115).

Beta-Lactams

UK

No data were available for *E. coli* isolates from seafood in the UK.

Europe

No data were available for *E. coli* isolates from seafood in main European exporting countries trading with the UK.

North, Central and South America

In the USA, a study in 2006 assessed occurrence of drug-resistant *E. coli* isolates from farmed versus wild shrimp in South Carolina; only one of seven isolates of *E. coli* from wild shrimp samples was resistant to ampicillin (113). Due to the small number of isolates tested, it is not possible to assess variation in resistance levels between wild and intensively farmed shrimp.

Asia

A study in 2004 in Vietnam observed 30% resistance to both ampicillin and amoxicillin but only 5% resistance to clavulanate-amoxicillin in *E. coli* isolates (n= 20) from shellfish at market level; this study did not draw a distinction between MDR (described below) and non-MDR isolates (114).

In China, in 2008 resistance to ampicillin was very high (78.9%) but resistance to cefotaxime was low (2.3%) and no resistance to ceftiofur was observed in *E. coli* isolates from 300 farmed fish at retail level; these estimates were based on a convenience sample (112). This study assessed occurrence of ESBL-producing *E. coli* isolates in the same sample (see MDR section below).

Fluoroquinolones

UK

No data were available for *E. coli* isolates from seafood in the UK.

Europe

No data were available for *E. coli* isolates from seafood in main European exporting countries trading with the UK.

North, Central and South America

No data were available for *E. coli* isolates from seafood in main American exporting countries trading with the UK.

Asia

A study in China in 2010 sampling 300 farmed fish obtained from 15 different markets in the city of Guangzhou, noted resistance to ciprofloxacin and nalidixic acid in 4.1 and 16% of *E. coli* isolates, respectively (112).

In Vietnam, four studies assessed occurrence of fluoroquinolone resistance in *E. coli* isolates from fish (115), shellfish (including shrimp) (114, 117) and non-specified seafood (116). One of these studies by Quoc Phong *et al* (2015) focused on the occurrence of ESBL-producing *E. coli* in shrimp and is discussed in the MDR section. In Vietnam, lower resistance to fluoroquinolones (enrofloxacin and ciprofloxacin, both at 10%) were reported compared to nalidixic acid (25%) in *E. coli* isolates from shellfish at retail level though no differentiation was made on resistance patterns from MDR (described below) and non-MDR isolates (114).

Macrolides

UK

No data were available for *E. coli* isolates from seafood in the UK.

Europe

No data were available for *E. coli* isolates from seafood in main European exporting countries trading with the UK.

Asia

Resistance to azithromycin in *E. coli* isolates was not assessed in any of the eligible studies conducted in China and Vietnam.

Colistin

None of the eligible studies assessed colistin resistance in *E. coli* isolates from seafood.

Multidrug resistance (MDR) in *Escherichia coli* isolates

UK

No data were available for *E. coli* isolates from seafood in the UK.

Europe

No data were available for *E. coli* isolates from seafood in main European exporting countries trading with the UK.

North, Central and South America

No data were available for *E. coli* isolates from seafood in main American exporting countries trading with the UK.

Asia

In Vietnam, a study conducted in 2004 detected MDR in 35% of *E. coli* isolates (n= 20) from shellfish (n= 50) at market level (114) which were commonly resistant to; ampicillin, tetracyclines, chloramphenicol, nalidixic acid streptomycin and trimethoprim. In the same year, Van *et al* (2007) detected two MDR isolates from seafood sampled at retail level but no other *E. coli* isolates were assessed (116). These isolates were resistant to; amoxicillin-clavulanic acid, ampicillin, cephalothin, tetracyclines, gentamicin, sulfamethoxazole, trimethoprim, streptomycin, nalidixic acid (n=1); amoxicillin, ampicillin, tetracyclines, chloramphenicol, sulfamethoxazole, streptomycin and nalidixic acid (116). Sarter *et al* (2007) assessed MDR in *E. coli* isolates from farmed catfish at capture level; the authors used a Multiple Antibiotic Resistance (MAR) Index for this purpose and reported 27.3% isolates as MDR that would not have been classified as such under the criteria applied in this systematic review as those isolates were only resistant to antimicrobials in two classes (i.e., oxytetracycline and sulfamethoxazole). A study in the same country by Quo Phong *et al* (2015) investigated the occurrence of ESBL-producing *E. coli* isolates from shrimp at a local market in Nha Trang in 2013; 18.3% of the isolates tested were ESBL producers and demonstrated resistance to ampicillin (100%), cefotaxime (100%), ciprofloxacin (27%), nalidixic acid (38%), streptomycin (27%), kanamycin (18%), gentamicin (9%), tetracyclines (75%), chloramphenicol (37%), florfenicol (9%) and sulfamethoxazole (56%). No resistance was observed to meropenem. The authors also reported MDR occurrence in ESBL-producing isolates at a 55% level (i.e., resistance to three or more antimicrobial classes other than β-lactams).

In China, a study by Jiang *et al* (2012) observed that three (1.5%) of 218 *E. coli* isolates from farmed fish obtained from 15 different markets in the city of Guangzhou were ESBL producers (112).

5. Vegetables and fruit

Twenty-seven studies assessed the occurrence of AMR in commensal bacteria from vegetables and fruit (Figure 14); of these, all were original scientific articles except three that were surveillance reports at either national (31, 57) or at European level (EFSA) (62).

