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# **The Pig-Salmonella Dynamic: Navigating Monophasic Variants, Control Challenges, and Genomic Interventions in the EU: A Scoping Review**

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## **1. Abstract**

Salmonellosis, a global public health concern, remains a pressing issue in the European Union (EU). Despite the predominant link to poultry, pigs are a significant source of infection. This scoping review delves into the understanding and implications of the monophasic variant of *Salmonella* Typhimurium (MST) in pigs within the EU between 2018 and 2023. It also explores the challenges associated with controlling *Salmonella* and discusses how genomic studies have been instrumental in refining current predictive models.

A rigorous screening process led to the inclusion of 35 studies. Key findings elucidate the growing epidemiological significance of MST in the pig sector and its increasing association with multi-drug resistance. The review also identifies gaps in country-specific data from 15 EU Member States, emphasizing a need for a comprehensive, EU-wide approach. The challenges tied to *Salmonella* control in pigs arise from varying contamination levels, the continuous evolution of *Salmonella* strains, and a pressing need to enhance surveillance measures.

Genomic studies have been pivotal in refining current predictive models, but a lack of real-time monitoring data is a hurdle. An integrated One Health approach, combining human, animal, and environmental health considerations, may offer a path forward. This research paper underscores the critical role of interdisciplinary efforts, routine audits, and engagement with the pork industry to devise targeted interventions. The study concludes by emphasizing the importance of real-time data collection and continuous refinement of existing strategies to effectively combat the threat of salmonellosis in the EU.



## 2. Plain Language Summary

Salmonellosis represents a disease caused by specific bacteria, and its symptoms typically include fever, stomach cramps, and diarrhea. This condition poses a global health challenge and has sustained prominence in the European Union (EU). Though we often associate salmonellosis with poultry, pigs have been a notable source of this infection in recent years.

One of the aims of the research discussed here is to shed light on a specific type of Salmonella bacteria found in pigs: the monophasic variant of Salmonella Typhimurium (MST). This scoping review specifically looked at literature from 2018 to 2023, focusing on pigs in the EU. A "scoping review" is a way to map out and understand all the research that has been done on a particular topic, and it helps give a big-picture view of what we know and what gaps might still exist. After a thorough search, 35 important studies were selected for this review. From these studies, a few key things stood out:

- The MST type of Salmonella is becoming more common in pigs, and it is worrisome because it is also becoming resistant to multiple drugs. When bacteria are resistant to drugs, the usual medicine might not work anymore, making the disease more challenging to treat.
- There is not enough information from some countries in the EU. Of all the EU member states, 15 had missing or incomplete data. It shows that there needs to be better coordination across the EU so that the countries can work together.
- Keeping Salmonella under control in pigs is a challenge. There are different levels of the disease in different places, and the bacteria keeps changing and evolving. On top of that, there need to be better ways to monitor the disease, known as 'surveillance'.
- Genomic studies - what are they? Simply put, our genes are blueprints for our bodies, and everything living has genes, including bacteria like Salmonella. Genomic studies look at these blueprints to understand how living things work and how they might change over time. We showed that these studies have helped predict how the disease might spread and how bad it might get. However, one big challenge remains: insufficient up-to-date or "real-time" data. Think of it like trying to predict the weather without the latest satellite images - it can get tricky.

A suggestion from this study is to use the "One Health" approach. This means looking at the health of people, animals, and the environment together since they are connected. To give an example, what affects pigs can affect humans and the environment they live in. So, by understanding all these aspects, there is a better chance of managing diseases like Salmonella.

To wrap up, this research highlights the need for everyone, from scientists to pork producers, to work together. By teaming up, they can better handle the Salmonella problem in the EU. It is essential to keep gathering information and using it to improve things.



### 3. Introduction

Salmonellosis, a significant global public health concern, continues to be a prime challenge within the European Union (EU). According to the **European Union One Health 2021 Zoonoses** report provided by the European Food Safety Authority (EFSA) and the European Centre for Disease Prevention and Control (ECDC), salmonellosis was still the second most prevalent foodborne disease in humans, with campylobacteriosis taking the first position. There were 60,050 (of which 43,720 infections were acquired in the EU) confirmed cases, corresponding to a notification rate of 15.7 per 100,000 population, marking an uptick from 2020 but a decrease from earlier years (European Food Safety et al., 2022). The gathering and analysis of data at the EU level for 2021 remained influenced by the ongoing effects of the COVID-19 pandemic and the control measures instituted by the Member States (MS), which included partial to full lockdowns (European Food Safety et al., 2022).

Despite poultry being the principal contributor, pigs are one species that deserve attention as a recognized source of salmonellosis (Chanamé Pinedo et al., 2022). Pigs are considered the second most crucial source of infections after poultry (European Food Safety et al., 2022). The five leading *Salmonella* serovars responsible for human infections in 2021 are distributed in this manner: *S. Enteritidis* (54.6%), *S. Typhimurium* (11.4%), monophasic *S. Typhimurium* (4,[5],12:i:-) (8.8%), *S. Infantis* (2.0%), and *S. Derby* (0.93%) (European Food Safety et al., 2022).

