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Comparative Assessment of Human Exposure to Antibiotic-Resistant *Salmonella* due to the Consumption of Various Food Products in the United States

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Comparative Assessment of Human Exposure to Antibiotic-Resistant *Salmonella* due to the Consumption of Various Food Products in the United States

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Antibiotic resistance (AR) was increasingly recognized as a global and national problem. Prevention efforts are hampered by a lack of complete understanding of how transmission pathways contribute to human AR exposure. Many reports have indicated the presence of antibiotic-resistant bacteria in foods at retail, suggesting that food consumption, animal-derived foods in particular, can represent a significant source of AR exposure among consumers. The presence of *Salmonella*, including antibiotic-resistant *Salmonella*, has been frequently reported in terrestrial animal-derived foods such as meat, poultry, and dairy products, as well as in aquaculture products. Identification of the significant food sources that harbor relatively substantial antibiotic-resistant *Salmonella* is the key for the design and implementation of effective and target AR mitigation strategies. Thus, a systematic evaluation of the relative contribution of different food sources to human antibiotic-resistant *Salmonella* was imperative.

This thesis aimed to gather qualitative and quantitative information about the contamination of antibiotic-resistant non-typhoidal *Salmonella* in various retail foods in the U.S. and identify knowledge gaps using systematic review (SR) and meta-analysis (MA) approaches. The data on resistant *Salmonella* concentration in foods has not been found. Resistant *Salmonella* prevalence in regulated commodities (beef, chicken, turkey, pork) and other food categories were conducted for major antibiotic classes. Generally,

poultry, pork, and turkey had a higher prevalence of resistant *Salmonella* than beef, while vegetables and imported foods (mainly spices in documented studies) had a lower prevalence. For antibiotics classes, tetracycline resistance was the most prevalent across major commodities. There is a moderate level of resistance to beta-lactam antibiotics, but the significance in clinical practice indicates a potential threats to public health.

Another objective was to develop a stochastic comparative exposure assessment model to estimate the relative contribution of various animal-derived food groups to overall foodborne exposure to cephem-resistant *Salmonella*. The model consists of four modules: retail, transport, storage, and preparation. Generally, the results showed that ground beef and chicken parts accounted for the largest proportion of total exposure to cephem-resistant *Salmonella* compared to pork cuts and ground turkey. The contamination level in products at retail and cooking temperature were the top influencing factors of the foodborne exposure for all food products evaluated in the present study.

Foodborne illness source attribution is the foundation of a risk-based food safety system. The present project provides a risk-based estimation of the degree to which different food categories are responsible for resistant *Salmonella* infections. With these estimates, target prevention measures can be designed and implemented to effectively mitigate the AR threat to public health attributable to the food consumption.

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CHAPTER 1. INTRODUCTION

1. Background and Objectives

It is increasingly recognized that antibiotic resistance (AR) is a devastating threat to public health in the U.S. and worldwide (CDC, 2013). It is projected that by 2050 the global deaths due to antibiotic-resistant infection will reach up to 10 million, costing 86 trillion USD, greater than those caused by cancer and the total from the deadliest infectious diseases (Anonymous, 2016). In the U.S., antibiotic-resistant bacteria and fungi cause at least an estimated 2.8 million infections and 35,900 deaths, costing more than 55 billion USD (CDC, 2019). Food products, particularly livestock-derived foods, nowadays are considered one of the major matrices facilitating the spread of AR between human and animal sources (Acar & Moulin, 2013). However, intervention efforts may be slowed down and the efficacy may be compromised due to a lack of comprehensive understanding of the transmission through various possible pathways (Knight et al., 2018).

Among foodborne pathogenic bacteria, non-typhoidal *Salmonella* enterica is the leading cause of foodborne infections, hospitalization, and death in the U.S. (Scallan, Griffin, Angulo, Tauxe, & Hoekstra, 2011). The presence of *Salmonella*, including antibiotic-resistant *Salmonella*, has been frequently reported in terrestrial animal-derived foods such as meat, poultry, and dairy products, as well as in aquaculture products (Miranda, Kehrenberg, Ulep, Schwarz, & Roberts, 2003). For public health protection, determining the relative contributions of different food groups to the overall antibiotic-resistant *Salmonella* exposure attributable to food consumption is crucial. Successfully identifying the most significant antibiotic-resistant *Salmonella* sources will play a critical role in

setting effective and efficient intervention strategies to limit the spread and development of antibiotic-resistant *Salmonella* in food supply chains.

The overall goal of the present project is to quantify the relative contribution of different food sources to human AR *Salmonella* using evidence-based systems approaches. Specifically, studies under the two specific objectives stated below were conducted.

- *Objective 1*. Characterize the distribution of antibiotic-resistant non-typhoidal *Salmonella* in various foods at retail and identify the knowledge gaps using systematic review (SR) and meta-analysis (MA) methods
- *Objective 2*. Estimate the relative contribution of different food groups to overall foodborne exposure to antibiotic-resistant *Salmonella* using the quantitative comparative exposure assessment method.

Results from the SR-MA study (Chapter 2) standalone present the epidemiological characteristics of antibiotic-resistant *Salmonella* of different food origins. In addition, the quantitative synthesis of contamination data in various foods facilitated the estimation of key input variables in the comparative exposure assessment model (Chapter 3). These results based on the integrated evidence-based, risk-based methods will support the regulatory agencies, industry professionals, and risk managers with scientific foundations in establishing performance standards and possible interventions at certain stages of the food supply chain to constrain the spread of antibiotic-resistant *Salmonella*.

2. Antibiotic resistance in the food supply chain

It has been evidenced that inappropriate use in human and animal husbandry of antibiotics has contributed to the rise of AR issues, creating detrimental effects on human

health through the development and transmission of antibiotic-resistant bacteria (ARB) in the anthropological environment, including both agriculture and aquaculture (White, Zhao, Simjee, Wagner, & McDermott, 2002). Figure 1.1 illustrates the possible ARB spreading pathways through the food supply chain (Bengtsson-Palme, 2017). Numerous studies have reported the contamination of ARB in food products at the retail stage, which indicates the potential of food consumption as an important exposure pathway to ARB (Mathew, Cissell, & Liamthong, 2007). Based on a recent report by CDC ranking the most urgent and serious AR threats in the U.S., food-related threats included carbapenem-resistant *Acinetobacter*, carbapenem-resistant *Enterobacteriaceae* (CRE), drug-resistant *Campylobacter*, Vancomycin-resistant *Enterococci* (VRE), extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, drug-resistant nontyphoidal *Salmonella*, drug-resistant *Salmonella* serotype Typhi, drug-resistant *Shigella*, and methicillin-resistant *Staphylococcus aureus* (CDC, 2019). Among the identified foodborne pathogens, non-typhoidal *Salmonella* enterica has been long identified as the leading cause of foodborne infections, hospitalization, and death in the U.S. (Scallan et al., 2011), which is well known harboured in livestock animals and commonly detected in a great variety of food products. The combination of the frequent presence of *Salmonella* in foods and the antibiotic-resistant properties presents a potential challenge for salmonellosis treatment due to the compromised effect of antibiotic therapy.

Attributable to the possibilities of over-and misuse of antibiotic drugs in veterinary settings, the selection of ARB, including antibiotic-resistant *Salmonella*, cannot be ruled out, which may subsequently escape from the primary production, survive the processing and preparation steps, and eventually pose risks to human health through possibly

contaminated food at the time of consumption (Aidara-Kane, 2012). Unfortunately, antibiotic-resistant *Salmonella* has been frequently detected in various foods. Up to date, monitoring efforts regarding foodborne ARB including *Salmonella* resistant to various antibiotic classes have primarily concentrated on food products from land animals. In 1996, the National Antimicrobial Resistance Monitoring Systems for Enteric Bacteria (NARMS) was established to track changes in the antibiotic resistance profiling of certain enteric bacteria in retail meat, including beef, pork, broiler chicken, and turkey (Food and Drug Administration). Compared to beef and pork, the overall prevalence of antibiotic-resistant *Salmonella* in poultry products from 1997 to 2018 shows a steady increase over time, particularly those of chicken origin (Food and Drug Administration).

Additionally, other food vehicles, such as produce and crop-based products, can also serve as sources of ARB exposure due to the introduction of contaminated cropping environment (e.g., manure amended soil and irrigation water) and/or the cross-selection of AR as a result of pesticide/herbicide application (Forsberg et al., 2012; Koutsoumanis et al., 2021; Rangasamy, Athiappan, Devarajan, & Parray, 2017). Antibiotic-resistant bacteria are reported in a variety of plant-based foods including leafy vegetables, tomatoes, beans, peppers, roots, and various fruits in the U.S., some of the isolates have multi-drug resistance (Liu & Kilonzo-Nthenge, 2017). Peng and coauthors also report that in integrated crop-livestock farms, where food animals and crops are produced nearby, the pre- and post-harvest prevalence of antibiotic-resistant *Salmonella* were higher compared to conventional farms (Reddy, Wang, Adams, & Feng, 2016). Furthermore, Watts and coauthors pointed out the emergence of antibiotic-resistant pathogens in aquaculture practices and resulting products, indicating the importance of

monitoring aquaculture supply chains (Watts, Schreier, Lanska, & Hale, 2017). Therefore, plant- and aquatic animal-based foods should be of concern as well as animal-based food products for integrated surveillance and risk/exposure assessment purposes.

3. Data collection for antibiotic resistance through SRMA

Systematic review and meta-analysis (SR-MA) are used to combine and analyze data collected from multiple existing studies conducted on similar topics (Ahn & Kang, 2018). Its usefulness has made SR-MA an appealing tool applied in the fields of human health and animal health. In the last decade, SR-MA has been recommended by inter-governmental food safety authorities as a robust tool to address food safety issues, particularly in the applications of microbial food safety risk assessment (EFSA, 2010; O'Connor & Sargeant, 2014). In general, a systematic review follows a well-designed protocol for collecting evidence, assessing the quality of sources, and synthesizing the data collected from relevant and qualified primary research (Armstrong, Hall, Doyle, & Waters, 2011). When sufficient information is available for quantitative evidence synthesis, meta-analysis can be followed to summarize the extracted data according to the proposed research questions, which may target different research focuses, such as the evaluation of the effectiveness of certain interventions (Onay B Dogan, Aditya, Ortuzar, Clarke, & Wang, 2022) or the determination of the contamination of a certain hazardous agent (Ortuzar et al., 2018).

Tremendous efforts have been devoted to understanding the scope of antibiotic-resistant *Salmonella* contamination in foods in the U.S., including the NARMS and numerous primary research studies documented in scientific literature covering various food types. However, to date, there is a lack of effort for systematically retrieving, appraising, and

synthesizing the available information on resistant *Salmonella* across different types of retail food in the U.S. covering both animal- and plant-derived food. Hence, in the present project (Chapter 2), we conducted an SR-MA to investigate the overall antibiotic-resistant *Salmonella* prevalence and concentration, as well as stratified by food types, and to provide unbiased estimates of the desired outcome together with the variability and uncertainty around the studied parameters. SR-MA findings can be applied to estimating the burden of foodborne diseases (Elias, Noronha, & Tondo, 2019; Naylor et al., 2016), identifying knowledge gaps and illustrating the direction of new research areas, and refining the parameterization process of risk assessments of antibiotic-resistant *Salmonella* in foods (Aiassa et al., 2015; Onay Burak Dogan, Clark, Mattos, & Wang, 2019; Jans et al., 2018).

4. Risk assessment as a robust tool for controlling foodborne antibiotic resistance

Risk assessment is a widely endorsed tool for a robust food regulatory system and food standards (FAO/WHO, 1995), and Codex Alimentarius Commission (CAC) published guidance for assessing the risks of foodborne antibiotic resistance (CAC, 2011). As a component of risk assessment, exposure assessment aims to evaluate the likely intake of hazardous agents via food as well as exposures from other sources if relevant. Besides as part of a risk assessment, it is not uncommon to undertake an exposure assessment stand-alone, especially in a situation when there is not enough information to support the establishment of a dose-response assessment or seek measures to mitigate exposure is sufficient from a risk management perspective (FAO and WHO, 2021). In the context of antibiotic resistance, no reliable dose-response models are available yet for microbial risk

assessment practices. Hence, a few published food safety risk assessments focused on the intake level of ARB at the time of food consumption as the major model outputs as a crude indicator or sentinel of public health concerns (Zhang, Schmidt, Arthur, Wheeler, & Wang, 2021; Zhang et al., 2022).

To identify the foods posing significant AR risks among consumers, comparative exposure assessment, a variant of the conventional approach, offers a unique opportunity to determine the relative contribution of different exposure routes (food products) to the overall exposure. This type of assessment was usually employed for comparison purposes to prioritize risk management strategies, which may compromise the true representation of public health risks but offer the flexibility to focus only on the elements necessary to make the comparison (Hald & Pires, 2011). Up to date, there are two comparative exposure assessments identified with a focus on foodborne antibiotic resistance. Evers and coauthors compared the exposure to ESBL-producing *E. coli* in various meat and poultry products among the Dutch population (Evers et al., 2017). Jans and coauthors conducted a comparative assessment of ARB in Swiss retail food (Jans et al., 2018), which delivered findings on a qualitative basis. There is no practices of comparative exposure assessment identified in the U.S. to mitigate AR exposure through the consumption of food products, which was therefore conducted as part of this project to fill the knowledge gap (Chapter 3).

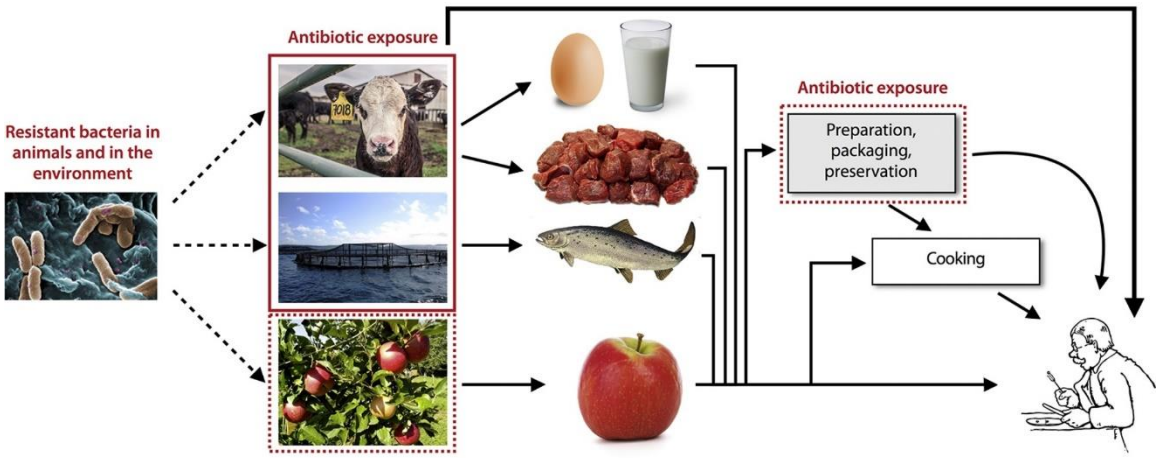

Figure 1.1 Antibiotic-resistant bacteria spreading pathways: Adapted from Bengtsson-Palme (2017)

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CHAPTER 2. ANTIBIOTIC-RESISTANT NON-TYPHOIDAL *SALMONELLA* IN VARIOUS FOODS AT RETAIL IN THE UNITED STATES: A RAPID SYSTEMATIC REVIEW AND META-ANALYSIS

1. Abstract

The rise of antibiotic resistance (AR) has become a significant public health threat in the United States and worldwide. Resistant bacteria in foods at retail have been frequently reported, indicating the potential of food consumption as an important exposure pathway. Among pathogenic bacteria, *Salmonella* is one of the leading causes of foodborne diseases in the United States. The present study aimed to comprehensively collect and critically review quantitative and qualitative information about the contamination of antibiotic-resistant non-typhoidal *Salmonella* in various foods at retail in the United States using systematic review (SR) and meta-analysis (MA) approaches. The CoCoPop framework (condition, context, and population) was followed to determine eligible citations from six electronic databases and grey literature. No data pertinent to resistant *Salmonella* concentration in foods were found. Meta-analyses of resistant *Salmonella* prevalence were performed by major commodities (beef, chicken, turkey, pork) and classes of antibiotics. From 11,839 retrieved citations, 45 were considered relevant. In addition, National Antimicrobial Resistance Monitoring System for Enteric Bacteria annual reports from 2002 to 2017 was included. In general, results showed a higher prevalence of resistant *Salmonella* in chicken, pork, and turkey, compared with beef, and lowest in vegetables and imported foods (data mainly available for spices). As for resistance to various antibiotic classes, tetracycline resistance was observed to be the highest among major commodities (39.67%-48.78%). Albeit a moderate level of resistance to beta-lactam antibiotics, the threat to public health can be profound due to their critical roles in clinical use. Surprisingly, resistance to macrolides, an important antibiotic class in veterinary settings, was considerably low for all major commodities,

which was however estimated based on less data currently available. Results of the present study will facilitate the application of quantitative microbial risk assessment in identifying and evaluating potential mitigation strategies for controlling human exposure to foodborne AR.