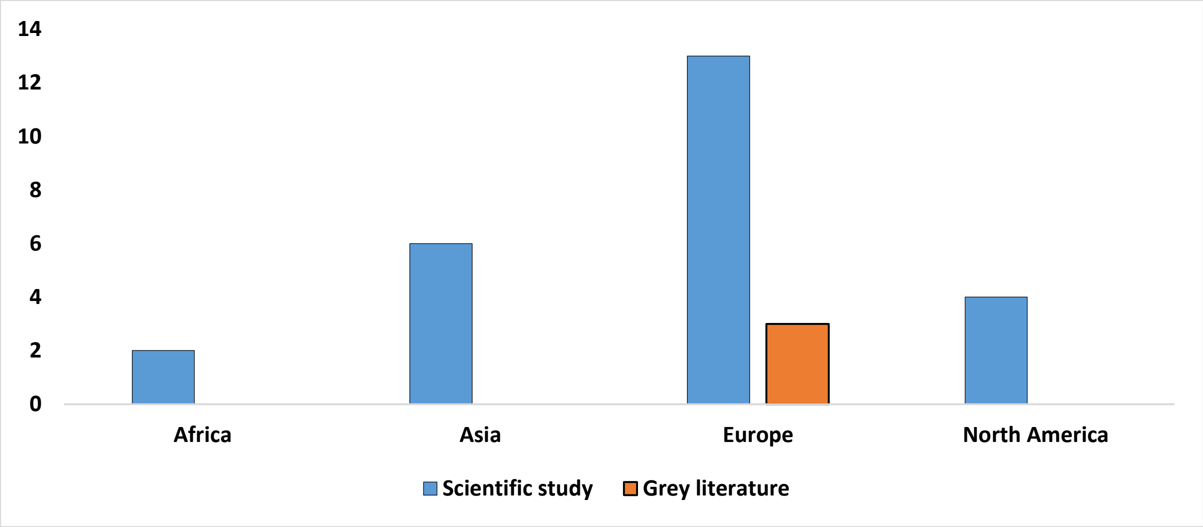

Figure 14- Distribution of eligible scientific and grey literature for antimicrobial resistance in fresh produce per continent.

Most studies applied convenience sampling; two of the three surveillance reports (31, 62) adopted a random sampling approach. Sixteen studies (57.1%) used clinical breakpoints whilst two studies (7.1%) adopted ECOFFs levels to assess resistance; the remaining 10 studies (35.7%) did not specify the breakpoints applied (Table 7).

Table 7- Standards applied in eligible studies assessing AMR in bacteria of interest from vegetables and fruit.

AMR Standards	Scientific studies	Grey literature	Total
Clinical and Laboratory Standards Institute (CLSI)	11	1	**12**
National Committee for Clinical Laboratory Standards (NCCLS)	2	1	**3**
The European Committee on Antimicrobial Susceptibility Testing (EUCAST)	2	1	**3**
German Institute for Standards (DIN)	2	0	**2**
Not specified	7	0	**8**
Total	**24**	**3**	**27**

[1] NB: NCCLS is a previous version of CLSI, as per 01/01/05.

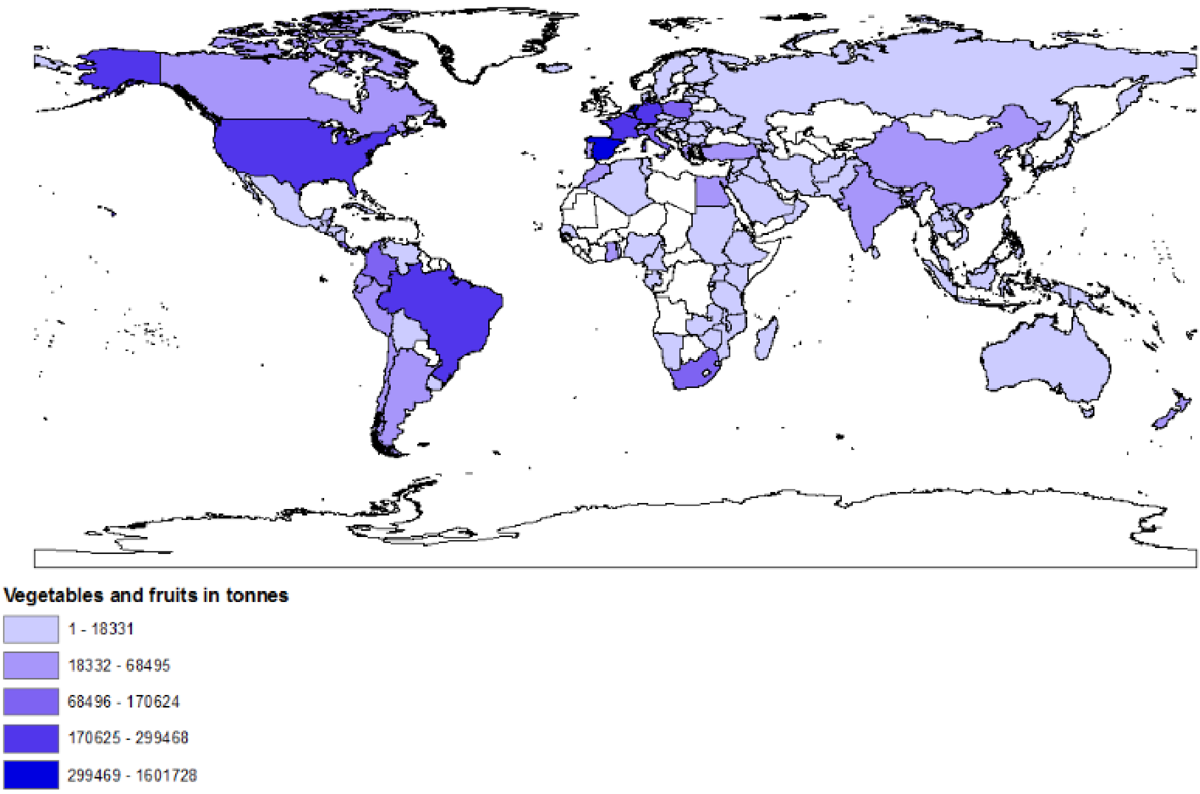

Figure 15- Exported fresh produce (in tonnes) per country into the UK in 2015 (Source: HM Revenue and Costumes, UK).

No eligible studies assessed the occurrence of AMR in commensal bacteria of interest from vegetables and fruit in the UK. The focus of this report was given to studies conducted in the main exporting countries of these food products to the UK (Figure 15); these countries include Spain (n=3) (118-120), the Netherlands (n= 4) (57, 62, 121, 122), Germany (n= 2) (123, 124) (Europe), the USA (n= 2) (60, 125) (Americas) and South Africa (n= 2) (126, 127) (Africa). There were no studies available for France, Turkey, and Brazil which are also major traders of fresh produce with the UK. Further information on AMR and MDR patterns observed in vegetables and fruit produced in other countries may be accessed in **Appendix 3**.

Enterococcus faecalis

No data were available for occurrence of AMR in *E. faecalis* isolates from fresh produce in the UK. A total of five eligible studies were identified across exporting countries; one in Spain (118), one in the Netherlands (57), one in Germany (123) and two for the USA (60, 125).

Beta-lactams

<u>UK</u>

No data were available for *E. faecalis* isolates from fresh produce in the UK.

Europe

The only study conducted in Spain (118) focused on MDR and is addressed in the MDR section below. In Germany, no ampicillin resistance was detected in a survey conducted between 2004 and 2005 in *E. faecalis* isolates from vegetables and fruit sold in farm shops (n= 702) and in large retail supermarkets (n= 299) (123); in this study only one isolate was found to be MDR (described below). In the Netherlands, similarly to the German study, no ampicillin resistance was detected in a sample of 96 *E. faecalis* isolates from vegetables and fruit at retail level sampled between 2010 and 2011 (57).

North, Central and South America

In the USA, no resistance to penicillin was observed in *E. faecalis* isolates from fruit (i.e., apples, tomatoes) or vegetables (i.e., cucumber lettuce, potatoes, sprouts) sampled between 2000 and 2001; only a convenience sample was taken and a reduced number of isolated assessed (between one and 20 isolates per group of fresh produce) (60, 125).

Fluoroquinolones

Not deemed relevant in Enterococci. Data on resistance to fluoroquinolones in *E. faecalis* isolates from vegetables and fruit at retail level per continent can be accessed in **Appendix 3**.

Macrolides

UK

No data were available for *E. faecalis* isolates from fresh produce in the UK.

Europe

In the Netherlands between 2010 and 2011, low prevalence of erythromycin resistance (6.3%) was observed in *E. faecalis* isolates from vegetables and fruit sold in that country, according to MARAN data (57).

North, Central and South America

No erythromycin resistance was detected in *E. faecalis* isolates from fruit and vegetables tested between 2000 and 2001 in the USA (60, 125).

Colistin

Not deemed relevant in Enterococci.

Multidrug resistance (MDR) in *Enterococcus faecalis* isolates

UK

No data were available for *E. faecalis* from fresh produce in the UK.

Europe

In the only study conducted in Spain, the main European exporter of vegetables and fruit to the UK, five MDR *E. faecalis* isolates were obtained from olives; these isolates were resistant to; rifampicin, tetracyclines and quinopristin/dalfopristin (118).

In Germany, a study conducted in 2004-2005 in local farm shops and large retailers, found one MDR *E. faecalis* isolate that was resistant to; fosfomycin, rifampicin, erythromycin, ciprofloxacin, doxycycline, sulfamethoxazole and nitrofurantoin in vegetables (123).

North, Central and South America

None of the eligible studies in the USA investigated occurrence of MDR in *E. faecalis* isolates from fresh produce.

Enterococcus faecium

Two studies assessed the occurrence of AMR and MDR in *E. faecium* isolates from Spanish vegetables and fruit (119, 120); one study was identified for this food item/ bacteria combination in the Netherlands (57), Germany (123) and the USA (125).

Beta-lactams

UK

No data were available for *E. faecium* isolates from fresh produce in the UK.

Europe

In Spain, ampicillin resistance was not detected in *E. faecium* isolates from a large variety of vegetables (green olives, beet, packed salad, lettuce, potatoes, sprouts, endives, broccoli, artichokes and celery) or fruit (tomatoes, strawberries, dates) at retail level; the authors sampled a reduced number of isolates (1-2 isolates per food item) and used a convenience sampling approach (119).

In the Netherlands, full susceptibility to ampicillin was observed in *E. faecium* isolates from vegetables and fruits at retail level between 2010 and 2011 but no information was provided on the types of fresh produce sampled and furthermore, a convenience sampling method was applied (57).

North, Central and South America

In the USA between 2000 and 2001, only one *E. faecium* isolate from vegetables as part of a survey in grocery shops in Athens (Georgia) was assessed for resistance to penicillin and was fully susceptible to this antimicrobial (125). The reduced number of isolates tested does not permit inferences to be made on the prevalence of resistance in *E. faecium* from fresh produce in that country.

Fluoroquinolones

Not deemed relevant in Enterococci. Data on resistance to fluoroquinolones in *E. faecium* isolates from fresh produce at retail level per continent can be accessed in **Appendix 3**.

Macrolides

UK

No data were available for *E. faecium* isolates from fresh produce in the UK.

Europe

Although a study in Spain assessed erythromycin resistance in *E. faecium* isolates, only a small numbers of isolates were tested (up to two isolates per type of vegetable or fruit sampled). It was therefore difficult to assess prevalence of resistance to macrolides (119). In the same country, another study identified a single isolate resistant to erythromycin in *E. faecium* from potatoes but no information was provided regarding sample size or year the survey was conducted (120).

In the Netherlands, 25.8% erythromycin resistance was observed in *E. faecium* isolates from fresh produce between 2010 and 2011 (57).

In Germany, a study by Schwaiger *et al* (2011) assessed MDR in domestic fresh produce; the results are described below in the relevant section.

North, Central and South America

A study conducted in the USA identified a single *E. faecium* isolate that was susceptible to erythromycin in fresh produce at retail level but the reduced number of samples and isolates tested (n= 1) does not permit inferences to be made on the prevalence of AMR in these food items in the USA (125).

Multidrug resistance (MDR) in *Enterococcus faecium* isolates

UK

No data were available for *E. faecium* isolates from fresh produce in the UK.