2. Introduction

Antibiotic resistance (AR) - the ability of a microbe to resist the microbicidal or microbiostatic effects of medication that once could successfully manage the microbe- is a global concern that has received targeted national attention (CDC, 2013) and government action in the United States. (PCAST, 2014). Antibiotic-resistant bacteria (ARB) are the microorganisms that have the property of AR. The Centers for Disease Control and Prevention (CDC) reported more than 2.8 million antibiotic-resistant infections and over 35,000 deaths in the United States each year (CDC, 2019). It has been evidenced that inappropriate use in human and animal husbandry of antibiotics has contributed to the rise of AR issues, creating detrimental effects on human health through the transmission of ARB in the anthropological environment, including food (White, Zhao, Simjee, Wagner, & McDermott, 2002).

Numerous studies have reported the contamination of ARB in food products at the retail stage, which indicates the potential of food consumption as an important exposure pathway to ARB. Among foodborne pathogenic bacteria, non-typhoidal *Salmonella* enterica is the leading cause of foodborne infections, hospitalization, and death in the United States. (CDC, 2013; Scallan et al.,2011). The existence of antibiotic-resistant (AR) *Salmonella* presents a challenge for salmonellosis treatment as a result of the compromised effect of antibiotic therapy. Unfortunately, antibiotic-resistant *Salmonella*

has been frequently detected in various foods. The emergency in antibiotic-resistant *Salmonella* has been wildly reported in terrestrial animal-derived food such as meat, poultry, dairy products, and aquaculture products (Miranda, Kehrenberg, Ulep, Schwarz, & Roberts, 2003). Attributable to the use and misuse of antibiotic drugs in veterinary settings, the development of ARB can be promoted, which may subsequently escape the primary production and pose risks to human health through the consumption of possibly contaminated food (Aidara-Kane, 2012). Additionally, other food vehicles, such as produce and crop-based products, can also serve as sources of ARB exposure due to the introduction of contaminated cropping environment (manure amended soil and irrigation water) and/or the cross-selection of AR as a result of pesticide/herbicide application (Forsberg et al., 2012; Koutsoumanis et al., 2021; Rangasamy, Athiappan, Devarajan, & Parray, 2017).

To develop and implement targeted prevention measures for effectively mitigating food-medicated AR health risks, regulatory agencies and the food industry need information about the relative contribution of various foods in causing resistant foodborne infections. To support the food source attribution analysis, essential information needed is the distribution of ARB in various foods. Tremendous efforts have been devoted to understanding the scope of ARB contamination in foods in the United States, including the National Antimicrobial Resistance Monitoring Systems (NARMS) mainly tracking AR enteric bacteria in retail meats (Karp et al., 2017) and numerous primary research studies documented in scientific literatures covering various food types. However, to date, there is a lack of effort for systematically retrieving, appraising, and synthesizing the

available information of AR across different types of retail food in the United States covering both animal- and plant-derived food.

To obtain a more complete view of the AR issue across different food systems, the present study aimed to (1) comprehensively collect and critically review data about the contamination of antibiotic-resistant non-typhoidal *Salmonella* in various food at retail in the United States using a systematic review (SR) and meta-analysis (MA) approaches, and (2) identify data gaps that require new research on the areas currently with limited knowledge. The results of this study will aid in the construction of a comparative exposure assessment model that allows for the attribution to food sources of antibiotic-resistant *Salmonella* exposure at the time of food consumption to direct the design of foodborne AR mitigation interventions.

3. Method

The present review was reported following the guidance elaborated in the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement (Liberati et al., 2009; Moher, Liberati, Tetzlaff, Altman, & Grp, 2009).

3.1 Research Question and Eligibility Criteria

This review was designed to address the question "What are the prevalence and/or concentration of antibiotic-resistant *Salmonella* from various types of food at the retail level in the United States?". Eligibility criteria were developed following the CoCoPop (condition, context, and population) framework covering the following concepts pertinent to the review question (Munn, Stern, Aromataris, Lockwood, & Jordan, 2018):

Condition (Co): ARB prevalence and/or concentration. Prevalence refers to the percentage of tested samples that are positive for antibiotic resistance. Concentration refers to the enumerable quantitative amount of ARB in the sample tested positive.

Context (Co): Studies related to the United States. Retail sectors were targeted. Though data from other countries with similar food safety standards as in the United States may represent a similar condition, no attempt was made to extend the scope in other countries beyond the United States.

Population (Pop): Any food at the retail level was targeted, covering various categories domestically produced and imported.

3.2 Search Strategy and Information sources

The search strategy integrated terms related to three main concepts as aforementioned. Key terms for each concept were combined using the Boolean operator "OR", and the concepts were combined using the Boolean operator "AND" (Table 2.1). The search syntax was verified by ensuring a full capture of a list of 30 relevant articles that were obtained before the systematic search based on a hand search.

The last literature search was conducted in July 2020, covering both bibliographical databases and grey literature. Bibliographical databases contain Web of Science Core Collection (via Web of Science, 1900 to date of search), Biological Abstracts (via Web of Science, 1926 to date of search), MEDLINE® (via PubMed, 1964 to date of search), CABI: CAB Abstracts and Global Health (via Web of Science, 1910 to date of search),

BIOSIS Citation Index (via Web of Science, 1926 to date of search), and Scopus (via the University of Nebraska-Lincoln Scopus interface, 1959 to date of search). The grey literature research mainly focused on the NARMS reports archived at the United States Food and Drug Administration (FDA) and the Microbiological Data Program (MDP) at the United States Department of Agriculture (USDA). No restrictions were placed on the search beyond the inception dates of databases. Additionally, no restrictions were placed on language in the initial search, although only citations in English were selected during the screening process.

Citations documenting primary research studies are the focus of the present review. To maximize the capture of relevant information, review papers on the topic of interest were also retained; and a backward snowballing search was conducted by seeking relevant primary studies in the bibliographic lists of selected review papers.

Search results were uploaded to EndNoteX9 (Clarivate Analytics, Philadelphia, PA). Duplicated citations identified by Endnote deduplication function and hand search were removed.

3.3 Study Selection

Screening of relevant studies was managed using EndNote. Two levels of relevance screening were conducted, i.e., a preliminary screening based on title and abstract and an advanced one based on full texts. The preliminary screening was conducted to rapidly exclude articles irrelevant to our research questions. The advanced screening was conducted to further confirm the studies relevance based on full texts. The relevance screening was performed using a rapid approach that utilized recognized techniques in conventional systematic review but just involved one reviewer in the process. To

strengthen the validity of the screening process, the reviewer conducted each screening step twice blind to the previous relevant decision.

The following questions were used for both preliminary and advanced screenings, which were adopted from the systematic review conducted by (Sargeant et al., 2019) with modifications suitable for our context.

(1) Is the study available in English? YES, NO, UNCLEAR

(2) Does the study primarily focus on *Salmonella*? YES, NO, UNCLEAR

(3) Are the study samples food at the retail level? YES, NO, UNCLEAR

(4) Are the study samples taken from the United States? YES, NO, UNCLEAR

(5) Does the study report the prevalence and/or concentration of *Salmonella* or anything about antibiotic-resistant characteristics? YES, NO, UNCLEAR

In the preliminary screening, studies were excluded only when the reviewer answered NO for more than two questions. When conflicts occur, studies were retained for the verification based on full texts. In the advanced screening, full texts were retrieved to confirm the study's eligibility for inclusion. The aforementioned five questions involved only YES or NO options as answers. Conflicts occurring at this step were resolved by consulting a second reviewer. Studies with all questions answered YES were moved for data extraction.

Studies involving samples collected on-farm were excluded considering these samples represent intermediate product units with further processing and not ready for the market. Albeit the possibility as end products, studies of raw milk collected on-farm were excluded due to a similar reason. However, studies sampling farm market food (including raw milk) were included because of their direct access to consumers. Data were extracted from articles after the complete screening phases, where affirmatively the answers to all questions were obtained.

3.4 Data Collection

Following a similar rapid approach, data were extracted by a single reviewer and stored in a standardized data extraction form based on an Excel spreadsheet. To strengthen the accuracy of extracted information, collected data were verified by the same reviewer. In the present review, a study refers to a single published article or report, while a trial refers to a reported result from a study where the prevalence or concentration could be computed for a particular sampling event. Hence, one study may provide data for multiple trials. Data related to bibliographic characteristics were extracted at the study level, while those related to sampling design, testing techniques and outcome measures were at the trial level. In summary, the extracted information is listed as follows.

Bibliographic characteristics: First author and publication year.

Sampling and testing information: Country/states where and year/month (or season) when the study was conducted, food type (e.g., pork, beef, turkey, chicken, vegetable, and imported food), food properties (e.g., organic, conventional, and no antibiotics),

sample volume (i.e., size of a sampling unit), sample size (i.e., number of sample collected for testing) and *Salmonella* and AR detection and quantification (if possible) methods.

Outcome measurement: Prevalence of antibiotic-resistant *Salmonella* resistant to particular antibiotic classes including a number of samples positive for AR among total sample size tested, and/or concentration of antibiotic-resistant *Salmonella* including mean, quantifiable variation and number of samples upon which the statistics were computed, and prevalence of antibiotic-resistant *Salmonella* by food type

3.5 Risk of Bias Assessment

Quality assessment was evaluated for individual studies using the appraisal tool for prevalence studies with modified questions (Broen, Braaksma, Patijn, & Weber, 2012; Munn, Moola, Lisy, Riitano, & Tufanaru, 2015; Munn et al., 2018) (Appendix). Three aspects of the research quality were considered by the adjusted tool: (1) the representativeness of the sample, (2) the deliberating of a study designed for prevalence measurement, and (3) the sufficiency of statistical power. A collection of samples was considered representative when two criteria were met. First, samples were food at the retail level or characterized *Salmonella* strains were isolated from retail food samples. Second, at least one of the following should have applied: the entire source population was sampled, the sample was randomly selected, or systematic sampling was implemented. A study was considered deliberately designed when prevalence and/or concentration estimation was stated as one of the objectives or provided as a by-product

outcome in the study that was conducted using the minimal inhibitory concentration (MICs) method which is the same mentioned in the NARMS report. Finally, a sample size of 140 (Plishka, Sargeant, Greer, Hookey, & Winder, 2021) was used to assess whether a study was sufficiently powered by an author-defined estimate of expected prevalence (10% or 90%) and allowable error (5%) (Naing, Winn, & Rusli, 2006).

3.6 Definition

To better represent results and conclusion, some definitions are proposed: (1) A study (citation or article) is referred to a single unique publication that was collected, analyzed and reported by authors. (2) Within a citation, trials represent the result from a study where the prevalence or concentration could be computed for a particular sampling event. (3) Sample size refers to the number of samples included. Thus, a study can be visited multiple times and contains multiple trials.

3.7 Synthesis of Results

Random-effects meta-analyses using DerSimonian and Laird methods were performed by outcomes (Jackson, White, & Thompson, 2010). To synthesize prevalence data, Freeman-Tukey double arcsine transformation (Barendregt, Doi, Lee, Norman, & Vos, 2013) was used to transform data in order to stabilize the large variation between studies (Lin & Xu, 2020; Schwarzer, Chemaitelly, Abu-Raddad, & Ruecker, 2019). This transformation was designed to take into account possibly very low or high prevalence estimates such as above 0.8 or below 0.2. A composite prevalence estimate was computed as a back-transformed percentage for each outcome. Heterogeneity was assessed using I^2 and Cochran's Q test (Higgins JPT, 2011). Data analysis was performed

in R (Team, 2020), with codes adopted from Wang (N. Wang, 2018; W. Wang et al., 2018) with modification.

Due to the lack of studies enumerating foodborne AR *Salmonella*, no qualitative or quantitative analysis was conducted for concentration estimates.

3.8 Additional Analyses

No additional analyses were conducted.

4. Result

4.1 Study Selection Process

A total of 11,839 studies from the six databases were retrieved. In addition, NARMS reports from 2002 to 2017 were included (Figure 2.1). After deduplication, 5,519 studies were screened at the title and abstract level, out of which 4,637 were irrelevant to the research topic and 610 were not conducted in the United States. When a publication was identified as a summary of or a portion of NARMS data from 2002 to 2017, the publication was precluded from avoiding repeatedly counting data from the same studies. As a result, 272 studies were further assessed for eligibility based on full texts and 227 of them were excluded for various reasons shown in Figure 2.1. Finally, data were extracted from 45 qualified studies for descriptive analyses. Four studies were further precluded from the quantitative analyses, i.e., meta-analysis, due to their different detection method from the remaining majority, resulting in a total of 41 studies being quantitively analyzed.

4.2 Descriptive Analyses

The key characteristics of the 45 studies are summarized in Table 2.2. Identified studies were conducted between 1988 and 2017, and most of them (36/45, 80%) were conducted in the second half of the time span, i.e., after 2002. The increasing number of studies over time is primarily attributable to the initiation of the NARMS program for retail food in 2002, from which 16 reports were included in this review. As for the sampling regions, around half of them (17/45, 37.8%) were sampled from multiple representative locations in the United States and South (12/45, 26.7%), followed by Midwest (6/45, 13.3%), Northeast (2/45, 4.4%) and West (2/45, 4.4%).

In total, 97 trials from the 45 studies were included, covering 8109 samples or *Salmonella* isolates from retail food tested for AR profile. At the trial level, the majority of trials focused on chicken (24/97, 24.7%) and turkey (21/97, 21.6%), followed by beef (18/97, 18.6%) and pork (17/97, 17.5%), and the least for mixed food with multiple types listed without differentiation (6/97, 6.2%), imported food mainly spices and seafood (5/97, 5.2%), and vegetable (2/97, 2.1%).

Across different food types, resistance was tested against 16 antibiotics in 9 classes by following the CLSI guidelines (CLSI, 2017), including aminoglycosides, β-lactam/β-lactamase inhibitor combinations, cephems, folate pathway inhibitors, macrolides, penicillins, phenicoles, quinolones, and tetracyclines. Forty-one studies (41of 45, 91.1%) applied broth microdilution for antibiotic susceptibility testing, while the remaining four used other methods such as disk diffusion. When reported (41/45 studies), all studies followed the resistant breakpoints in accordance with the CLSI guidelines (CLSI, 2017).

It is worth mentioning that all identified studies reported antibiotic-resistant *Salmonella* prevalence in retail food, but few covered enumeration information, which represents a significant data gap hindering the development of evidence-based control strategies for foodborne AR control. Hence, the following quantitative analyses and interpretations were regarding the prevalence of antibiotic-resistant *Salmonella* incidence in retail food samples only.

4.3 Prevalence of antibiotic-resistant *Salmonella* by Resistance Type

Except for NARMS reports, most scientific articles did not annotate *Salmonella* serotypes. Hence serotype-specific subgroup meta-analyses were not conducted. All studies reported the overall antibiotic-resistance *Salmonella* prevalence but one-third of the scientific articles (8/25, 36%) did not stratify the prevalence results based on antibiotic classes or agents. Hence, class- and agent-specific pooled prevalence was estimated with a smaller set of trials.

Meta-analyses were conducted to estimate the overall prevalence of antibiotic-resistant *Salmonella* in food, the prevalence by antibiotic class and by antibiotic agent (Table 2.2). The estimated overall prevalence was 57.3% (95% CI: 52.2-62.3%), referring to the proportion of samples detected with *Salmonella* resistant to at least one antibiotic agent examined in the identified trials. Stratified by antibiotic class, *Salmonella* in food bears the highest pooled prevalence against tetracyclines (44.2%, 95% CI: 39.7-48.8%), followed by penicillins (ampicillin as the test agent, 24.0%, 95% CI: 20.1-28.2%), while macrolides (azithromycin as the test agent, 0%, 95% CI: 0-0%) and quinolones (0%, 95% CI: 0-0.3%) was the antibiotic class of lowest prevalence. Regarding specific antibiotic agents, prevalence estimates were arbitrarily categorized into high- (greater than 20%),

medium- (5-20%), and low-incidence groups (lower than 5%). Resistance to streptomycin, ampicillin, sulfisoxazole and tetracycline represented high incidence, gentamicin, kanamycin, amoxicillin-clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, and cephalothin belonged to the medium group, while the low-incidence group comprised trimethoprim-sulfamethoxazole, ciprofloxacin, nalidixic acid, and azithromycin.

In general, an estimate of I^2 value greater than 25% is considered an indication of significant between-trial variability (Punch, 2013). Based on the 25% rule of thumb, most meta-analyses (19/21 meta-analyses, 90.5%) showed high between-trial heterogeneity (I^2 range: 42-94%) except for those for ciprofloxacin and azithromycin, which, however, covered fewer food types than other resistance prevalence.

4.4 Prevalence of antibiotic-resistant *Salmonella* by Food Type

Meta-analyses were performed for different food types, regardless of resistance profile, and the distribution of reported prevalence from individual primary trials, together with the pooled prevalence estimates with 95% CI can be read in Figure 2.2. Overall, 57.3% of food samples tested positive for *Salmonella* isolates with AR properties. Chicken and turkey samples are of the highest prevalence of antibiotic-resistant *Salmonella*, with pooled estimates of 61.7% (95% CI: 57.3-66.0%) and 72.8% (68.5-77.0%), followed by pork 59.2% (95% CI: 49.7-68.5%), mixed meat products 57.6% (95% CI: 45.7-69.1%), and beef 33.2% (95% CI: 26.0-40.7%). The food was marked as mixed meat sampled from multiple animal sources including pork, beef, chicken, and turkey, but results were reported without differentiating the sources. The pooled prevalence of mixed meat was corroborated, as its estimates fell between the range of the four animal-derived food types. Vegetables and imported food have been investigated in a smaller set of primary trials,

reported a lower incidence of antibiotic-resistant *Salmonella* compared to other animal-derived food, with a mean prevalence estimate of 5.8% (95% CI: 2.6-9.9%) and 3.3% (95% CI: 0.3-8.1%), respectively. Imported food has a unique AR *Salmonella* distribution mainly because it consists of two types of food – frozen seafood and spice. The heterogeneity remained high within most of the groups (I^2 range: 51-89%) except for beef (11%) and vegetables (0%).