Europe

In Spain, MDR isolates were reported in a survey conducted in vegetables and fruits at retail level; these were isolated from green olives, beet, packed salads, lettuce, endives, sprouts, broccoli, celery, dates and strawberries. MDR isolates were resistant to tetracyclines, rifampicin and erythromycin. Only a small number of *E. faecium* isolates were reported and it was not possible to determine the prevalence of MDR isolates, as a convenience sampling approach had been adopted by authors; year the survey was conducted was not provided (119).

In Germany, in a study Schwaiger *et al* (2011) between 2004-2005, six MDR isolates were detected from 1,001 vegetables produced at national level and purchased from 11 supermarkets and 13 farm shops. Five MDR isolates were from supermarkets and one from a farm shop. The five isolates from commercial shops were resistant to; erythromycin, doxycycline, enrofloxacin, fosfomycin, rifampicin and streptomycin whilst the single isolate from farm retail was resistant to ciprofloxacin, mezlocillin, fosfomycin, rifampicin and linezolid (123).

North, Central and South America

In the USA, the single *E. faecium* isolate tested in a study conducted by McGowan *et al* (2008) from fresh produce at retail level between 2000 and 2001 was sensitive to vancomycin (i.e. not VRE) (125).

Escherichia coli

No eligible studies assessed the occurrence of AMR in *E. coli* isolates from fresh produce in the UK.

Four studies investigated the occurrence of AMR in *E. coli* isolates from vegetables in the Netherlands (57, 62, 121, 122). In other countries, two studies were conducted for the same purposes in Germany (123, 124) and two others in South Africa (126, 127).

Beta-lactams

UK

No data were available for *E. coli* isolates from fresh produce in the UK.

Europe

In the Netherlands, a survey conducted between 2010 and 2011 detected low prevalence (1.5%) of ampicillin resistance and full susceptibility to cefotaxime in *E. coli* isolates from vegetables and fruits according to MARAN data (57). In 2012, the Netherlands reported to EFSA low levels of resistance to ampicillin (2.3%) and full susceptibility to cefotaxime of *E. coli* isolates (n= 128) from vegetables and fruit (n=

170) (62). The remaining studies conducted in the Netherlands focused on occurrence of MDR and are discussed in the relevant section below.

Both studies performed in Germany (123, 124) investigated the occurrence of MDR strains in vegetables and fruit and therefore are discussed in the MDR section below.

Africa

In South Africa, two studies assessed the occurrence of AMR (126, 127), though one of these focused on MDR (126) and therefore is discussed in the relevant section below. In the remaining study, conducted in onions (n= 60) at processing (i.e., packaging) and retail levels in 2012 only three out of five *E. coli* isolates demonstrated resistance to ampicillin, one of which was a MDR strain (discussed below); no resistance to clavulanate-amoxicillin or to cefotaxime was observed but only a convenience sample was taken (127).

Fluoroquinolones

UK

No data were available for *E. coli* isolates from fresh produce in the UK.

Europe

Low prevalence levels of resistance to ciprofloxacin and nalidixic acid (both at 1.5%) were reported in isolates from vegetables and fruit sampled in the Netherlands, according to MARAN (57). Similar prevalence levels (2%) for ciprofloxacin resistance in *E. coli* isolates were observed by the same country in 2012 (62).

Africa

Findings for the two studies conducted in South Africa (126, 127) concerned MDR *E. coli* isolates from fresh produce; findings are provided below in the relevant section.

Macrolides

UK

No data were available for *E. coli* isolates from fresh produce in the UK.

Europe

No data were available for *E. coli* isolates from fresh produce in main European exporting countries.

Colistin

UK

No data were available for *E. coli* isolates from fresh produce in the UK.

Europe

In the Netherlands, all *E. coli* isolates (n= 120) from vegetables and fruit were sensitive to colistin in 2012, as reported by this country to EFSA (62).

Multidrug resistance (MDR) in *Escherichia coli* isolates

UK

No data were available for *E. coli* isolates from fresh produce in the UK.

Europe

Very low numbers of MDR isolates were detected in *E. coli* isolates from vegetables and fruit in exporting countries. Due to the scarcity of scientific evidence and numbers of *E. coli* isolates tested, caution is required when interpreting these findings.

In the Netherlands, Veldman *et al* detected in 2011 a small number of ESBL-producing *E. coli* isolates (n= 6) in imported herbs (n= 50) from Thailand (122); these isolates were resistant to ampicillin, cefotaxime, ciprofloxacin, streptomycin and trimethoprim. As no information was provided regarding the total number of *E. coli* isolates tested it was not possible to assess the extent of ESBL-producers in these food items. Another study in the same country in 2012 assessed 1,216 vegetables from both conventional and organic production systems, and found a single MDR isolate (ESBL-producing *E coli*); this isolate was found in celery imported from Spain (121).

In Germany, a study conducted by Boehme *et al* (year of study unknown) observed three MDR *E. coli* isolates, mainly isolated from sprouts, that were resistant to ampicillin, mezlocillin, streptomycin, tetracyclines, sulfamethoxazole and co-trimoxazole (124). A further study conducted between 2004 and 2005 reported nine MDR isolates that were resistant to ampicillin, cefaclor, cefoxitin, cefuroxime, mezlocillin and streptomycin from a sample of 1,000 vegetables purchased from both large retailers and farm shops. One of the MDR isolates also exhibited resistance to colistin (123).

Africa

In South Africa, two studies reported MDR isolates; a study that sampled 19 lettuces in 2011 noted that nine out of 10 *E. coli* isolates were ESBL/ Amp-C producers (126). In the second study the following year, only one isolate detected in onions was MDR and exhibited resistance to tetracyclines, ampicillin, enrofloxacin, nalidixic acid and trimethoprim-sulfamethoxazole (127).

Conclusions

This systematic review assessed AMR evidence on the foods and bacteria of interest across over different 58 different countries (including the UK) across all five continents. Overall 304 studies published between 1999 and May 2016 were included (with exception of FSA surveys conducted after that period that were included at request of FSA); from those, 253 (83.2%) were original scientific articles (including four reviews) whilst 51 (16.8%) were (surveillance) reports. For the purpose of this report we focused on the resistance levels observed in British food but also in the main exporting countries trading with the UK, in order to ascertain AMR levels in domestically and imported food products to which British consumers could be exposed to. AMR data if food produced in other countries can be accessed in **Appendix 3**.

This systematic review focused on assessing evidence for resistance against specific antimicrobial groups deemed as critically important in human medicine (β-lactams, fluoroquinolones, macrolides and polymyxins). We considered only a limited number of foodborne pathogens (*Salmonella* spp., *Campylobacter* spp.) and of commensal bacteria across a limited range of food items (pork and poultry meat, dairy, seafood and fresh produce). This meant that resistance to other antimicrobial substances that are widely used in animal production (e.g., tetracyclines) were not assessed, unless when in the context of MDR isolates. Nevertheless, the selections of bacteria-species combinations considered were consistent with scientific evidence (16) and was supported by a panel of experts consulted prior to the preparation of this review. From this review were excluded meats from other animal species (e.g., cattle), food items (e.g. eggs) that are also part of the diet of British consumers, and that can act as a source of resistant bacteria, as shown in recent national and multi-country outbreaks (128). This was due to the limited timeframe for this study and also due to the feedback provided by experts as mentioned above.

Most of the studies included in this review had a high risk of bias due to non-random, convenience sample selection, small sample size and other limitations. Very few studies provided sufficient detail regarding the sampling strategy and justified the sample size, except for international (EFSA) and national surveillance reports that applied random sampling and followed standardised protocols. This affected the representativeness and comparability of data reported and also prevented adequate comparisons across years, within and between countries. We therefore identify an urgent need for more harmonisation in study and surveillance design in studies and surveillance programs assessing AMR at retail level. EFSA previously stated in its annual report on AMR that '*the main issues when comparing AMR data originating from different countries are the use of different laboratory methods and different interpretative criteria of resistance*' (49). Similarly, the variation in criteria of resistance (e.g., clinical versus epidemiological breakpoints or ECOFFs, use of CLSI versus EUCAST guidelines), within and between countries and microbial classes severely limits adequate comparisons and assessment of trends in resistance.

There was a paucity of recent data for domestically-produced and imported food in the UK. Also the lack of surveillance data (apart from that available through the FSA surveys and EFSA's surveillance reports) did not allow the detection of trends in

AMR in food; these data would be relevant for risk assessment of exposure of British consumers.

For exporting countries in Europe, AMR trends were available mainly for Nordic countries (e.g., Denmark) and The Netherlands, where AMR data have been systematically collected for surveillance purposes for many years (DANMAP, MARAN). For these and other European countries, evidence was available through both national and EFSA annual integrated surveillance reports for AMR in foodborne zoonoses and indicator bacteria in animals, humans and food. Data for these reports were only collected in a harmonised manner from 2011 onwards following EFSA recommendations (17, 129), which made it difficult for comparison of trends of AMR between and within countries prior to 2011. Surveillance data were key to identify changing trends of resistance in critically important antimicrobials, such as the rise observed over the last decade to ampicillin resistance in Gram-negative bacteria (i.e., *Salmonella* spp. and *E. coli*) from Danish pork and the rise in fluoroquinolone resistance currently observed in *C. jejuni* in poultry in the Netherlands.

There is heterogeneity in AMR prevalence observed across different countries that otherwise would not be detected if not for surveillance and research efforts focused on food at retail level. This heterogeneity could be due to variations in animal production and food processing practices. There is a lack of AMR data for particular food groups; these are milk and dairy products, seafood and fresh produce. This is particularly worrying as there is evidence of national and multi-state/multi-national outbreaks of foodborne disease associated with these foods (130-132). No surveillance programmes, to our knowledge, assess AMR in milk, dairy products or seafood in a systematic manner. Fresh produce is covered by some surveillance systems in European countries (the Netherlands and Denmark) and overall surveillance at European level by EFSA but only in recent years. This lack of AMR data at retail level is a concern as international trade had been identified in the past as of key importance for the dissemination of resistant bacteria in 1999 by the ACMSF (133). This same committee recognised that implementation of restrictions on antimicrobial use in food-producing animals in the UK would not be effective in tackling of AMR until the risk of AMR entry through food imports had been addressed (133).

It was difficult to assess patterns of MDR as different criteria have been used to categorise MDR. Results were therefore often not comparable both between local and international studies. Furthermore, most studies reported MDR results selectively and it was therefore not possible to extrapolate findings to determine and compare the prevalence of MDR in food groups within and between countries.

The main findings and gaps of knowledge identified for each of the main food groups are summarised below.

<u>Pork</u>

UK

There was limited, dated scientific evidence in respect of AMR available for British pork or imported pork sold in the UK. Only a small number of MDR isolates have been analysed. MDR phenotypes in *S.* Typhimurium often involved resistance to ampicillin, streptomycin, tetracyclines and nalidixic acid. This is particularly relevant as an increase of prevalence of MDR in *S*. Typhimurium DT104 isolates and of foodborne infections caused by these pathogens in humans had already been reported in 1999 (133). No evidence was available on the occurrence of ESBL-producers in the UK or in exporting European countries in isolates from pork meat; there is a paucity of data assessing the occurrence of these isolates in food according to EFSA (134).

Exporting countries

The paucity of data from UK-produced pork is in contrast with the more extensive data available from the two main pork exporting European countries trading with the UK (i.e., Denmark, the Netherlands). Denmark has reported an increase in ampicillin resistance but a very low prevalence of fluoroquinolone resistance in salmonella isolates. All isolates tested in recent years were susceptible to colistin. Furthermore, no MDR isolates were observed, according to DANMAP data. Ampicillin resistance was also on the rise in *E. coli* isolates from Danish pork meat; low prevalence rates of resistance to 3GCs as well as to fluoroquinolones were observed in *E. coli* isolates from Danish pork meat (135, 136).