4.5 Prevalence of antibiotic-resistant *Salmonella* by Resistance and Food Type

There was a considerable dispersion in the number of trials among the food-resistance type combinations. Hence, hierarchical subgrouping meta-analyses were conducted on the suitable combinations presenting sufficient trials. As a result, subgrouping meta-analyses were performed in four major retail commodities (beef, pork, chicken and turkey) for all nine antibiotic classes, results of which are summarized in Figure 2.3. In general, the ranking orders of the prevalence of resistance against antibiotic classes are relatively consistent across commodities. Tetracyclines, penicillins, and aminoglycosides resistance are the top three for all major commodities, among which turkey and chicken took the lead. Lower in the ranking lists are folate pathway inhibitors, beta-lactam, cephems and phenicols, among which resistance prevalence in chicken/turkey products and beef/pork products tended to be comparable. As an exception, pork and beef bear lower resistance prevalence against most antibiotic classes except for phenicol. At the bottom of the ranking list were quinolones and macrolides, which was the case for all four commodities.

4.6 Multi-resistance prevalence of *Salmonella* and Food type

Studies that include multi-resistance *Salmonella* data were extracted and conducted meta-analysis individually. The overall prevalence estimate was 46.9% (95% CI: 42.2% - 51.6%), lower than the overall antibiotic-resistant *Salmonella* prevalence estimate of 57.3%, which was reasonable. The subgrouping meta-analysis was conducted by food type. Chicken and turkey samples are two of the highest prevalence of multi-resistance *Salmonella*, with pooled estimates of 52.8% (95% CI: 48.4-57.1%) and 59.2% (95% CI: 53.9-64.5%), followed by pork 43.9% (95% CI: 35.7-52.3%) and beef 24.5% (95% CI: 18.3-31.2%). The other food types were excluded due to the lack of enough studies. Overall, the order was similar to the overall antibiotic-resistant *Salmonella* prevalence.

4.7 Risk of Bias in Individual Trials

Results from the risk of bias analysis by domain were presented in the Supplementary Appendix. Except for the 16 NARMS annual reports, most of the studies published as scientific articles (16/25) were not randomly collect the sample from retail food directly, one of the articles collected samples systematically and others (8/25) collected samples by convenience or directly from *Salmonella*. The prevalence of antibiotic resistance to *Salmonella* extracted from some articles (5/25) was a by-product of another study design. Besides, some articles' (9/25) detection methods were not followed by the recommended ways published by the Clinical and Laboratory Standards Institute (CLSI/NCCLS). Most of the datasets from articles (18/25) were not sufficiently powered to detect a 10 % prevalence with a 5% allowable error.

5. Discussion

It has been widely perceived that the use of antibiotic agents during the preharvest stage of food production was associated with the selection and distribution of antibiotic-resistant pathogens in food (Torrence, 2016). Overall, these were corroborated by the results of this review, though the causality cannot be concluded solely based on the data presented herein. Based on the 2019 summary report on antibiotics approved for use in food-producing animals (Administration, December 2020), of 10 medically important antibiotic classes, the reported domestic sales and distribution data showed tetracyclines ranked the highest (67%), followed by penicillins (12%). Assuming the sale estimates are viable for tracking actual use, antibiotic usage estimates correspond to the top 2 antibiotic classes of resistant *Salmonella* estimated in this review. Surprisingly, resistance to macrolides, an important antibiotic class used in veterinary settings which accounted for 8%, was considerably lower for all major commodities. The discrepancy could be caused by the scarcity of relevant data currently available. None of the scientific articles covered *Salmonella* resistance to macrolides in their study scope, and the measurement of azithromycin resistance was not covered in NARMS reports until 2010. To increase confidence in the interpretation of resistant foodborne pathogens, it is imperative to gather more balanced information across antibiotic classes to understand the up-to-date epidemiological situation. Information on antibiotic exposure status throughout the food production chain prior to the sample collection could have shed light on the different frequencies of resistance among food samples. Although this key information was purposely designed to be extracted, due to the lack of reporting in the vast majority of relevant studies, comparisons and conclusions need to be made with caution.

Animal-derived food is perceived to be at higher risk of being contaminated with antibiotic-resistant pathogens, as livestock animals were the most immediate recipients of antibiotic administration. In general, the results illustrated a consistency in the perception. Higher prevalence was estimated for turkey, chicken, beef and pork, while vegetables are among the lowest. However, it should be noted that only two studies were identified reporting relevant data for fruits and vegetables (Liu & Kilonzo-Nthenge, 2017). Although extracted from limited studies, data should be representative, as they were collected through USDA MDP which implemented one of the largest national monitoring programs to collect contamination data of foodborne pathogens in fresh fruit and vegetables in the United States. The observation of a low incidence of resistant *Salmonella* in fresh fruit and vegetables might be driven by the rare detection of *Salmonella* contamination in this food type. Based on the MDP monitoring data, only 123 of 82,582 samples (0.15%) of domestic fresh produce samples collected over an 11-year period (2002-2012) were positive for *Salmonella* (Reddy, Wang, Adams, & Feng, 2016), which is considerably lower than the prevalence of *Salmonella* observed among retail meat samples (Broadway et al., 2021). However, without a thorough assessment of dynamic changes in the resistant *Salmonella* population from retail through transport, storage, and preparation, to consumption, the relative significance of fresh produce compared to animal-derived food products on the public health risk of antibiotic-resistant salmonellosis is still uncertain. With the aid of a quantitative microbial risk assessment model, the implication of retail-level contamination data on health risk can be supported with stronger scientific evidence (Evers et al., 2017; Zhang, Schmidt, Arthur, Wheeler, & Wang, 2021).

Besides, a few articles focused on imported food; and data were extracted and synthesized mainly for spices, indicating a rare presence of antibiotic-resistant *Salmonella*. The finding was in accordance with the general consideration that spices are under the category of low-moisture food commonly characterized as low-risk commodities for microbial contamination due to their lack of favourable properties for microbial proliferation (Van Doren et al., 2013). In addition to spices, seafood was another type of import food that has been investigated for the contamination of antibiotic-resistant *Salmonella* (F. Wang et al., 2011). Unfortunately, the incidence estimate is not reliable, given the extremely small sample size involved (0/1, 0.0%, 95% CI: 0.0-79.3%). However, the role of seafood products attributing antibiotic-resistant *Salmonella* infection, particularly imported seafood, cannot be neglected. Based on national surveillance of *Salmonella* contamination in seafood implemented by the United States Food and Drug Administration (FDA) over a 9-year period, *Salmonella* was detected in 7.2% and 1.3% of import and domestic products, respectively (Heinitz, Ruble, Wagner, & Tatini, 2000). Among import shellfish consumed raw, the incidence of *Salmonella* can be high up to 3.4%, at a comparable level to meat and poultry products (Broadway et al., 2021). These findings indicate possible threats to food safety associated with importing seafood and urge more large-scale studies to continuously track the microbiological safety and presence of antibiotic-resistant pathogens in seafood.

The hierarchical subgroup analyses considering both food type and resistance type were able to be conducted for four major animal-derived food products, i.e., chicken, turkey, pork, and beef, because of their higher abundance of relevant, available data. In terms of the total mass of medically important antibiotics used for food-producing animals, the

most recent estimates by the FDA showed that 41%, 42%, 10%, and 3% are intended for use in cattle, swine, turkeys, and chickens, respectively (Administration, December 2020).

6. Limitations

The number of studies included in subgroup meta-analyses was relatively small, resulting in a loss of accuracy. Besides, many studies did not specify the laboratory method clearly, making the results difficult to evaluate. In some articles, the sampling year, the sampling place, and other information are also lacking. Inconsistencies in the time and place of sampling may increase heterogeneity. It is important that future studies specify this information so that these factors can be considered when analyzing the data. The raw data was not specified individually in some articles. Additionally, some articles didn't get *Salmonella* directly from retail food but from the FDA or another agency that did sampling. While this may be a great way to reduce bias in extracting and culturing *Salmonella*, inconsistencies will also influence the results.

Numerous studies included in the meta-analysis did not consider if their sample size was sufficient. More than half of the articles did not sample enough data to guarantee adequate power. Most articles paid less attention to the sampling process and usually just sampling by convenience. Random sampling was rarely mentioned and most of the articles did not clearly describe the sampling method which makes it hard to determine the risk of bias in an individual study and makes the data less reliable.

In this review, some areas were not evaluated, such as food status (organic, conventional) and could be explored in the future. A study can be conducted to analyze the impact of sampling place and date in the future. After considering food type and antibiotic class,

heterogeneity remained high in most subgroup analyses. The methods and sampling process of future studies should follow the specific recommendations in NARMS reports.

7. Conclusions

The study aimed to comprehensively collect and critically review quantitative and qualitative information about the contamination of antibiotic-resistant non-typhoidal *Salmonella* in various foods at retail in the United States. In general, results showed a higher prevalence of resistant *Salmonella* in chicken, pork, and turkey, compared with beef, and lowest in vegetables and imported foods (data mainly available for spices). As for resistance to various antibiotic classes, tetracycline resistance was observed to be the highest among major commodities (39.67%-48.78%). Albeit a moderate level of resistance to beta-lactam antibiotics, the threat to public health can be profound due to their critical roles in clinical use. Surprisingly, resistance to macrolides, an important antibiotic class used in veterinary settings, was considerably lower for all major commodities, which however was estimated based on less data currently available. The results of the present study will facilitate the application of quantitative microbial risk assessment methods in identifying and evaluating potential mitigation strategies for controlling human exposure to foodborne AR. The information to make a quantitative comparison between the subgroups and evaluation of quality should be recorded in future studies.

Table 2.1 Search terms used in the present review for search formation listed by search concept

Concept	**Search Terms**
Condition (ARB prevalence/concentration)	(non-typhoidal *Salmonella* OR non-typhoid *Salmonella* OR *Salmonella*) AND (antimicrobial-resistant OR antibiotic-resistant OR drug-resistant OR antimicrobial OR antibiotic OR drug OR resistant OR resistance OR tolerance OR susceptibilities OR foodborne)
Context (U.S.)	USA OR US OR U.S. OR United States OR United States of America
Population (any food at retail)	(retail OR store OR grocery OR sale OR wholesale OR foodservice suppliers OR shop OR market OR food supply) AND (food OR meat OR poultry OR cattle OR beef OR dairy OR milk OR sheep OR goat OR pork OR broiler OR turkey OR seafood OR produce OR fresh produce OR fruit OR vegetable OR grain OR beans OR legume OR oil OR sugar)

Table 2.2 Meta-analyses of antibiotic-resistant *Salmonella* prevalence in food by resistance type

Antimicrobial Class	Antimicrobial Agents	Number of trials analyzed	Pooled Prevalence	95% CI of Pooled Prevalence	Heterogeneity (I^2)
All classes		8115	57.3%	52.2%-62.3%	94%
All classes (Multi)		6950	46.9%	42.2%-51.6%	92%
Aminoglycosides	All agents	19580	15.7%	13.4%-18.2%	94%
	Gentamicin	7253	7.4%	5.0%-10.1%	91%
	Kanamycin	5386	8.5%	6.2%-11.0%	84%
	Streptomycin	6941	33.9%	29.7%-38.1%	90%
β-Lactam/β-Lactamase Inhibitor Combinations	Amoxicillin–Clavulanic Acid	6738	10.7%	8.0%-13.6%	90%
Penicillins	Ampicillin	7016	24.0%	20.1%-28.2%	92%
Cephems	All agents	19304	8.9%	7.3%-10.5%	91%
	Cefoxitin	6880	8.4%	6.1%-10.9%	89%
	Ceftiofur	4686	11.4%	8.0%-15.3%	91%
	Ceftriaxone	6946	7.4%	5.1%-10.1%	91%
	Cephalothin	792	13.3%	3.2%-27.2%	94%
Folate Pathway Inhibitor	All agents	13874	10.6%	8.0%-13.5%	95%
	Sulfisoxazole	6940	26.8%	23.5%-30.3%	86%
	Trimethoprim-Sulfamethoxazole	6934	0%	0%-0.2%	50%
Phenicols	Chloramphenicol	6940	2.2%	1.3%-3.4%	74%
Quinolones	All agents	13905	0%	0%-0%	42%
	Ciprofloxacin	6952	0%	0%-0%	0%
	Nalidixic acid	6952	0%	0%-0.3%	59%
Tetracyclines	Tetracycline	6996	44.2%	39.7%-48.8%	91%
Macrolides	Azithromycin	2712	0%	0%-0%	0%

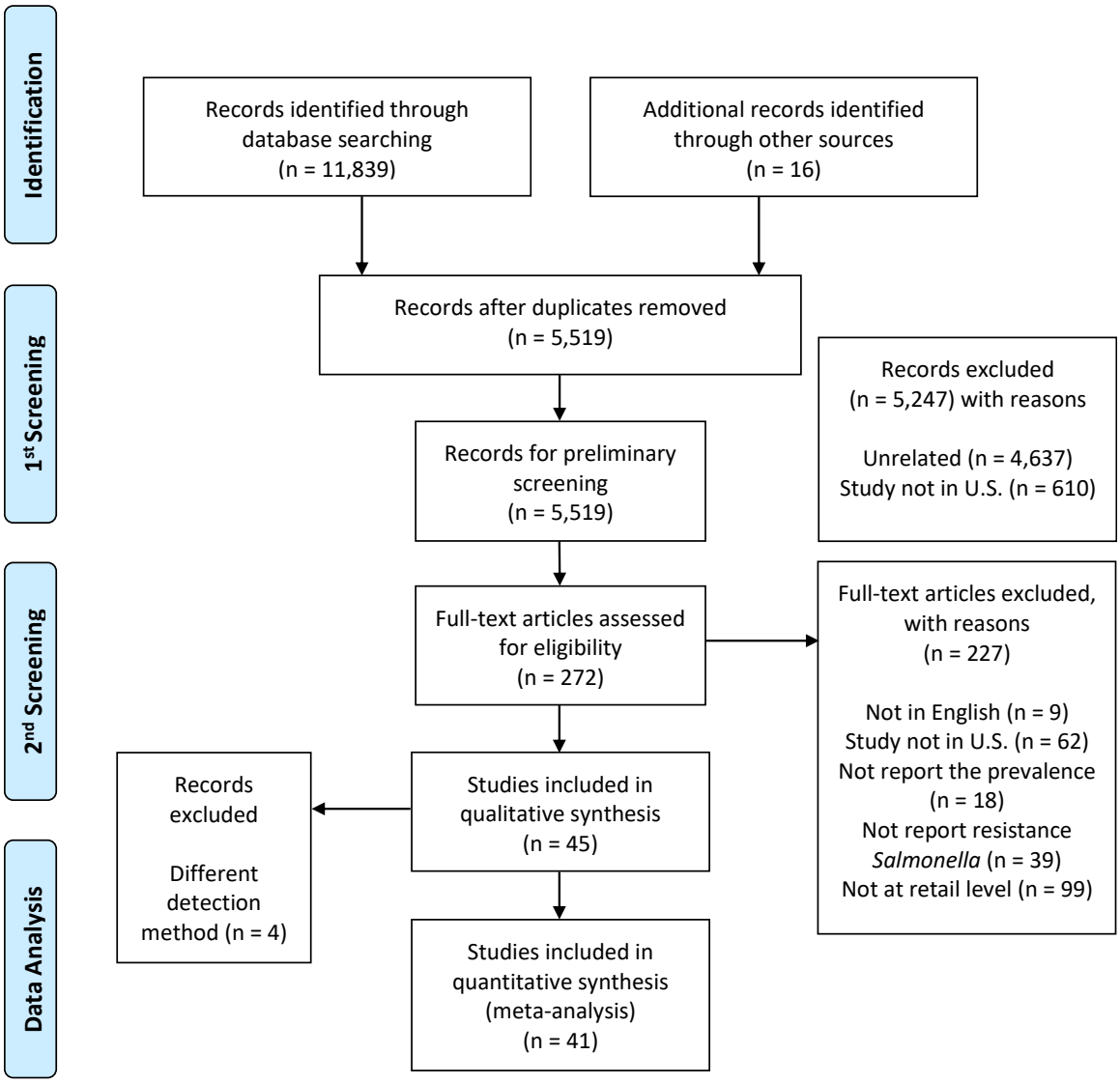

Figure 2.1. Systematic review flow chart detailing study selection process with reasons of exclusion.