In the Netherlands, there were limited data on resistance to antimicrobials in *Salmonella* spp.. MARAN's surveillance data focused on commensal bacteria in pork; low prevalence of ampicillin resistance was observed in both *E. faecalis* and *E. faecium* isolates. In contrast high levels resistance to erythromycin in *E. faecalis* and *E. faecium* were down to 12.7% in 2014 from 34% in 2006; low prevalence levels of resistance to 3GCs were also detected (57-59).

In German pork, limited evidence was available in AMR bacteria of interest. Low prevalence of ampicillin resistance was detected in *S.* Derby but this result was based on a single study in 2007 (36); no resistance to colistin was observed. In commensal bacteria, *E. coli* presented the highest levels of clavulanate-amoxicillin resistance whilst no resistance to 3GCs (cefotaxime, ceftiofur) was observed. Resistance to fluoroquinolones was very low but the figures date back to 2004 and may not be representative of current trends (37).

In the USA ampicillin resistance has increased since 2002 in *Salmonella* spp. isolates but no resistance to cefotaxime was observed in n 2013. There was also no resistance to (fluoro)quinolones, according to NARMS data but this surveillance system follows a convenience sampling approach. For commensal bacteria, low levels of ampicillin in *E. faecalis* and to penicillins were stated for both *E. faecalis* and *E. faecium* isolates from pork meat. Erythromycin resistance was at low levels in *E. faecalis*. In *E. coli*, amoxicillin-clavulanic and ampicillin resistance levels were low in 2013. As in other countries, resistance to 3GCs and to fluoroquinolones was below 1.5%. No resistance to azithromycin was observed in *E. coli*. Nevertheless, up to 14% of *E. coli* isolates from USA pork were MDR according to NARMS data but no data were provided regarding phenotypes.

<u>Poultry</u>

UK

Poultry meat (including chicken and turkey) was the food group for which there was more evidence available for the UK. Most of the available data was from studies conducted in 2006 (apart from the FSA surveys conducted focused on AMR in *Campylobacter* spp.), or earlier and therefore findings should be interpreted carefully.

In *Campylobacter jejuni*, resistance to ciprofloxacin and nalidixic acid have increased steadily since 2001; these were at 50% and 51.5% whilst in 2005, these levels were at 15% and 22%, respectively in isolates from conventionally-produced poultry meat. Low levels of erythromycin resistance were reported in *C. jejuni*. Prevalence of MDR has increased in recent years from 19.1% in 2008 up to 43.4% in 2014-2015 in *C. jejuni* isolates from chicken meat at retail level in the UK; the most common phenotype was ciprofloxacin, nalidixic acid, tetracyclines and trimethoprim (n= 71). Data were scarce for AMR occurrence in commensal bacteria from British poultry meat. In 2000, high prevalence rates of penicillin resistance were observed in *E. faecalis* and *E. faecium* isolates, respectively. Resistance to erythromycin was high in *E. faecalis* and in *E. faecium* isolates from chicken and turkey in 2002. MDR was not investigated in commensal bacteria in British poultry meat at retail level. No data were available for AMR and MDR prevalence in *E. coli* isolates from British poultry meat.

Exporting countries

High levels of resistance to ciprofloxacin and nalidixic acid were reported in *C. jejuni* from poultry in the Netherlands and in Poland (up to 100%). Other exporting countries outside Europe, such as Argentina, Brazil and Chile, also observed similar high prevalence of fluoroquinolone resistance in isolates from poultry meat. In contrast, the USA observed lower prevalence rates of ciprofloxacin and nalidixic acid resistance in *C. jejuni* isolates from chicken and turkey meat, respectively.

In contrast to the UK, low erythromycin resistance in *C. jejuni* was reported in Netherlands and in the USA (< 10%); no resistance was reported to this antimicrobial in Polish poultry. Higher prevalence rates of erythromycin resistance were noted in *C. jejuni* isolates from Argentinian (20%) and Brazilian (68.8%) poultry meat; in contrast, Chile reported similar rates to those observed in European countries.

No MDR isolates were detected in *C. jejuni* from Dutch poultry meat through surveillance up to 2013, whilst in Poland, MDR levels up to 45% were noted but no information was provided on common phenotypes.

In commensal bacteria, data were limited in exporting countries. Low prevalence of ampicillin resistance (1.8%) was noted but an increase of erythromycin resistance to 51.8% was detected in *E. faecalis* isolates from Dutch poultry in 2013 in comparison to previous years, according to MARAN data. In the same country, ampicillin resistance has sharply decreased in *E. faecium* isolates from poultry meat between 2002 and 2013 (i.e., 16% down to 6%).

In the USA, a downwards trend was observed between 2002 and 2013, with ampicillin resistance being reduced from 44.2 to 9.9% in *E. faecium* isolates and erythromycin resistance from 45% to 35% in *E. faecalis* isolates from poultry meat. In contrast, in Brazil in 2004, high prevalence of erythromycin resistance (90.2%) was detected in *E. faecalis* isolates from poultry but it was not possible to ascertain if these high prevalence levels have been maintained over time.

No MDR isolates were detected in enterococci from Dutch poultry meats at retail level. In the USA, MDR levels up to 79% were observed in *E. faecalis* and *E. faecium* isolates from poultry meat, respectively. There was no information on the phenotypes observed. High prevalence of MDR (92.5%) were detected in Enterococci, particularly in *E. faecium* isolates from turkey meat in 2009 but have since decreased (79.2% in 2013) but remain at substantial levels (39).

In the Netherlands, there was a reduction in ampicillin resistance in *E. coli* isolates in 2014, with the highest levels of resistance at 66% observed in turkey meat. Cefotaxime resistance has also decreased since 2002 according to MARAN data. Colistin resistance was higher in turkey meat than in chicken meat but still below 5%; no trends were reported.

In Poland, high prevalence levels of ampicillin resistance of up to 100% were observed and to a lesser extent, to cefotaxime at 41.7% in *E. coli* isolates from poultry meat, but no resistance was detected against carbapenems; this evidence was provided from a single study and therefore should be interpreted with care.

In Europe, there is currently scarce data on levels of carbapenem resistance in isolates from poultry meat; EFSA recommends active surveillance and target surveys to assess prevalence of carbapenem resistance in *Salmonella* spp. and *E. coli* isolates from poultry meat in EU Member States (137).

In the USA in 2010, high prevalence of resistance to β-lactam antimicrobials was observed in *E. coli* isolates from poultry meat produced in conventional systems, particularly to ampicillin (up to 58%), 3GCs (up to 90%) and to fluoroquinolones (up to 98%). These findings are higher than those reported by other studies in the same country, conducted years earlier. No resistance to erythromycin was detected in *E. coli* isolates from USA poultry meat.

It was not possible to ascertain prevalence of MDR in *E. coli* isolates from poultry meat from exporting countries. In the Netherlands, in a study focused in ESBL-producing *E. coli*, low levels of colistin resistance (< 2%) were observed. This finding is worrying, as a EFSA scientific opinion reported increasing trends of ESBL-producers in poultry meat across Europe and in other countries, worldwide (134); the acquisition of resistance to colistin by ESBL-producers could pose a serious risk to consumers and should be further investigated. It was not possible to evaluate trends of ESBL-producers in this systematic review due to the limited data but this should be further investigated. In the USA, data from 2002 estimated prevalence of MDR between 10 and 26% in *E. coli* isolates from poultry meat.

<u>Dairy products</u>

UK

It was not possible to assess the frequency of AMR in commensal bacteria in milk and other dairy products at retail level in the UK due to the lack of scientific evidence between 1999 and 2016.

Exporting countries

In exporting countries, limited dated data were available for most exporting countries.

No amoxicillin resistance was detected in *E. faecalis* isolates from French cheese in 2005. High levels of resistance to ampicillin (up to 44%), ciprofloxacin (92%) and erythromycin were reported in milk and cheese in *E. faecalis* in Turkey in 2005. High prevalence rates of resistance to ampicillin and ceftriaxone were also observed in *E. faecium* from the same food in the same year. A recent study in Turkey assessed MDR in *E. faecium* isolates from dairy milk and reported the absence of VRE. It was not possible to assess MDR across countries due to the paucity of data.

There was no AMR evidence available in European countries (apart from MDR) in *E. coli* in dairy products; in the USA, ampicillin resistance of up to 80% and to a lesser extent, to ceftriaxone (30%) and full susceptibility to fluoroquinolones were observed in *E. coli* isolates from raw cow's milk. Only a reduced number of MDR isolates were assessed in dairy production in both European countries and in the USA and therefore it was not possible to extrapolate findings. No data were available for other exporting countries such as the RoI, Canada, New Zealand, Israel or from the United Arab Emirates (UAE).

<u>Seafood</u>

UK

It was not possible to assess the prevalence of AMR in commensal bacteria in farmed and wild fish and shellfish in the UK due to the lack of scientific evidence and surveillance.

Exporting countries

For exporting countries, scarce data were available and only for AMR occurrence in *E. coli* isolates from seafood at harvest and retail levels for countries outside Europe. In Asian countries, the highest prevalence rates of ampicillin resistance (up to 79%) were observed in farmed fish in China, compared to Vietnam (30%). In China, resistance to cefotaxime was low and no resistance was observed to ceftiofur whilst resistance to ciprofloxacin and nalidixic acid were relatively low in isolates from farmed fish. In Vietnam, similar prevalence rates were observed to ciprofloxacin but there was a higher prevalence of resistance to nalidixic acid in *E. coli* isolates from farmed seafood (fish and shrimp) at retail level.

Occurrence of MDR was reported in China and Thailand; China detected a low prevalence of ESBL-producers (1.5%) in *E. coli* isolates from farmed fish at market level, whilst studies in Vietnam detected higher rates of ESBL-producers at 18.3% in isolates from farmed shrimp at retail level.

Vegetables and fruit

UK

It was not possible to assess AMR in commensal bacteria in domestically-produced vegetables and fruit due to the lack of scientific evidence. There was also a paucity of data on frequency of AMR in imported fresh produce in the UK.

Exporting countries

Amongst the main exporting European countries trading with the UK, only the Netherlands assessed AMR in vegetables and fruit as part of their MARAN surveillance programme. Limited evidence was available for Spain, currently the main exporter of fresh produce to the UK at European level and to a lesser extent, for Germany. Outside Europe, limited evidence was available for the USA and South Africa. No data were available for France, Turkey and Brazil.

The most reliable data was that published by the MARAN surveillance program, as studies in other countries only assessed small number of isolates or evidence pertained to AMR findings was dated (USA) and therefore findings are difficult to interpret and compare across countries.

In *E. coli*, low levels of ampicillin, ciprofloxacin and nalidixic acid resistance, and no resistance to either cefotaxime or colistin were detected in Dutch vegetables at retail level.

It was not possible to assess MDR across countries due to the paucity of data.

General recommendations

- The time of sample collection was not reported in several of the studies included in this review. Authors should state the years of sample collection to allow temporal trends in AMR to be followed;
- Findings have shown that there is often no standardisation in the selection of antimicrobials for susceptibility testing panels. If possible, recommendations by relevant competent organisations or authorities should be developed and/or followed (if available) regarding relevant antimicrobials per antimicrobial class to be tested for particular foodborne pathogens and commensal bacteria;
- There was a large variation of the reporting of MIC breakpoints and interpretative criteria (e.g., CLSI, EUCAST) followed by researchers across scientific studies to assess resistance. Most studies outside the EU applied clinical breakpoints, taking into account the risk of therapeutic failure in humans. For the purpose of assessing emergence of acquired resistance in bacterial isolates in food, it would be more adequate to follow epidemiological cut-off values (ECOFFs) as these have lower MIC values than clinical breakpoints as it has been previously recommended by the ACMSF;
- It was not possible to assess AMR in other food items (i.e., beef and veal meats and eggs); additional systematic reviews should o be conducted to assess

prevalence levels and trends of AMR and MDR in relevant foodborne and commensal bacteria in these food items;

- No standardised definition for MDR is being used across scientific literature and grey literature. There are definitions proposed by Magiorakos *et al*. (2012) and by EFSA (2015), but these are very recent. There is therefore a need for the scientific community to develop common definitions acceptable to the majority to allow easier and quicker comparison of data on MDR between scientists as well across countries;
- There was evidence of variation of AMR levels and trends in bacterial-food groups combinations across countries, as observed, e.g. resistance to fluoroquinolones in *C. jejuni* across European, North and South American countries and across types of food sampled (e.g. chicken versus turkey meat). It is important to interpret these findings in the context of antimicrobial use (AMU) and animal and food production practices.