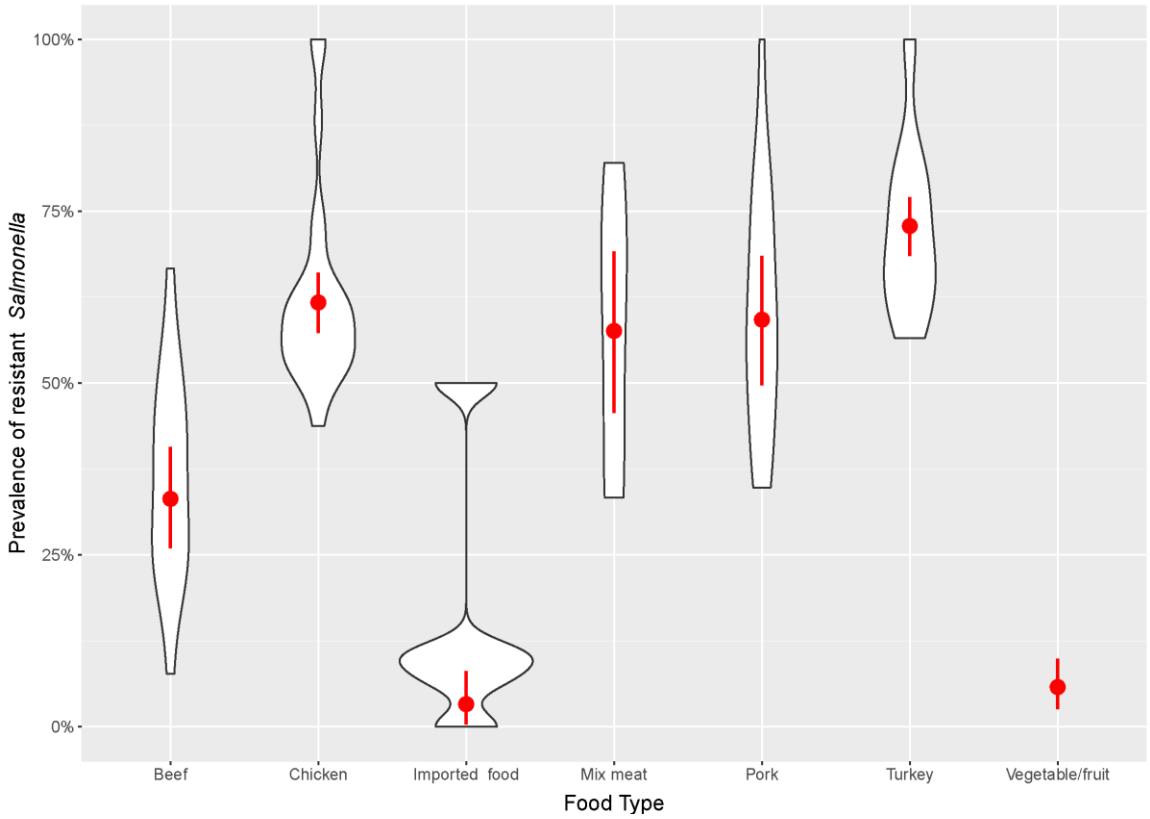

Figure 2.2. Violin chart for the distribution of resistance *Salmonella* prevalence in food

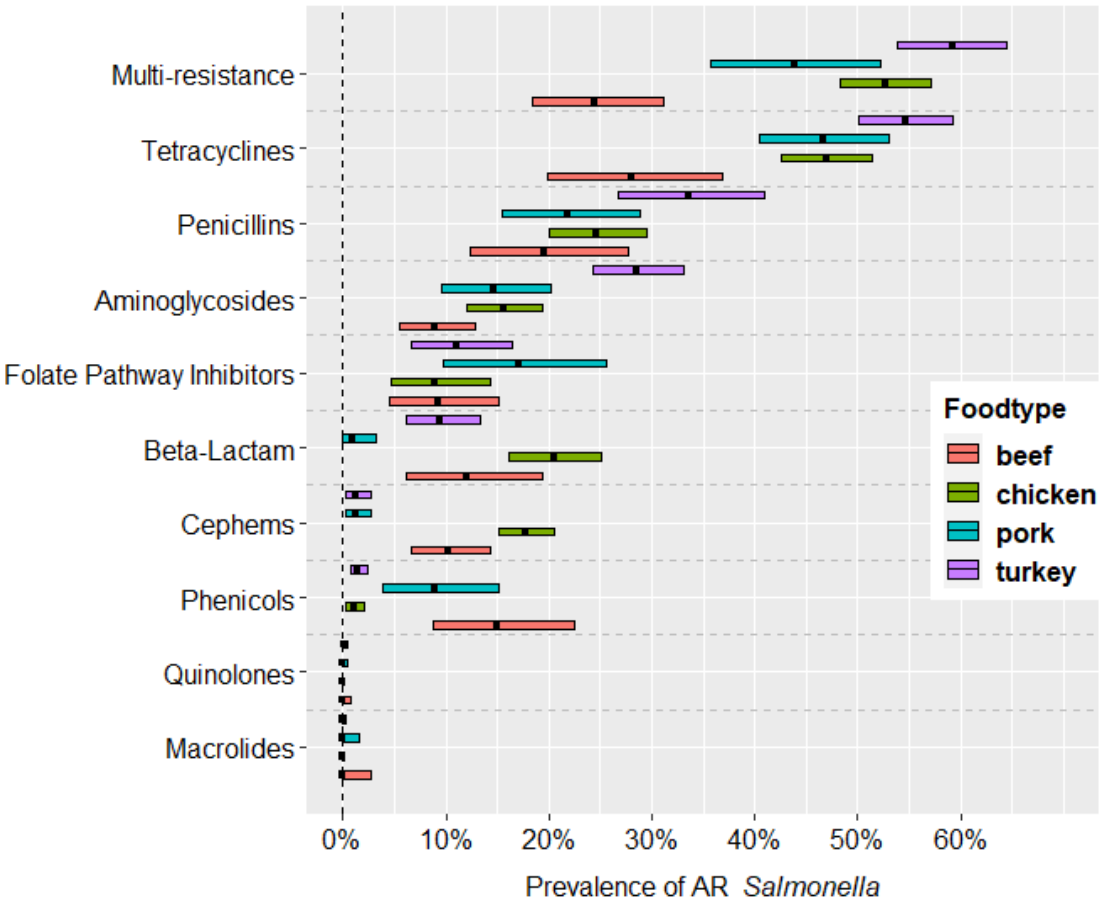

Figure 2.3. Prevalence of antibiotic-resistant *Salmonella* for antibiotics in food

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9. Supplementary Appendix

Risk of Bias Assessment Criteria

Criteria #	*Question*
1	The final sample should be representative of the target population. There is no point if the article did not mention relevant information. (0 point) high 1. Samples are randomly collected, and the randomization process is clearly reported. (4 points) low 2. Samples are randomly collected but the process is not reported. (3 points) low 3. Samples are systematic collected. (2 points) some concerns 4. Data are collected directly from *Salmonella* isolates or samples are collected by convenience. (1 point) some concerns
2	The objective of the study was designed to measure the prevalence of antibiotic resistance of *Salmonella* (2 points) (low) or the prevalence was just the by-product of another study design? (1 point) (some concerns) The study was complied with the certificated method (NCCLS) (2 points) (low), if not: 1 point (some concerns) The domain will be considered as high if both questions' answers are some concerns, the domain will be considered as low if both questions' answers are low. The domain will be considered as some concern if one question' answer was low and the other was some concerns
3	General description of the method and results should include: Was the study adequately powered to detect a 10% prevalence with a 5% allowable error in an adequate sample size? (4 points- sample size larger than 140, 2 points- sample size between 70 with 140)

JBI Critical Appraisal Checklist for Studies Reporting Prevalence Data was modified for this review. A cut-off sample size of 140 was used to determine if a study was adequately powered.

Characteristic information of the articles included in meta-analysis

Citation	Author	Year	Year(s), month(s) the study was conducted	Sampling place	Commodity of interest	Detection method	Sampling randomization	Outcome measure	Individual antibiotic test result
1	Aarestrup, F. M.	2007	NR	NR	Imported food	MIC	NR	Prevalence	Y
2	Berrang, M. E.	2006	NR	Northeast Georgia	Chicken	MIC	NR	Prevalence	Y
3	P.J.Carter	2002	2001 June to2001 November	Iowa	Turkey,pork,beef	MIC	NR	Prevalence	N
4	Sheng Chen	2004	1998-2000	Washington, D.C.	Chicken, turkey, pork, and beef	MIC	NR	Prevalence	Y
5	Cui, Shenghui	2005	September 2002 and August 2003	Maryland	Chicken	MIC	R	Prevalence	Y
6	Gad	2018	2009	Oklahoma	Turkey, chicken	MIC	NR	Prevalence	Y
7	Khaitsa, M. L.	2007	2006 January 10 to 2006 March 31	Midwestern United States	Turkey	MIC	NR	Prevalence	Y
8	Lestari	2009	2006-2007, October to September(every week)	Louisiana	Chicken	MIC	R	Prevalence	Y
9	Mazengia, E.	2014	2011 April-2012 April	Seattle, WA	Chicken, turkey	MIC	NR	Prevalence	Y
10	Peng, Mengfei	2016	2012-2014, June and August	Maryland and the Washington DC	Poultry, vegetable	MIC	NR	Prevalence	N
11	Pires, A. F. A.	2020	2018 February to July	Northern California	Meat and vegetable	MIC	NR	Prevalence	Y
12	R.Sudler	2000	NR	Maryland	Mixed meat	MIC	NR	Prevalence	N
13	Wang, Fei	2011	2009 July-2010 January	NR	Imported Seafood	MIC	R	Prevalence	Y
14	White, David G.	2001	1998, June and September	Washington, D.C.	Chicken, turkey, pork, and beef	MIC	NR	Prevalence	Y
15	SHANKER P. REDDY	2016	2002-2012	11 States	Vegetable	MIC	R	Prevalence	N
16	Zhao, Tong	2002	NR	New York, San Francisco, Philadelphia, Denver, Atlanta,Houston, and Chicago	Beef	MIC	R	Prevalence	Y
17	Bokanyi, R. P. Jr	1988	1988	Columbus, Ohio	Chicken	MIC	NR	Prevalence	N
18	Brundage, M. A.	1990	1990	Columbus, Ohio	Turkey	MIC	NR	Prevalence	N
19	deGraft-Hanson, J. A.	2005	NR	Morgantown, WV	Chicken	MIC	Sysmetric	Prevalence	N
20	M'Ikanatha, N. M.	2008	2006-2007	Pennsylvania	Chicken	MIC	NR	Prevalence	N
21	Liu, Siqin	2013	Summer and fall of 2014	Davidson County，Tennessee	Vegetable	MIC	NR	Prevalence	N
22	Van Doren, J. M.	2013	October 1,2006 and September 30 2009	NR	Imported dried spice	MIC	R	Prevalence	Y
23	Zhao, S.	2006	2001	NR	Imported foods(aquatic food and spices, herbs, and flavorings)	MIC	R	Prevalence	Y
24	Zhao, S. H.	2003	2000	NR	Imported foods(seafood,fresh produce)	MIC	R	Prevalence	Y
25	Fakhr, M. K.	2006	2003	Fargo, North Dakota	Turkey	MIC	NR	Prevalence	Y
26	NARMS	2002	2002	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
27	NARMS	2003	2003	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
28	NARMS	2004	2004	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
29	NARMS	2005	2005	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
30	NARMS	2006	2006	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
31	NARMS	2007	2007	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
32	NARMS	2008	2008	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
33	NARMS	2009	2009	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
34	NARMS	2010	2010	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y

35	NARMS	2011	2011	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
36	NARMS	2012	2012	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
37	NARMS	2013	2013	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
38	NARMS	2014	2014	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
39	NARMS	2015	2015	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
40	NARMS	2016	2016	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
41	NARMS	2017	2017	U.S.	Chicken, turkey, pork, and beef	MIC	R	Prevalence	Y
42	Bokanyi, R. P. Jr	1990	July, 1987 to January, 1988	Columbus, OH	Chicken	disk	R	Prevalence	Y
43	Kiessling	2002	1999-2000	NR	NR	disk	NR	Prevalence	Y
44	Kilonzo-Nthenge,	2013	Unknowm	Tennessee	beef,turkey, chicken	disk	NR	Prevalence	Y
45	Liu, Siqin	2017	2014	Davidson County，Tennessee	iceberg lettuce	disk	R	Prevalence	Y

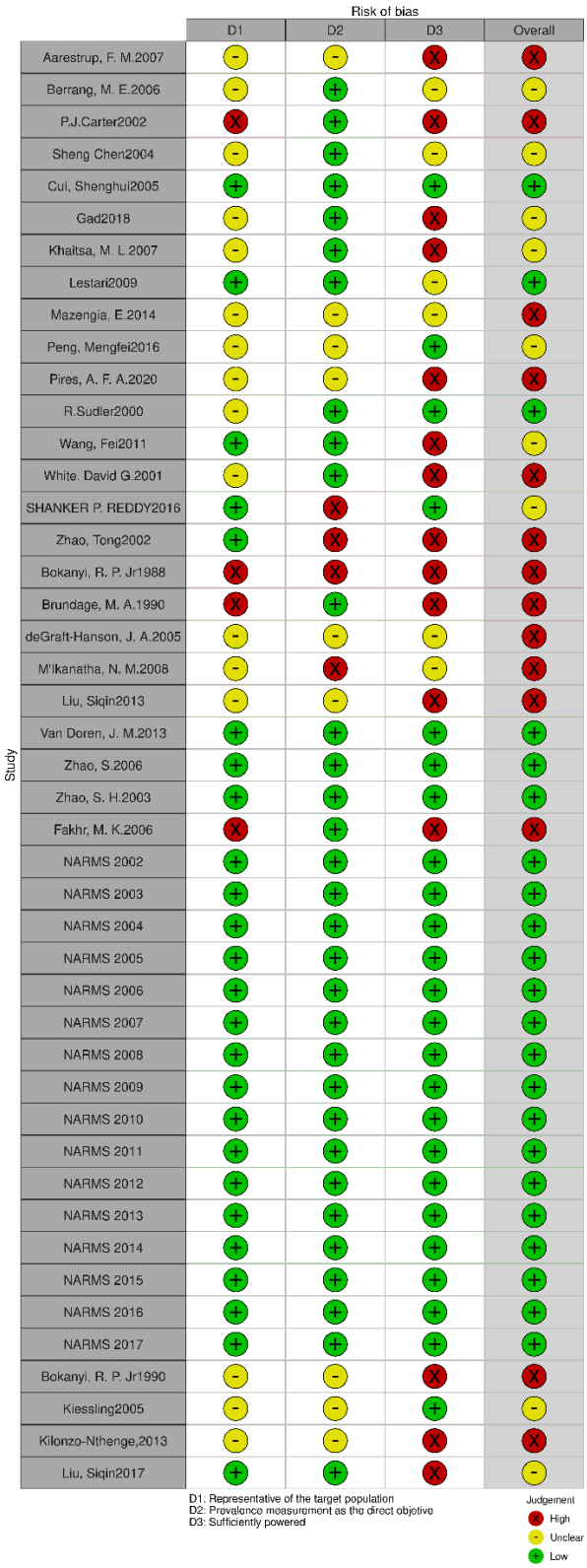

Table. 1 Overall risk of bias traffic light table for all studies

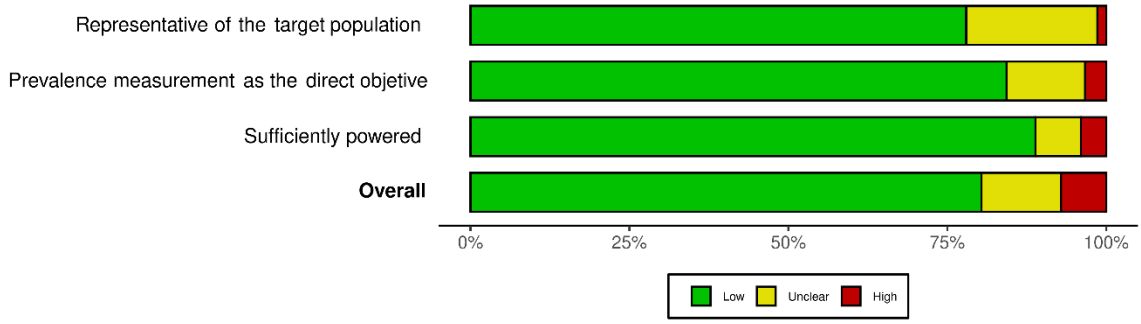

Table. 2 Overall summary result for risk of bias analysis

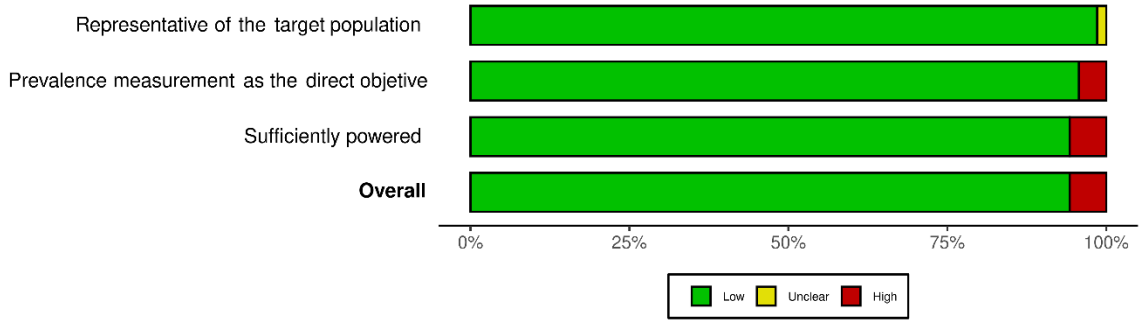

Table. 3 Summary of risk of bias results for beef

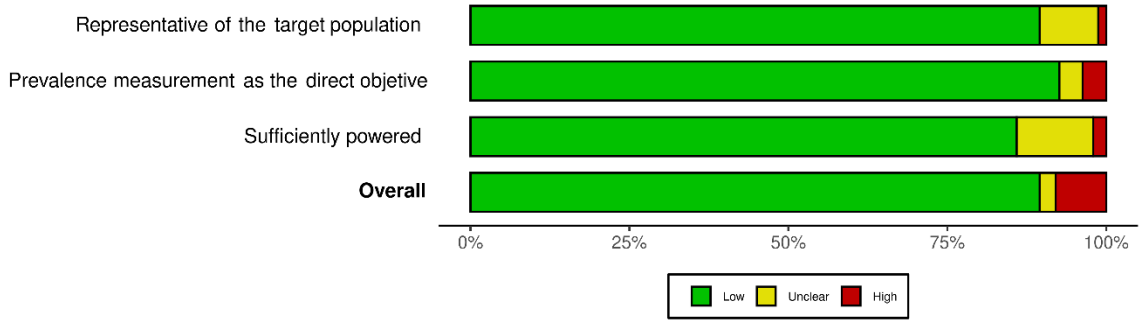

Table. 4 Summary of risk of bias results for chicken

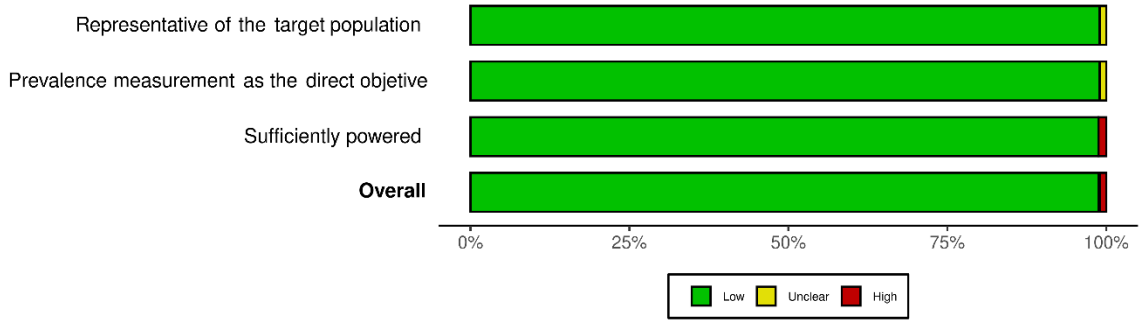

Table. 5 Summary of risk of bias results for imported food

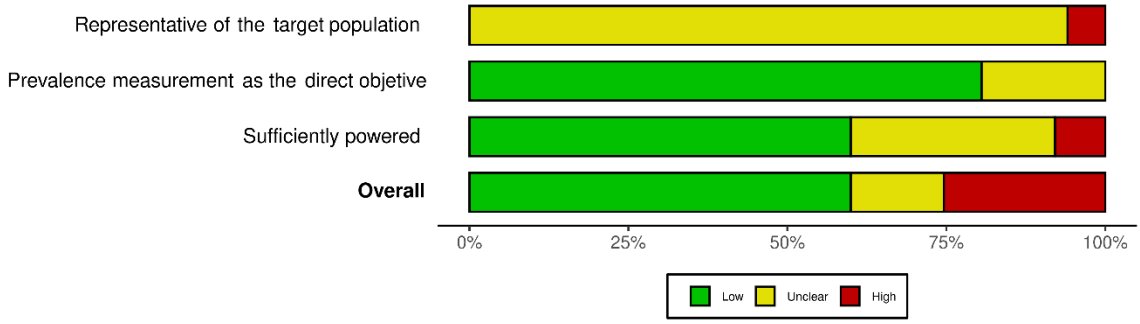

Table. 6 Summary of risk of bias results for mixed meat

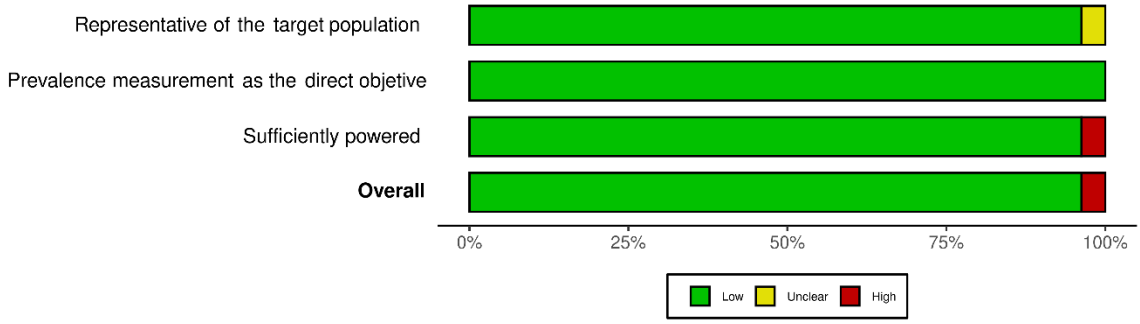

Table. 7 Summary of risk of bias results for pork

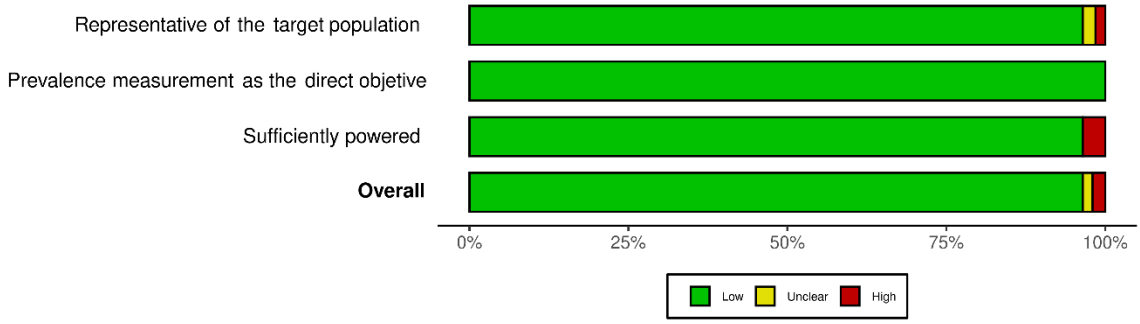

Table. 8 Summary of risk of bias results for turkey

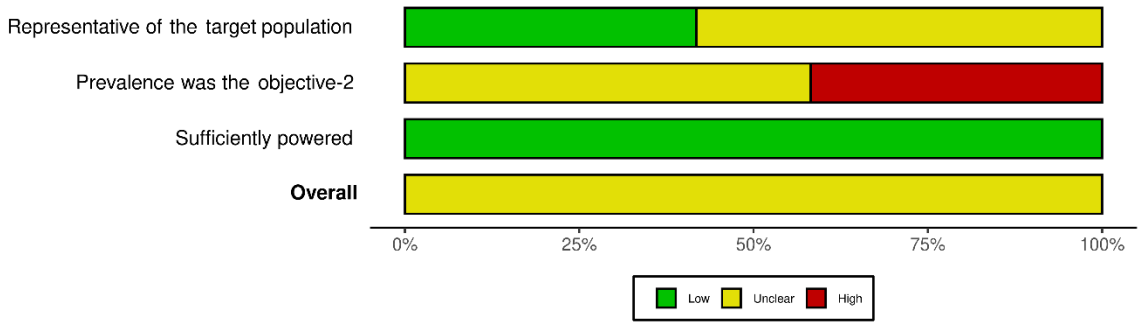

Table. 9 Summary of risk of bias results for vegetable and fruit

CHAPTER 3. COMPARATIVE EXPOSURE ASSESSMENT OF CEPHEM-RESISTANT *SALMONELLA* THROUGH THE CONSUMPTION OF VARIOUS ANIMAL-DERIVED PRODUCTS IN THE UNITED STATES.

1. Abstract

The rise of antibiotic resistance (AR) has become a serious threat to public health in the United States and worldwide. The detection of antibiotic-resistant bacteria in foods at retail has been frequently reported, indicating the potential of food consumption animal-derive food in particular as a significant source of AR exposure. *Salmonella* is one of the most common pathogenic foodborne bacteria in the United States. *Salmonella* resistance to cephem has become a major public health problem across the world since cephems are one of the key drugs for the treatment of salmonellosis. As a well-known systems approach to food safety protection, the quantitative microbial risk assessment (QMRA) can be used to identify influencing risk factors, evaluate and prioritize potential control strategies that can be implemented along the food supply chain for the risk mitigation purpose. The objective of this study is to develop a stochastic comparative exposure assessment model to estimate the relative contribution of various animal-derived food groups to overall foodborne exposure to antibiotic-resistant *Salmonella.* The model consists of four modules, i.e., retail, transport, storage and preparation. In general, results showed ground beef and chicken parts accounted for the highest percentage of the overall exposure to resistant *Salmonella*, compared with pork cuts and ground turkey. Sensitivity analysis illustrated that *Salmonella* contamination in products at retail and cooking temperature were the two main factors influencing the exposure amount for all food products evaluated in the present study. However, few studies or databases focused on *Salmonella* enumeration as one of their major outcome measurements, which is a significant data gap preventing a reliable comparison of the relative importance among foods. Our findings are expected to contribute to a better understanding of the source

attribution to foodborne antibiotic-resistant *Salmonella*, which will assist policymakers, government and industry food safety experts, and risk managers in establishing performance standards and possible interventions at certain stages of the food supply chain to constrain the spread of antibiotic-resistant *Salmonella*.

2. Introduction

Globally, *Salmonella* represents an important genus of public health importance and has been responsible for thousands of deaths due to foodborne illnesses annually (Lee, Runyon, Herrman, Phillips, & Hsieh, 2015; Scallan et al. 2011). Antibiotic resistance (AR) is being increasingly acknowledged as a worldwide and national issue (CDC, 2019a). Combining the pathogenic and resistant traits, antibiotic-resistant non-typhoidal *Salmonella*, a serious threat, causes more than 212,500 infections and 70 deaths each year in the U.S (CDC, 2019b). However, a lack of understanding of the relationship between exposure pathways and AR-related public health slowed down prevention efforts (Knight et al., 2018). Food products are now widely regarded as one of the most important contributors to the transfer of antibiotic-resistant bacteria (ARB) originating from agriculture practice to humans, such as due to the use of antibiotics during livestock husbandry (Acar & Moulin, 2013). The reports of the occurrence of antibiotic-resistant *Salmonella* in animal-derived foods are not uncommon, which has been demonstrated to associate with the occurrence of foodborne outbreaks (Moher, Liberati, Tetzlaff, Altman, & Grp, 2009).

Quantitative microbial risk assessment (QMRA), as a well-recognized tool for evaluating and prioritizing control strategies in risk management (FAO/WHO, 1995), can be used to identify influencing risk factors, evaluate and prioritize potential control strategies that

can be implemented along the food supply chain for the risk mitigation purpose (Cummins, 2008). A risk assessment does not necessarily require an exposure assessment. In some situations, exposure assessment may be a stand-alone process, for example, in cases where there is not enough information available to conduct a dose-response assessment (i.e., a Hazard Characterization) or when risk management only pertains to quantifying or reducing exposure (WHO). Comparative exposure assessment focuses on determining the relative contribution of different exposures (food products) to a population's daily exposure. These findings will contribute to a better understanding of the source contribution of foodborne antibiotic-resistant *Salmonella*. Policymakers, government and industry food safety experts and risk managers will be able to set performance standards and identify possible interventions at certain stages of the food supply chain to inhibit the spread of antibiotic-resistant *Salmonella* based on the results.

In humans and animals, cephems are used to treat major bacterial infections (Wong, Yan, Chan, Biao, & Chen, 2014). The incidence of cephem-resistant *Salmonella* in humans and food-producing animals has been increasing in recent years (Eguale et al., 2017; Qiao et al., 2017). Cephem resistance is usually caused by the production of extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC β-lactamase (pAmpC), both of which degrade extended-spectrum cephems. *Salmonella* strains that produce ESBLs and pAmpC pose a major threat to global public health as it results in treatment limitations in humans (Nguyen et al., 2016; Seiffert, Hilty, Perreten, & Endimiani, 2013).

To date, the relative importance of the transmission routes in the food chain is still unknown. Therefore, we aim to develop a stochastic comparative exposure assessment model to estimate the relative contribution of food groups, in particular animal-derived

foods, to overall foodborne exposure to antibiotic-resistant *Salmonella* in the United States.

3. Material and method

3.1 Model overview

Unified quantitative exposure assessment models were constructed and applied to describe the dynamic changes of cephem-resistant *Salmonella* in the retail-to-table continuum of four major the U.S. Department of Agriculture (USDA) regulated animal-derived food products, including chicken parts, ground turkey, pork cuts, and ground beef. The models covered the major stages largely relevant to consumers' behavior and practices, i.e., retail, storage at home, preparation, and consumption. For each food-specific model, the final output was the total annual exposure amount of resistant *Salmonella* per person through the consumption of food products of interest in the unit of $\log_{10}$ CFU/person-year. By integrating four food-specific models, relative attribution of different foods to the per-person annual exposure was estimated. When comparing and visualizing the dynamic changes in the resistant *Salmonella* throughout different stages, the contamination was quantified as $\log_{10}$ CFU to reflect a common situation in food handling and consumption. A conceptual model which the quantitative models were built upon was provided in Figure 3.1. A Monte Carlo simulation by Latin Hypercube Sampling with 100,000 iterations was conducted to capture the uncertainty and variability of the model outputs using @Risk, version 8.2. (Palisade, Newfield, NY, USA). The number of iterations was determined depending on the convergence criteria of a 95%

confidence level with a tolerance level of 3% in terms of the exposure output means for all products modeled.

3.2 Exposure assessments

3.2.1 Retail

Food-specific exposure models started with the retail module. The contamination of cephem-resistant *Salmonella* in foods at retail was the major model input. In the present model, the contamination of resistant *Salmonella* was estimated given the concentration of *Salmonella* regardless of resistance classification and the proportion of being cephem-resistant among the total *Salmonella* strains tested for AR. Regarding the *Salmonella* concentration, fitted distributions used in published risk assessment models were used for chicken parts and ground turkey products, and the summary statistics in USDA Food Safety and Inspection Service (FSIS) reports and other nation-scale surveillance studies were used to generate empirical non-parametric distributions for pork cuts and ground beef. For resistant *Salmonella* proportion, it was estimated through a systematic review and meta-analysis study (details in Chapter 2) for a representation in the U.S. Since the *Salmonella* concentration distributions used in the present model covered both detected (enumerated levels) and non-detected data (not enumerable, but with presence/absence determined), a separate prevalence variable was not considered.

Concentration distributions were fitted based on nationally representative data. Specifically, the parameters for ground turkey and chicken parts were extracted from two risk assessment models by Lambertini et al. For ground turkey, the concentration was modeled as a combination of discrete and cumulative distribution leveraging on two

USDA FSIS sampling programs, i.e., sampling for ground and other comminuted turkeys in 2015-2016, and not-ready-to-eat comminuted poultry exploratory sampling in 2013-2015 (Lambertini, Ruzante, Chew, Apodaca, & Kowalcyk, 2019; Lambertini, Ruzante, & Kowalcyk, 2021). For chicken parts, a cumulative distribution for *Salmonella* concentration was fitted to the FSIS 2012 baseline survey of *Salmonella* in chicken parts (Lambertini et al., 2019; Lambertini et al., 2021)). The concentration parameters for ground beef were estimated through the study conducted by the U.S. Department of Agriculture Agricultural Research Service Meat Animal Research Center, in which beef samples were collected in a 13-month period from three food service supply establishments covering multiple cattle harvesting facilities in the Pacific, west south-central and south Atlantic regions according to the U.S. Census Bureau Divisions (Zhang, Schmidt, Arthur, Wheeler, & Wang, 2021). For pork cuts, FSIS market hog baseline microbiological data was used to fit a cumulative distribution. A transformation factor was applied to translate the *Salmonella* level quantified in MPN/cm^2 to CFU/g. The factor was calculated as the ratio of dressed hog weight (N. P. P. Council) to the total carcass surface area (Hurnik & Lewis, 1991).

3.2.2 Transport from retail to consumers' home

Growth kinetics of *Salmonella*. The growth of *Salmonella*, including antibiotic-resistant *Salmonella*, is possible when the products fall into the growth temperature zone. Due to the lack of microbial kinetics investigation focusing on resistant strains, a growth model developed for generic *Salmonella* was used, assuming a similar kinetic curve between the resistant and susceptible subpopulations. To predict microbial growth over time, the growth rate (*GR*) and maximum population density (*MPD*) as a function of exposure

temperature are two key factors to consider. The lag phase (*L*) was not considered in the present model, as it was assumed that the time between the initial contamination at the harvesting and processing facilities was sufficiently large so the bacterial population already became acclimated to the product conditions at the post-harvest stages (Bollaerts et al., 2009).

The modeling of *GR* and *MPD* were conducted separately for foods of different animal origins if allowable, i.e. pork, beef and poultry (combing chicken and turkey), following the approach used by Gurman et al. (2018). Unfortunately, the differentiation between different food processing types, such as cuts versus ground meat, was not allowed due to the data limitation. Data used for *GR* and *MPD* modeling were primarily from Combase and Combase premium (Baranyi & Tamplin, 2004), the largest data resources for quantitative and predictive food microbiology, and from a study by Ingham et al. (2007) that comprehensively investigated the *Salmonella* growth for foods of all animal origins interested in the present study. When searching in Combase databases, relevant studies were retrieved by applying the following filtering criteria to exclude the conditions that could inhibit the growth of *Salmonella*, including "temperature < 60," and "no information" for the conditions "CO_2", "N_2", "O_2", "acetic_acid", "citric acid", "benzoic acid", "lactic acid", "modified atmosphere", "lauricidin", "HCl" and "dried". In summary, 1,447 *Salmonella* growth experiments, including 99 for pork, 253 for beef, and 1,095 for poultry, were identified and used for the modeling of *GR* and *MPD*.