Recommendations for improved surveillance

- The use of convenience, non-probabilistic sampling of food samples at retail level was widespread across scientific studies and surveillance programmes; this affected the accuracy of the resistance estimates presented in this report. Where possible, researchers and policy-makers should promote the adoption of random sampling and adequate study design for epidemiological studies and when implementing surveillance systems, respectively for determination of AMR in the food chain as indicated in the EFSA guidelines[9];
- Identification of a core set of relevant antimicrobials for prospective testing for the different bacteria/food-group combinations should be established for the UK and for surveillance in general to permit comparison within and across studies as indicated in the susceptibility panels provides by EFSA guidelines;
- Surveillance priorities could be set according to risk, using a risk-based approach taking into account the importance of antimicrobials for human and animal health and existing evidence of AMR mechanisms (genotype and phenotype) in the bacteria of interest (e.g. colistin resistance in Gram negative bacteria). This would be in line and updated from the previous recommendations by the ACMSF in their 1999[10] report on Microbial Antibiotic Resistance in Relation to Food Safety;
- Considering that commensal bacteria such as enterococci isolated from foods and clinical samples are becoming resistant to an increasing number of antimicrobials, continued surveillance of their incidence and emerging resistance (including MDR) is important in order to identify foods that can present a risk for British consumers, and ensure effective treatment of human enterococcal infections. In this respect, both the ACMSF 1999 report on Microbial Antibiotic Resistance in Relation to Food Safety and, more recently, the EFSA have

[9] EFSA's Technical report: Manual for reporting on antimicrobial resistance within the framework of Directive 2003/99/EC and Decision 2013/652/EU for information deriving from the year 2015: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2016.EN-990/pdf
[10] https://acmsf.food.gov.uk/committee/acmsf/acmsfsubgroups/amrwg

recommended the inclusion of commensal *E. faecium*, *E. faecalis* and *E. coli* as indicator bacteria in AMR surveillance programmes[11];

- There is a lack of information on AMR bacteria in foods of animal origin other than meat at retail level. In recent years, there have been growing numbers of outbreaks associated with milk and dairy products (cheese, butter, yogurt), seafood (fish and shellfish) and fresh produce (fruit, vegetables and salads) at national and international levels but there is scarce, scattered evidence of resistance and MDR occurrence in foodborne and commensal bacteria in these food products and its implications for public health. These gaps should be addressed also using a risk-based approach following evidence of resistance in food items as well as the extent of expected consumer exposure using consumption and import volumes.

- There is a particular lack of surveillance data of AMR occurrence in seafood, including those produced under aquaculture systems and those from natural marine or fluvial ecosystems. There is regrettably, a lack of antimicrobial use data for fish and shellfish produced under intensive production systems. Surveillance of both antimicrobial use and AMR in aquatic species was recommended in the ACMSF 1999 Report on Microbial Antibiotic Resistance in Relation to Food Safety[12]. More recently the Food and Agriculture Organisation (FAO), the World Organisation for Animal Health (OIE) and WHO have supported the recommendations in this report.

- Efforts should be made to continue to monitor resistance trends (AMR and MDR) in *Campylobacter* spp. strains and commensal bacteria from both imported and domestically-produced poultry meat in the UK; differentiation should be made for different types of poultry meat sampled (i.e., chicken and turkey meat) and types of production systems due to variations observed in farming management practices.

- AMR and MDR evidence in bacterial isolates from pork meat in the UK is limited and dated. Research and surveillance should be developed to monitor AMR and MDR levels in foodborne pathogens (e.g., *Salmonella* spp.) and commensal bacteria from imported and domestically-produced pork meat in the UK.

Recommendations for risk assessment

- Data on AMU in food-producing animals in the UK by species will be important to explain the occurrence and dynamics of AMR, resistance genes and MDR phenotypes in a defined geographical area. Although other drivers of resistance occurrence such as biocide and heavy metals use may also be important, their effects have not been quantified. AMU is generally accepted as the most direct risk factor for AMR. Furthermore, more complete information should be collected

[11] EFSA's Technical report: Manual for reporting on antimicrobial resistance within the framework of Directive 2003/99/EC and Decision 2013/652/EU for information deriving from the year 2015: http://onlinelibrary.wiley.com/doi/10.2903/sp.efsa.2016.EN-990/pdf

[12] https://acmsf.food.gov.uk/committee/acmsf/acmsfsubgroups/amrwg

on the type of production system from which food samples originate to assess the impact of animal husbandry practices as risk factors or resistance;

- There is a need for more studies to quantify the contribution of both domestic and imported foods to AMR occurrence. Information on country of origin for imported products should be collected. Priorities should be set according to the importance of a food item in terms of exposure of consumers. Consumption data will be essential for assessing the risk of exposure of British consumers;

- Further research and surveillance is needed to establish and quantify the risk of transmission of resistance against critically important antimicrobials from foods of animal (including meat, seafood and dairy products) and non-animal origin (e.g. vegetables and fruit) to humans. This is essential to develop interventions along the food chain to protect British consumers. The international method of choice is microbiological risk assessment, according to the Codex Alimentarius[13].

[13] Codex Alimentarius microbiological risk assessment: ftp://ftp.fao.org/docrep/fao/005/Y1579e/Y1579e.pdf

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