Using the retrieved data, the relationship between *GR* and temperature was modeled based on the modified Ratkowsky equation (Ratkowsky, Lowry, McMeekin, Stokes, & Chandler, 1983; Shi, Reddy, Chen, & Ge, 2016; Zwietering, De Koos, Hasenack, De Witt,

& Van't Riet, 1991) as below in Equation (1):

$$GR = \left(cc * (T - T_1) * \left(1 - exp\left(k * (T - T_2)\right)\right)\right)^2 \qquad \text{Eq. (1)}$$

where *GR* is the development rate ($\log_{10}$ CFU/g/h), *T* is the temperature (°C), T_1 and T_2 are the theoretical minimum and maximum temperatures beyond which *Salmonella* growth is generally considered not possible, *cc* and *k* are regression constants. The parameters in the Ratkowsky equation were estimated separately for pork, beef and poultry products. The parameter estimation was conducted in statistical software R version 4.0.3 (Team, 2021) using 'devRate' package (Rebaudo, Struelens, & Dangles, 2018).

The relationship between *MPD* and temperature was modeled using the equation created by Zwietering, Cuppers, De Wit, and Van't Riet (1994) with modifications by Oscar (2005), as shown below in Equation (2):

$$MPD = \frac{a(T-T_{min2})(T-T_{max2})}{(T-T_{min})(T-T_{max})} \qquad \text{Eq. (2)}$$

where the *MPD* represents the maximum population density ($\log_{10}$ CFU /g), T_{min2} and T_{max2} are the theoretical temperature (˚C) that the *MPD* is speculated to be 0 $\log_{10}$ CFU /g, T_{max} is a temperature higher than T_{max2} and T_{min} is a temperature smaller than T_{min2}.

Due to insufficient data, all pork, beef, and poultry data were combined to model *MPD*. Therefore, the equation relating *MPD* and temperature shared the same parameters across all products. Estimated growth model parameters for *MPD*s and *GR*s are listed in Table 3.1.

In general, the contamination level of resistant *Salmonella* after a certain stage where

Salmonella proliferation is possible can be calculated using Equation (3) based on the estimation of *GR* and *MPD* and the temperature and time during the stage.

$$N_i = Min[10^{log_{10}(N_{i-1})+GR(T=T_i)*t_i}, MPD(T=T_i)] \qquad \text{Eq. (3)}$$

where N_{i-1} is the initial concentration, specifically referring to the concentration at retail before transport here, and N_i is the concentration after a certain stage or after transport here. T_i and t_i are the temperature and time during a certain stage, which is detailed in the section below for the transport stage. If the resulting bacterial amount was larger than *MPD*, *MPD* became the concentration estimate instead.

Transport conditions. Transport time and temperature were extracted from the 2007 United States cold temperature database (EcoSure, 2008). The sampling project collected data from primary shoppers of over 900 households geographically dispersed across the country and include different types of fresh meat products. The transport temperature was measured upon arrival home before placing products into the refrigerator. The data were fitted using a normal distribution with a mean of 8.39 ˚C and a standard deviation of 2.48˚C truncated at -5.56 and 24.4 ˚C, the recorded minimum and maximum temperatures. The transport time data were fitted using a normal distribution with 1.17 hours as mean value and 0.43 hours as standard deviation, truncated at 0.3 and 3.7 hours.

3.2.3 Home storage

The *Salmonella* growth model (Eq. 1-3) was also used in this stage, where the T_i represents the storage temperature and t_i represents the storage time, which was also extracted from the EcoSure database. In the database, storage temperatures were provided as the frequency of measurements by every increment of 1.67 ˚C with a minimum of -5

˚C and a maximum of 20˚C. Hence, the variability around temperature at this stage was described using an empirical cumulative distribution. In terms of the storage duration in homes, no data were reported in the EcoSure database. As a substitute, the storage time from a risk assessment of *Salmonella* in broiler chicken was used, the variability of which was described as a Pert distribution, with the minimum as 0 days representing an immediate use, the most likely of 2 days, and the maximum of 5 days. (Organization, 2002). It was well supported by the observations in a survey of consumer food-handling practices from grocer to home (Godwin & Coppings, 2005).

3.2.4 Cooking

To simulate the impact of cooking on microbial changes, a thermal inactivation model which requires D-value, the decimal reduction time, as well as the cooking time and temperature, is needed. Previous studies demonstrated limited differences in the heat resistance of multidrug-resist versus non-multidrug-resist *Salmonella* (Stopforth, Suhalim, Kottapalli, Hill, & Samadpour, 2008), which supports the feasibility of using the thermal inactivation model for generic *Salmonella* as a substitute for resistant strains. Numerous studies have investigated the inactivation kinetics of *Salmonella* under varying cooking conditions. To minimize the bias introduced by inter-study heterogeneity, a study that included all four types of meat was used here (Horn, Olsen, Hasell, & Cook, 2015). Linear regressions between D-value and cooking temperature applied at 95^{th} percentile observations reported in that study were used in our model for a more conservative estimation of the log reduction as a result of thermal inactivation.

The cooking temperature and time for ground beef were extracted from Junna et al. risk assessment article where the ground turkey was using the same data (Sampedro, Wells,

Bender, & Hedberg, 2019; Zhang, Schmidt, Arthur, Wheeler, & Wang, 2021). The cooking temperature and time for pork cuts and chicken parts, it was extracted from another risk assessment article focusing on the whole chicken(Oscar, 2004). A maximum of 7-log reduction was applied to avoid unrealistically simulated values.

3.2.5 Cross-contamination.

Modeling of the cross-contamination primarily considers the situations of inappropriate food handling. Two mishandlings that frequently occur during the food preparation and service were modeled: (i) raw meat contaminates hands, which then contaminates cooked meals, and (ii) raw meat contaminates cutting boards (or kitchen tools) which then contact cooked meals. The raw meat and poultry were modeled as the primary contamination sources, and cross-contamination from and to other foods served in the same meals was not considered. Variables characterizing the contamination transfer phenomenon were adopted from a published risk assessment, in which parameters were quantified using systematic review and meta-analysis approaches (Smadi & Sargeant, 2013). Due to the limited data availability, studies investigating the cross-contamination process for all types of meat and poultry products were collectively used in the meta-analysis. Hence the same transfer coefficients were used for pork, beef, and poultry in the present model (Table 3.3). The output of this module estimated the concentration of cephem-resistant *Salmonella* at the time of consumption ($\log_{10}$ CFU/g).

3.2.6 Consumption

The exposure level of cephem-resistant *Salmonella* was estimated as per-serving and annual per-capita exposure for ground beef, pork cuts, chicken parts, and ground turkey,

respectively. Per-serving exposure (log_{10} CFU/serving) referred to the ingested dose of resistant *Salmonella* through one portion of meal, which was computed based on the concentration at the time of consumption (log_{10} CFU/g) and the serving size (g). Annual per-capita exposure (log_{10} CFU/person-year) estimated the total intake for a random person throughout a year, which was computed as the sum of per-serving exposure across all servings in a year (Table. 3.2). The exposure attribution to a specific food was calculated as the percentage of the mean food-specific annual per-capita exposure out of the total across all foods.

Serving size of 85 g was chosen to estimate the per-serving exposure level based on the recommended healthy portion size of protein foods for the general adult (USDA). Based on USDA reports, the average annual per capita consumptions were 12.2 kg for beef, 8.5 kg for pork, 37.0 kg for chicken, and 2.28 kg for turkey (N. C. Council, 2020). For the specific processing types modeled in the present study, ground beef accounts for 46% of the total annual beef consumption, and pork cuts, chicken parts and ground turkey account for 36.1%, 97.2%, and 32.9% of their respective totals. As a result, approximately a total of 144, 100, 435, and 27 servings of ground beef, pork cuts, chicken parts, and ground turkey are consumed on average annually in the U.S.

3.3 Other analysis

Sensitivity analysis was applied to obtain quantitative information about the most important parameters affecting the final exposure amount. This will help risk managers focus on the most critical input variables for the design of intervention strategies. Two measurements were used for this purpose. In both measurements, per-serving exposure was the output focused. To preliminarily identify higher influencing variables, the

Spearman's rank correlation coefficient was calculated using @Risk. All the coefficients are between 1 to -1, with the value 1 indicating a complete positive correlation and -1 for a complete negative correlation, and 0 meaning no correlation between input parameter and output result. Correlation coefficients greater than 0.15 were usually considered the main influence factors and were selected for advanced analysis.

In advanced sensitivities analysis, the change in output means was used to quantify the ability of an input variable to change the exposure estimation. Briefly, to evaluate a specific input, 100,000 simulated data for the input from the baseline were grouped into 20 bins with 5000 data in each, ranging from the input's lowest to highest values. The output mean was calculated for each bin of the target input. The difference between the maximum and minimum values of the 20-output means indicated the input's impact on the output mean. These steps were repeated for the stochastic input variables selected in the preliminary analysis.

4. Results and discussion

4.1 Exposure estimation and relative attribution

As shown in Figure 3.2, at the serving level, the exposure to cephem-resistant *Salmonella* in $\log_{10}$ CFU/serving was highest for ground beef at -4.37 (95% CI: -7.59 ~ 1.54), followed by chicken parts at -4.81 (95% CI: -8.24 ~ -0.27), ground turkey of -5.02 (95% CI: -8.02 ~ 1.34), and pork cuts of -6.37 (95% CI: -8.90 ~ -1.39) in the U.S. Considering the consumption frequency in a year, the annual per capita exposure in $\log_{10}$ CFU/person-year showed a similar ranking, but chicken parts ranked the highest as of -2.17 (95% CI: -5.61 ~ 2.37), followed by -2.21 (95% CI: -5.43 ~ 3.70) for ground beef, -3.60 (95%

CI: -6.59 ~ 2.77) for ground turkey, and -4.37 (95% CI: -6.90 ~ 0.61) for pork cuts. As a result, the consumer exposure to cephem-resistant *Salmonella* was the highest through chicken parts, which accounted for 51.0% of the total exposure, and the second through ground beef (46.8%). In comparison, pork cuts and ground turkey were less significant, attributing 0.321% and 1.91% of the total exposure, respectively.

There was no available empirical data regarding cephem-resistant *Salmonella* in food products of interest at the time of consumption to be compared against for a thorough model prediction validation. However, the estimated ranks are consistence with epidemiological observations. Using the USDA microbiological surveillance data, it was estimated that *Salmonella* concentrations considering both non-detects (samples without detectable contamination) and detects (samples above detection limits and/or quantifiable) are relatively high in ground beef (-1.3 $\log_{10}$ CFU/g ± 0.02 for 90% CI) and chicken parts (-2.2 $\log_{10}$ CFU/g ± 0.01 for 90% CI), in comparison to ground turkey and pork cuts. Regarding cephem-resistant *Salmonella*, our systematic review and meta-analysis showed chicken and beef products harbor the highest and second-highest percentage of *Salmonella* isolates conferring cephem resistance at 19.8% and 13.5%, respectively. In addition, chicken is the most consumed animal-derived protein source in the U.S., followed by beef. Hence, the higher attributions of chicken parts and ground beef to cephem-resistant *Salmonella* exposure were well supported. On the contrary, though ground turkey at retail was estimated with a *Salmonella* concentration (-2.1 $\log_{10}$ CFU/g ± 0.01 for 90% CI) similar to Chicken parts, the percentage of *Salmonella* isolates from ground turkey being cephem resistant was low (7.74%). Together with the lowest annual consumption amount of ground turkey among the four products, it resulted in a low

exposure through this route. Due to similar reasons, a relative rank of pork cuts was estimated within expectations.

4.2 Changes in contamination along the chain

Fig. 3.3 shows the dynamic changes that occur over the phases of the food consumption chain. To be comparable, the unit of measurements at different phases was $\log_{10}$ CFU /serving. Note that estimated concentration included both non-detects and detects, and prevalence was therefore not necessary to consider. It was estimated that the mean concentration of resistant *Salmonella* slightly increased during transport and storage without a noticeable difference in comparison to the initial contamination level at retail, which is due to the consideration of the low possibility of elevated temperatures falling into the range suitable for *Salmonella* growth. Cooking caused the largest drop in contamination before consumption. Almost all the bacteria were thermally inactivated by heating. Thus, basically the majority of resistant *Salmonella* left in the cooked meal was modeled as a result of the cross-contamination due to inadequate non-compliance with good hygienic practices during food handling and practices.

Given the conditions considered in the present model, it was estimated that the transport and storage stages did not change the relative comparison in per-serving contamination of cephem-resistant *Salmonella* among the four food products, which is consistent with the initial concentration at retail. However, interpretations should be made with caution because of a recognized pitfall in the growth modelling. It was challenging to identify data to quantify the growth kinetics of *Salmonella* in different foods. In the present model, the *GR* and *MPD* that were used for the description of *Salmonella* growth under specific temperatures over time were estimated using data retrieved from the worldwide largest

food microbiology database designed for predictive modeling purposes. Whenever possible, different *GR* and *MPD* values were estimated separately for foods with different animal origins. However, due to the lack of disclosing processing types, such as ground meat versus intact meat cuts or carcass parts, it was not possible to differentiate kinetic parameters based on such processing types, which may nevertheless influence differently on microbial changes. In general, intact animal muscle tissues are considered sterile. Therefore microbial contamination is assumed primarily on the surface of meat cuts or carcass parts. On the contrary, for comminuted products or mechanically tenderized products, the disruption of muscle structure and/or commingling effect cause internal contamination. In addition, higher fat contents can be expected in ground meat than meat cuts. These disparities may result in varying consequences on microbial adaptation and proliferation in different food types. Future studies are highly recommended to investigate the fate of *Salmonella* specifically in meat and poultry products processed differently, and this information is imperative for improving quantitative microbial exposure and risk assessment models.

At the time of consumption, the relative ranks are slightly shuffled, which may be explained by the different considerations in the cross-contamination phenomenon between ground meat and intact parts. The tremendous decrease in per-serving contamination at the moment of consumption reflects the combined effect of both thermal inactivation and the occurrence of cross-contamination. Due to the large log reductions ranging from 4.63 to 5.23 log estimated due to cooking, the resulting contamination at the time of consumption can be mainly from cross-contamination. As aforementioned, contamination on chicken parts and pork cuts are assumed to be located on the exterior

surface, while ground beef and ground turkey carry contamination distributed throughout the whole unit. To represent this, only 10% of total *Salmonella* cells located in the surface layer of a portion of raw ground meat were assumed transferable, while all *Salmonella* on a portion of pork cuts and chicken parts can be transferred. Mathematically, this consideration created a 1-log difference in decreases between ground meat and intact portions. As a result, per-serve contamination became lower for ground turkey than chicken parts at consumption, and the disparity between ground beef and chicken parts shrank (Figure 3.3).

4.3 Identification of significant input variables

Figure. 3.4 integrated the Spearman's rank correlation coefficients of the stochastic input variables at different stages within the food chain. Cooking temperature and *Salmonella* amounts in the products at retail were the two most influencing factors for all four types of food. The highest correlation coefficients were estimated for cooking temperature, ranging from -0.66 ~ -0.84, followed by the retail *Salmonella* contamination ranging from 0.36 ~ 0.51. The next group of variables with a coefficient value close to 0.15 are cooking time and variables related to cross-contamination (particularly the probability of unwashed board used for both raw meat and cooked meal). Among the remaining variables, no strong association with the output was observed. These results were also illustrated and supported by the tornado chart in Figure 3.5. When the cooking temperature was arbitrarily changed within its range, it resulted in a 6- to 7- log difference in the per-serving exposure estimates. Since the cooking temperature is one of the main influencing factors, it's critical to control the cooking time and temperature, making sure the inner temperature reaches the recommended temperature. The mainly

resistant *Salmonella* exposure comes from cross-contamination. Thus, all the cooking tools used during preparation must be cleaned next time. Changing *Salmonella* concentration at retail resulted in a 3- to 4- log difference in the per-serving exposure estimate. As another main influencing factor, it illustrated the importance of controlling bacteria contamination during pre-harvest and transportation. As for pork cuts and chicken parts, changing the cooking time and using a board for raw meat resulted in around 1-log difference in the per-serving exposure.

For the *Salmonella* growth during both transport and storage stages, the temperature seems playing a more critical role in influencing per-serving exposure, compared to the duration experienced in a particular stage, as the temperature is a determining factor of *MPD* in a positive correlation. The influence caused by temperature change was amplified by the corresponding change in *MPD*. For example, when the storage temperature changes from 3 ˚C to 4 ˚C, the *MPD* will increase by almost two $\log_{10}$ CFU/g. However, the effect of stage temperature can be limited by stage time. For example, because of the shorter duration of transport, resistant *Salmonella* usually cannot reach the *MPD* at the end of this stage. On the contrary, the relatively long time of storage allows for a great opportunity for microbial proliferation to reach the *MPD* during storage. Hence, the correlation coefficient is higher for storage than transport temperature. However, a longer storage duration did not result in dramatically high contamination at this stage, mainly because a large proportion of storage temperatures was lower than the minimum growth temperature. In order to limit the *MPD* value to a lower number, it's critical to keep the storage temperature lower than 4 ˚C.

4.4 General discussion of the model development

Current *Salmonella* concentrations for different food were extracted from different kinds of literature. However, the best way to estimate the *Salmonella* concentration for different food should be by accessing the raw baseline microbiological data from the Food Safety and Inspection Service (FSIS) for all different meat types. Then selecting the best distribution curve to fit the raw data such as lognormal distribution. However, there will be an extremely long waiting period to receive the raw concentration data through the FSIS database.

The lag phase would reduce *Salmonella* growth in food. Without considering the lag phase may make our exposure estimates higher than it actually is, which provides a more conservative assessment for the safety consideration. However, to improve the prediction accuracy, it can be added to the model in the future.

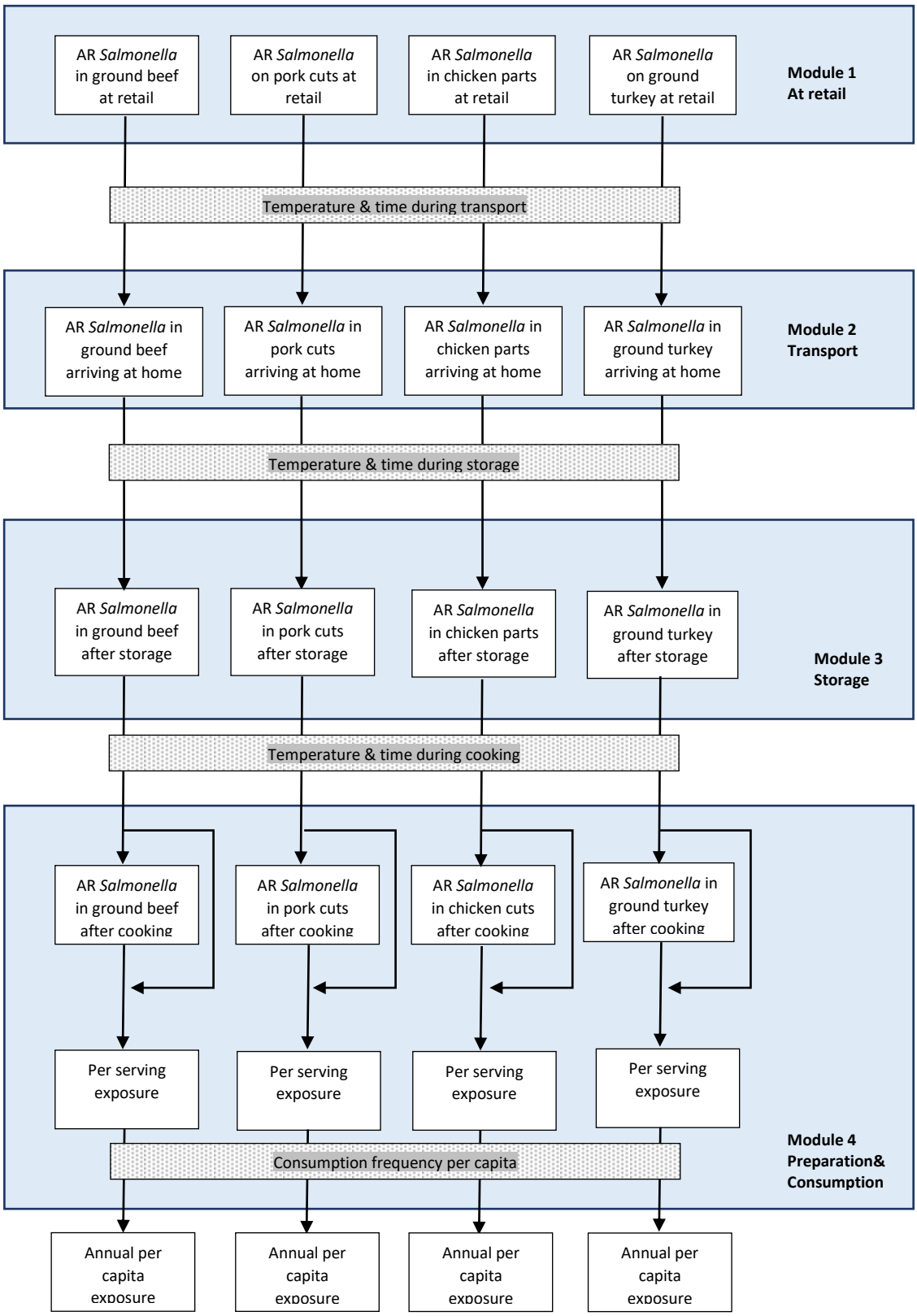

Figure 3.1. Conceptual model for the comparative exposure assessment

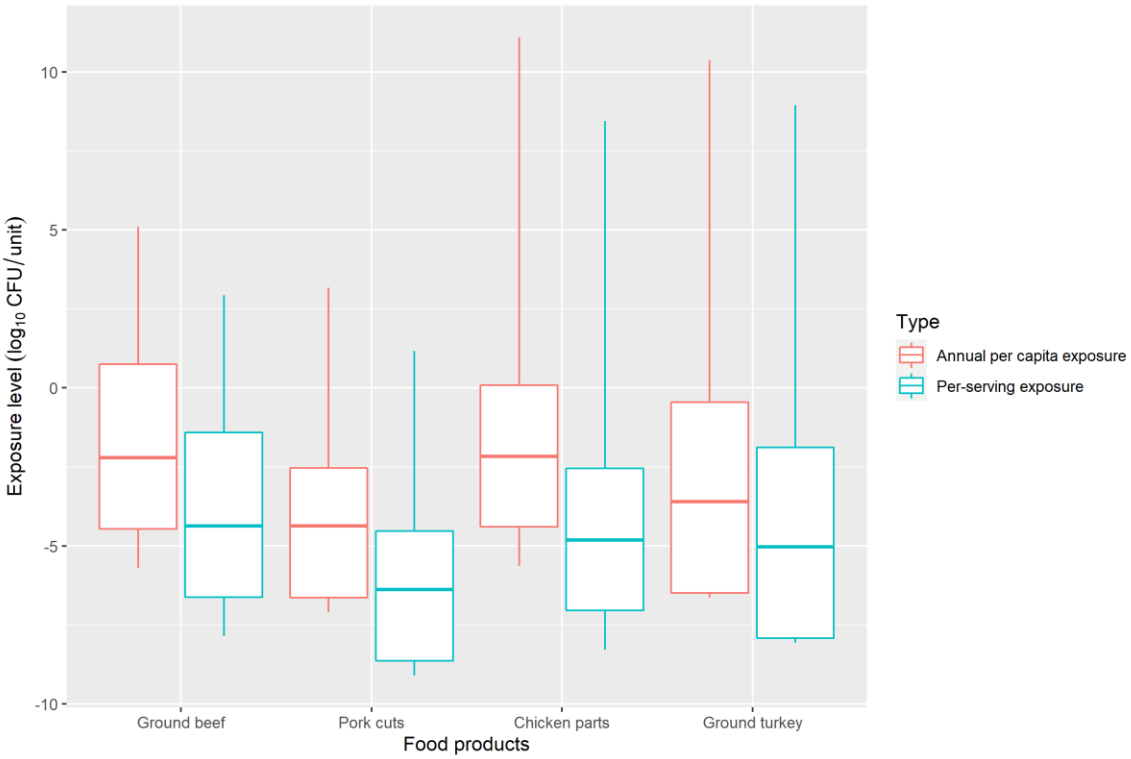

Figure 3.2. Box plot presenting the distributions of simulated per-serving exposure and annual per capita exposure to cephem-resistant *Salmonella* through the consumption of four different food products based on 100,000 iterations (per serving exposure in $\log_{10}$ CFU/serving; annual per capita exposure in $\log_{10}$ CFU/person-year)

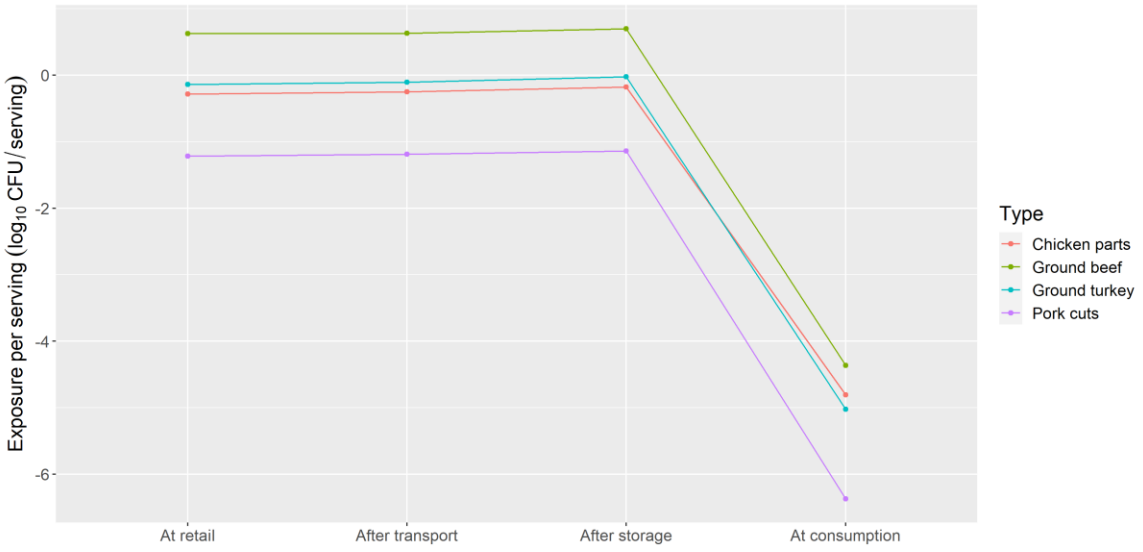

Figure 3.3. Cephem-resistant *Salmonella* dynamic changes over the food chain

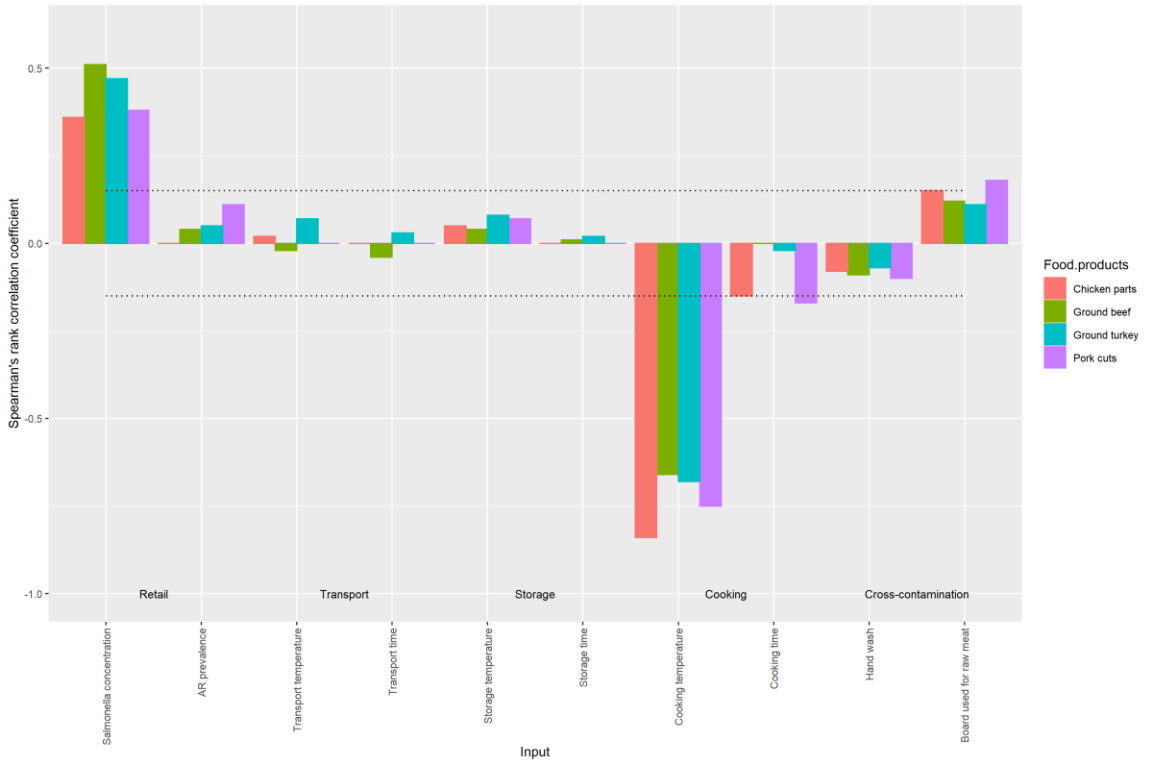

Figure 3.4. Spearman's rank correlation coefficients for various stochastic input variables modeled at different stages. The dash lines represent values of 0.15 and -0.15

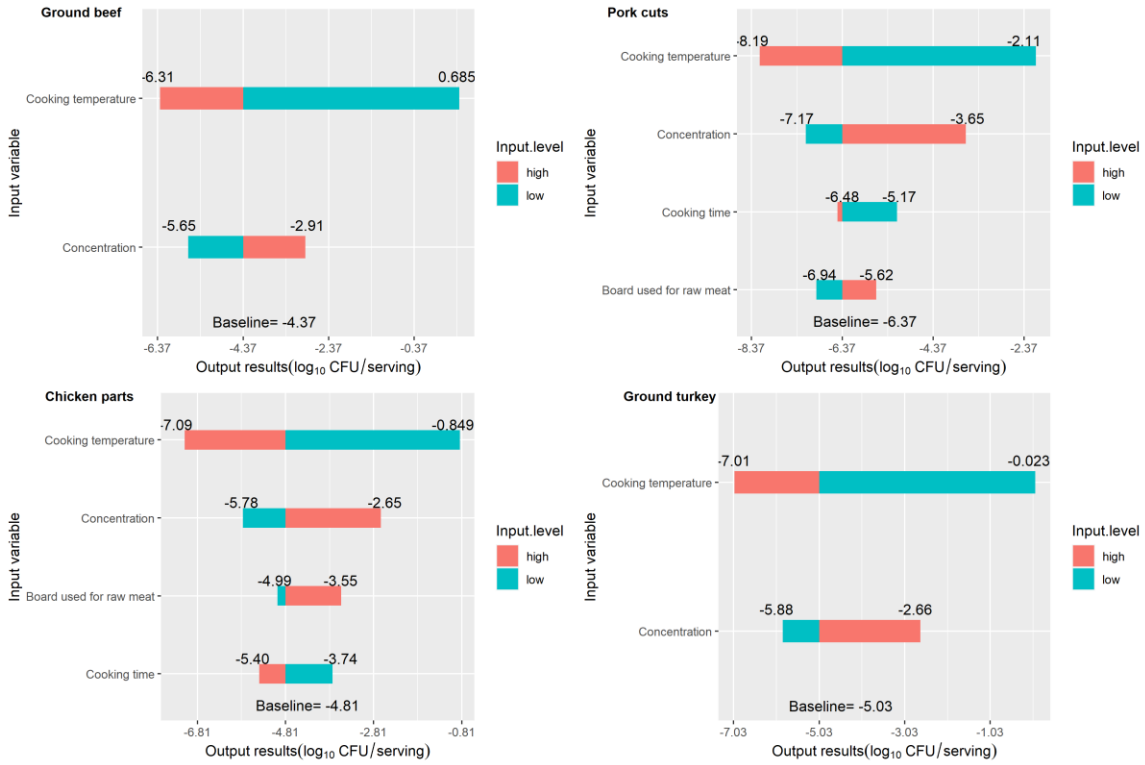

Figure 3.5. Tornado chart for different food products showing the effect of identified influencing input variables on the change in output mean, based on per-serving exposure estimation. The red bar represents the output means at a relatively higher input value, while the green bar represented at a relatively lower input value. The baseline value was per-serving exposure level estimated by keeping the distributions as listed in Table 3.2 in the simulation

Table 3.1 List of parameters for estimating growth rate and maximum population density

Parameter		Beef	Pork	Poultry
Growth model				
GR	cc	0.069 (95% CI: -1.17 – 1.317)	0.027 (95% CI:0.003 – 0.051)	0.029 (95% CI:0.025 – 0.032)
	T1	7.912 (95% CI:1.267 – 14.555)	4.966 (95% CI: -6.484 – 16.416)	4.456 (95% CI:2.074 – 6.838)
	k	0.007 (95% CI: -0.209 – 0.224)	0.106 (95% CI: -0.494 – 00.708)	0.151 (95% CI:0.109 – 0.0.193)
	T2	49.5	49.5	49.5
MPD	a	11.824 (95% CI: 4.754 – 18.894)		
	Tmin	-2.533 (95% CI: -22.558 – 17.492)		
	Tmax	53.118 (95% CI: 38.6 – 61.63)		
	Tmin2	3.839 (95% CI: -3.751 – 11.428)		
	Tmax2	50.313 (95% CI: 42.594 – 58.031)		
Thermal inactivation model				
D	Slope	0.1516		0.1672
	Intercept	10.034		11.132

Table 3.2 Contribution of different food types to the exposure of cephem-resistant *Salmonella* at the moment of consumption per person per year

Meat type	*Cephem-resistant Salmonella Prevalence*	*Cephem-resistant Salmonella exposure per serving (log CFU/serving)*	*Total number of consumed portions*	*Total exposure (log CFU)*	*Percent (%)*
Beef	0.134	-4.37	144	-2.21	46.8
Pork	0.0385	-6.37	100	-4.37	0.321
Chicken	0.198	-4.81	435	-2.17	51.0
Turkey	0.0774	-5.03	26.9	-3.60	1.91

Table 3.3 Model description and parameters

Variable	Description	Unit	Distribution/calculation				Reference
			Ground Beef	Chicken Parts	Ground Turkey	Pork Cuts	
	At retail						
C_sal_g	Concentration of *Salmonella* per gram	CFU/g	Discrete(Discrete(10^(Uniform(-1.8, 0.9), 0,{10/191}, {181/191}), Discrete(10^(Cumulative (1.8, 1.9, {0.9}, {0.95})), 0,{20/179}, {159/179}), {0.901}, {0.099})	Discrete(Lognorm(0.15, 0.35, Turncate2(0.0066,2.42)), 0.0033, 100, 0, {444/2496}, {201/2496}, {12/2496}, {0.738})	Discrete(Discrete(Lognorm (18.2, 63.9, Turncate2(0.03, 240)), 0.015, 0, {0.0725}, {0.0725}, {0.855}), Discrete(Lognorm(1.2, 4.9, Turncate2(0.03, 240)), 0.015, 0, {0.195*(78/151)}, {0.195*(73/151)}, {0.805}), {1361/4284}, {2923/4284})	Discrete(Cumulative(0,100, 0, 0.025 x 0.2979, 0.25 x 0.2979, 2.5 x 0.2979, 25 x 0.2979, 250 x 0.2979, {0}, {0.717}, {0.774}, {0.943}, {0.981}, {1}, 0, {53/1960}, {1-53/1960})	36,7,15 ,19
P_sal	Prevalence of cephem-resistance *Salmonella*	%	Beta(76+1,570-76+1)	Beta(1738+1,8772-1738+1)	Beta(544+1,7040-544+1)	Beta(24+1,647-24+1)	
C_sal	Concentration of *Salmonella* per gram	Log CFU/g	Log10(C_sal_g x P_sal)	Log10(C_sal_g x P_sal)	Log10(C_sal_g x P_sal)	Log10(C_sal_g x P_sal)	
	At transportation						
Tem_trans	Transport temperature	˚C	(Normal(47.1, 6.9, turncate(22,76))-32)*5/9	(Normal(47.1, 6.9, turncate(22,76))-32)*5/9	(Normal(47.1, 6.9, turncate(22,76))-32)*5/9	(Normal(47.1, 6.9, turncate(22,76))-32)*5/9	9
tim_trans	Transport time	h	Normal(70, 26, turncate(18,222))/60	Normal(70, 26, turncate(18,222))/60	Normal(70, 26, turncate(18,222))/60	Normal(70, 26, turncate(18,222))/60	
GR	Maximum growth rate	log10 CFU/g*h	(c_1*(Tem_trans - T_min1)*(1-EXP(c_2*(Tem_trans-T_max1))))^2	(c_1*(T_trans - T_min1)*(1-EXP(c_2*(Tem_trans-T_max1))))^2	(c_1*(T_trans - T_min1)*(1-EXP(c_2*(Tem_trans-T_max1))))^2	(c_1*(T_trans - T_min1)*(1-EXP(c_2*(Tem_trans-T_max1))))^2	13
MPD	Maximum population density function with respect to temperature	log10 CFU/g	((c_mpd*(Tem_trans-T_min2)*(Tem_trans-T_max2))/((Tem_trans-T_submin)*(Tem_trans-T_supmax)))	((c_mpd*(Tem_trans-T_min2)*(Tem_trans-T_max2))/((Tem_trans-T_submin)*(Tem_trans-T_supmax)))	((c_mpd*(Tem_trans-T_min2)*(Tem_trans-T_max2))/((Tem_trans-T_submin)*(Tem_trans-T_supmax)))	((c_mpd*(Tem_trans-T_min2)*(Tem_trans-T_max2))/((Tem_trans-T_submin)*(Tem_trans-T_supmax)))	
N_tra	cephem resistance *Salmonella* amount after growth	CFU/g	MIN(10^(C_sal+GR*tim_trans), 10^(MPD))	MIN(10^(C_sal+GR*tim_trans), 10^(MPD))	MIN(10^(C_sal+GR*tim_trans), 10^(MPD))	MIN(10^(C_sal+GR*tim_trans), 10^(MPD))	13
N_aftran	cephem resistance *Salmonella* amount after transport	Log CFU/g	If(C_sal > N_tra, log10(C_sal), log10(N_tra)	If(C_sal > N_tra, log10(C_sal), log10(N_tra)	If(C_sal > N_tra, log10(C_sal), log10(N_tra)	If(C_sal > N_tra, log10(C_sal), log10(N_tra)	

At storage						
Storage temperature	˚C	(Cumulative(20, 70, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, {0.005}, {0.035}, {0.105}, {0.235}, {0.515}, {0.832}, {0.932}, {0.972}, {0.992}, {0.994}, {0.996}, {0.998}, {0.999}, {1})-32)*5/9	(Cumulative(20, 70, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, {0.005}, {0.035}, {0.105}, {0.235}, {0.515}, {0.832}, {0.932}, {0.972}, {0.992}, {0.994}, {0.996}, {0.998}, {0.999}, {1})-32)*5/9	(Cumulative(20, 70, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, {0.005}, {0.035}, {0.105}, {0.235}, {0.515}, {0.832}, {0.932}, {0.972}, {0.992}, {0.994}, {0.996}, {0.998}, {0.999}, {1})-32)*5/9	(Cumulative(20, 70, 26, 29, 32, 35, 38, 41, 44, 47, 50, 53, 56, 59, 62, 65, {0.005}, {0.035}, {0.105}, {0.235}, {0.515}, {0.832}, {0.932}, {0.972}, {0.992}, {0.994}, {0.996}, {0.998}, {0.999}, {1})-32)*5/9	
Storage time	h	Cumulative(0,120,{24,72},{0.38,0.85})	Cumulative(0,120,{24,72},{0.38,0.85})	Cumulative(0,120,{24,72},{0.38,0.85})	Cumulative(0,120,{24,72},{0.38,0.85})	
Maximum growth rate	log10 CFU/g*h	(c_1*(Tem_stor - T_min1)*(1-EXP(c_2*(Tem_stor-T_max1))))^2	(c_1*(Tem_stor - T_min1)*(1-EXP(c_2*(Tem_stor-T_max1))))^2	(c_1*(Tem_stor - T_min1)*(1-EXP(c_2*(Tem_stor-T_max1))))^2	(c_1*(Tem_stor - T_min1)*(1-EXP(c_2*(Tem_stor-T_max1))))^2	
Maximum population density function with respect to temperature	log10 CFU/g	((c_mpd*(Tem_stor-T_min2)*(Tem_stor-T_max2))/((Tem_stor-T_submin)*(Tem_stor-T_supmax)))	((c_mpd*(Tem_stor-T_min2)*(Tem_stor-T_max2))/((Tem_stor-T_submin)*(Tem_stor-T_supmax)))	((c_mpd*(Tem_stor-T_min2)*(Tem_stor-T_max2))/((Tem_stor-T_submin)*(Tem_stor-T_supmax)))	((c_mpd*(Tem_stor-T_min2)*(Tem_stor-T_max2))/((Tem_stor-T_submin)*(Tem_stor-T_supmax)))	13
cephem resistance *Salmonella* amount after growth	CFU/g	MIN(10^(N_aftran+GR_stor*tim_stor), 10^(MPD_stor))	MIN(10^(N_aftran+GR_stor*tim_stor), 10^(MPD_stor))	MIN(10^(N_aftran+GR_stor*tim_stor), 10^(MPD_stor))	MIN(10^(N_aftran+GR_stor*tim_stor), 10^(MPD_stor))	
cephem resistance *Salmonella* amount after storage	Log CFU/g	If(N_aftran>N_afstor_2, log10(N_aftran), log10(N_afstor_2)	If(N_aftran>N_afstor_2, log10(N_aftran), log10(N_afstor_2)	If(N_aftran>N_afstor_2, log10(N_aftran), log10(N_afstor_2)	If(N_aftran>N_afstor_2, log10(N_aftran), log10(N_afstor_2)	
At cooking						
cooking temp	˚C	Weibull(7.03, 78.1, Shift(-3.07), Truncate2(23,99))	Pert(55, 62, 70)	Weibull(7.03, 78.1, Shift(-3.07), Truncate2(23,99))	Pert(55, 62, 70)	36,39, 40
Cooking time	min	If(T_cook>65.6, Uniform(10, 12), Uniform(8, 10))	Pert(15, 30, 45)	If(T_cook>65.6,Uniform(10, 12), Uniform(8, 10))	Pert(15, 30, 45)	
Decimal reduction time	min	10^(-0.1516*T_cook+10.034)	10^(-0.1672*T_cook+11.132)	10^(-0.1672*T_cook+11.132)	10^(-0.1516*T_cook+10.034)	14
Log reduction due to cooking	log	MIN(7,t_cook/D_value)	MIN(7,t_cook/D_value)	MIN(7,t_cook/D_value)	MIN(7,t_cook/D_value)	
Concentration of bacterial after cooking	logCFU/g	LOG10(N_afstor)-log_cook	LOG10(N_afstor)-log_cook	LOG10(N_afstor)-log_cook	LOG10(N_afstor)-log_cook	

Cross-contamination

Num	Number of resistance *Salmonella* Transfer from raw meat to hands	CFU/g	10^(N_afstor)	10^(N_afstor)	10^(N_afstor)	10^(N_afstor)
Prop_CH	Proportion transferred from raw meat to hands (Bacterial transfer rate)	proportion	Pert(0.011,0.065,0.261)	Pert(0.011,0.065,0.261)	Pert(0.011,0.065,0.261)	Pert(0.011,0.065,0.261)
XCH	Probability of transfer from raw meat to hands	CFU/g	IF(Num = 0,0,Prop_CH)	IF(Num = 0,0,Prop_CH)	IF(Num = 0,0,Prop_CH)	IF(Num = 0,0,Prop_CH)
Num_C1	Number on hands	CFU/g	IF(XCH=0,0,Num x Prop_CH)	IF(XCH=0,0,Num x Prop_CH)	IF(XCH=0,0,Num x Prop_CH)	IF(XCH=0,0,Num x Prop_CH)
Num_H	Number left on raw meat		Num - Num_C1	Num - Num_C1	Num - Num_C1	Num - Num_C1
HW_Prob	Transfer from hands to cooked meat Probability that hands are not washed after handling raw meat	Proportion	Pert(0.003,0.006,0.0105)	Pert(0.003,0.006,0.0105)	Pert(0.003,0.006,0.0105)	Pert(0.003,0.006,0.0105)
HW	Were hands washed? (1 = y, 0 = n)	Proportion	Binomial(1,1-HW_Prob)	Binomial(1,1-HW_Prob)	Binomial(1,1-HW_Prob)	Binomial(1,1-HW_Prob)
Prop_HC	Proportion transferred from hands to cooked meat (Bacterial transfer rate)	Proportion	Pert(0.001,0.089,0.529)	Pert(0.001,0.089,0.529)	Pert(0.001,0.089,0.529)	Pert(0.001,0.089,0.529)
Num_CC1	Number on cooked meat from raw meat via hands	CFU/g	IF(HW = 1,0,Num_C1*Prop_HC)	IF(HW = 1,0,Num_C1*Prop_HC)	IF(HW = 1,0,Num_C1*Prop_HC)	IF(HW = 1,0,Num_C1*Prop_HC)
Prop_CB	Proportion transferred from raw meat to cutting board (Bacterial transfer rate)	Proportion	Pert(0.03,0.075,0.309)	Pert(0.03,0.075,0.309)	Pert(0.03,0.075,0.309)	Pert(0.03,0.075,0.309)
XCB	Probability of transfer from raw meat to cutting board	CFU/g	IF(Num=0,0,Prop_CB)	IF(Num=0,0,Prop_CB)	IF(Num=0,0,Prop_CB)	IF(Num=0,0,Prop_CB)
Num_B	Number on board	CFU/g	IF(XCB = 0,0,Num*Prop_CB)	IF(XCB = 0,0,Num*Prop_CB)	IF(XCB = 0,0,Num*Prop_CB)	IF(XCB = 0,0,Num*Prop_CB)
Num_C1	Number left on raw meat	CFU/g	Num-Num_B	Num-Num_B	Num-Num_B	Num-Num_B
Brd_use_Prob	Transfer from cutting board (or plate) to cooked meat Probability that same board (or utensils) used for raw meat is used for cooked meat without washing	Proportion	Uniform(0.01,0.02)	Uniform(0.01,0.02)	Uniform(0.01,0.02)	Uniform(0.01,0.02)
Brd_use	Were boards used for raw foods? (1 = y, 0 = n)	Proportion	Binomial(1,Brd_use_Prob)	Binomial(1,Brd_use_Prob)	Binomial(1,Brd_use_Prob)	Binomial(1,Brd_use_Prob)

Prop_BC	Proportion transferred from boards to cooked meat	Proportion	Pert(0.105,0.194,0.424)	Pert(0.105,0.194,0.424)	Pert(0.105,0.194,0.424)	Pert(0.105,0.194,0.424)
Num_CC2	Number on cooked meat from raw chicken via board (or utensils)	CFU/g	IF(Brd_use = 0,0,Num_B*Prop_BC)	IF(Brd_use = 0,0,Num_B*Prop_BC)	IF(Brd_use = 0,0,Num_B*Prop_BC)	IF(Brd_use = 0,0,Num_B*Prop_BC)
Num_XC	Total number of resistant *Salmonella* via cross-contamination	CFU/g	Num_CC1+Num_CC2	Num_CC1+Num_CC2	Num_CC1+Num_CC2	Num_CC1+Num_CC2
Inges_CC	Ingestion via cross-contamination	CFU/g	IF(Num=0,0,Num_XC)	IF(Num=0,0,Num_XC)	IF(Num=0,0,Num_XC)	IF(Num=0,0,Num_XC)
N_total	Contamination before digestion	CFU/g	Inge_CC+10^(Cx_cook)	Inge_CC+10^(Cx_cook)	Inge_CC+10^(Cx_cook)	Inge_CC+10^(Cx_cook)
W_meat	Meat consumption per U.S. person per year	g	26489.7728*0.46	44089.1424*0.972	6939.9576*0.329	23586.784*0.361
N_final	Cephem resistance *Salmonella* contamination per person per year	logCFU/g	LOG10(N_total*W_meat)	LOG10(N_total*W_meat)	LOG10(N_total*W_meat)	LOG10(N_total*_meat)

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CHAPTER 4. OVERALL CONCLUSION

In the present thesis, two studies were covered with an overall goal of providing a quantitative and qualitative understanding of the relative contribution of different food sources to human antibiotic-resistant *Salmonella:* i) a systematic review (SR) and meta-analysis (MA) study characterizing the distribution of antibiotic-resistant non-typhoidal *Salmonella* in various foods at retail and identify the knowledge gaps, and ii) a comparative exposure assessment model estimating the relative contribution of different food groups to overall foodborne exposure to antibiotic-resistant *Salmonella*.

In general, chicken, pork, and turkey products were found more prevalent with resistant *Salmonella* compared to beef, while imported foods and vegetables were the least (data mainly on spices). When comparing antibiotic resistance among major antibiotic classes, tetracycline resistance occurred most frequently. Although the prevalence of *Salmonella* resistant to the beta-lactam antibiotics represented a moderate level, the critical role of this class in clinical treatment might indicate a potentially serious threat to public health. Resistance to macrolides, an important class of antibiotics used in veterinary settings, was less detected for all major food groups, which nevertheless needs be interpreted with caution due to a lack of sufficient data supporting the estimation The results of this study will assist in quantifying microbiological risk and developing mitigation strategies for foodborne AR control.

The comparative exposure assessment showed that consumers' exposure to cephem-resistant *Salmonella* were the highest through chicken parts, which accounted for 51.0% of the total exposure, followed by ground beef (46.8%). Pork cuts and ground turkey, on the other hand, were less significant, accounting for 0.3%and 1.9% of overall exposure, respectively. During the food consumption chain, the mean concentration of cephem-

resistant *Salmonella* was estimated to increase slightly during transit and storage. Almost all the resistant *Salmonella* were killed after cooking and most of the resistant *Salmonella* found in the cooked meal resulted from cross-contamination. Among all the input factors applied to the model, cooking temperature and *Salmonella* concentration at the retail stage were the two main influencing factors which can significantly change the final exposure estimates. In the future, to improve the prediction accuracy, the *Salmonella* concentration for different food at the retail stage can be replaced by selecting the best distribution to fit the raw baseline microbiological data accessed from the Food Safety and Inspection Service (FSIS).

In conclusion, the occurrence of AR *Salmonella* in various food products at the retail stage is not uncommon, particularly in animal-derived foods. Among animal-derived foods, poultry products generally harbor AR *Salmonella* at a relatively higher frequency. As for *Salmonella* resistance to antibiotics, tetracyclines and penicillin are more prevalent than other classes. For other antibiotics like cephems, quinolones and macrolides, the three highest priority critically important antibiotics classified by WHO, generating more data is necessary. Among them, cephems need to pay more attention since cephem-resistant *Salmonella* is more frequently detected based on the currently available data. Among specific animal-derived foods, chicken parts and ground beef contribute the most to foodborne cephem-resistant *Salmonella* exposure, followed by ground turkey, and least for pork cuts. More data need to be generated for characterizing cephem-resistant *Salmonella* or other AR *Salmonella* distribution in foods other than animal origins.