The most frequently identified serovar in pigs was the monophasic variant of *S. Typhimurium* (MST), accounting for 28.2% of cases (European Food Safety et al., 2022). Between 2015 and 2019, pork was the predominant food source of concern in Northern and Western Europe, and outbreaks caused by the monophasic variant of *Salmonella Typhimurium* (MST) were primarily linked to pork (Chanamé Pinedo et al., 2022). *Salmonella enterica* serovar *Typhimurium* with the antigenic formula 4,[5],12:i:- is recognized as a monophasic variant of *S. Typhimurium* that lacks the second phase flagellar antigen (Palma et al., 2018). This serovar was identified in 1997, and since then, it has been consistently associated with human infections and pig farming (Palma et al., 2018). Barilli et al. (2018) documented a strain of this variant that exhibited resistance to 12 antimicrobial agents, including the critically important ones like colistin and cefotaxime. Additionally, the strain carried an array of genetic markers for virulence. The prevalence of these strains is especially concerning due to their increasing multi-drug resistance (MDR). Given this backdrop, focusing on these strains becomes paramount. Their potential to resist multiple treatments and the extensive virulence factors they harbor present unique challenges in both treatment methodologies and preventive strategies. Furthermore, it is essential to note that interventions aimed at reducing the prevalence of these strains in the primary production stage might not be as effective as previously believed. Cargnel et al. (2020) highlighted that even with a hypothetical 95% reduction of seropositive pigs, there was not a substantial decline in the human incidence of salmonellosis due to



minced pork consumption. This raises questions about the best strategies to mitigate the risks posed by these strains and what evidence-based interventions could be most effectively implemented at these stages to limit the spread.

The intricacy of salmonellosis transmission and the variable effectiveness of interventions underscore the importance of accurate predictive models in this domain. Given the ever-evolving nature of the pathogen and all the complexities surrounding its transmission dynamics, it becomes imperative to ascertain the fidelity of these models compared to real data. These models play a dual role: they are instrumental in guiding our understanding of transmission pathways and risk and act as tools for policymakers to devise efficient and targeted interventions. However, the efficacy and accuracy of these models often come into question, especially when contrasted with limited empirical data from actual observations. A recent study by Mu et al. (2023) underscored this challenge, revealing a significant lack of real monitoring data. Their research was constrained by the absence of real-time monitoring data related to *Salmonella* spp. outbreaks, forcing them to rely on literature data and expert opinions to approximate the real-world scenario. Additionally, their modeling process was unable to address the speed of recovery due to this data shortfall, further emphasizing the need for comprehensive, real-time data to enhance the accuracy and reliability of such predictive models. Considering these challenges, this scoping review seeks to address three fundamental research questions:

- 1. How has the perception and understanding of the monophasic variant of *Salmonella* Typhimurium (MST) in pigs evolved, and what are its broader implications for public health?**
- 2. A) What challenges are faced in controlling *Salmonella* in pigs in the?  
B) What evidence suggests that pre-harvest interventions may lead to successful control of this pathogen?**
- 3. How accurately do current models reflect the real-world epidemiology of *Salmonella* transmission from pigs to humans in the EU, and what role do genomic studies play in refining these models for more effective interventions?**

The **aim** of this scoping review focused on pigs. It was conducted for the period between 2018 and 2023 within the European Union member states to critically assess the evolving understanding and perception of the monophasic variant of *Salmonella* Typhimurium, to identify and evaluate challenges faced in controlling *Salmonella* in the pig sector, the potential efficacy of pre-harvest interventions, and to determine the alignment between current models and real-world data in the context of *Salmonella* in pig production, while highlighting the contribution of genomic studies in enhancing the precision and effectiveness of these models for more tailored intervention. By delving deep into these areas, this review seeks to provide insights to inform



strategies and guide future research for more effective prevention and control of salmonellosis in the EU.

## 4. Methods

### 4.1. Data Sources and Search Strategy

The literature search was tailored to ensure a comprehensive understanding of recent developments in the field, following the guidance of Utrecht University, which emphasizes the use of recent literature spanning a maximum of five years, with at least two of those years being no older than three years. Consequently, the timeframe for the literature search was set explicitly between 2018-2023. An extensive literature search was executed using a combination of keywords that corresponded with the research questions. First, the focus was placed on the foodborne pathogen with terms like 'Salmonella OR salmonellosis'. Secondly, to align with the source of transmission in focus, terms such as "pig", "swine", or "pork" were used. Thirdly, terms like "Europe" OR "EU" were incorporated to narrow the scope geographically. A set of terms related to specific attributes and methodologies concerning the pathogen ('genome', 'serotype', 'mlst', 'sequence') and to stages of pork processing and inspection were integrated, using keywords such as 'slaughterhouse', 'abattoir', 'meat processing', 'meat inspection' and 'meat contamination'. Here are some representative examples of the search queries:

- ('salmonella'/exp OR salmonella OR 'salmonella enterica'/exp OR 'salmonella enterica') AND ('pig'/exp OR pig OR 'pork'/exp OR pork) AND ('europe'/exp OR europe) AND (genome OR serotype OR mlst OR sequence) AND (2018:py OR 2019:py OR 2020:py OR 2021:py OR 2022:py OR 2023:py)
- TITLE-ABS-KEY ("salmonella" OR "salmonella enterica" ) ) AND ( TITLE-ABS-KEY ( "swine" OR "pig" OR "pork" ) ) AND ( TITLE-ABS-KEY ( "europe" ) ) AND ( TITLE-ABS-KEY ( "genome" OR "serotype" OR "sequence" ) ) AND PUBYEAR > 2017 AND PUBYEAR < 2024
- (Salmonella OR "Salmonella Infections") AND (pork OR swine) AND Europe AND ("2018/01/01"[Date - Publication]: "2023/12/31"[Date - Publication]) AND "english"[Language] AND (slaughterhouse OR abattoir OR "meat processing" OR "meat inspection" OR "meat contamination")
- ("salmonella"[MeSH Terms] OR "salmonella" OR "salmonella enterica"[MeSH Terms] OR "salmonella enterica") AND ("swine"[MeSH Terms] OR "pig" OR "pork"[MeSH Terms] OR "pork") AND ("europe"[MeSH Terms] OR "europe") AND ("genome" OR "serotype" OR "mlst" OR "sequence") AND ("2018"[PDAT]: "2023"[PDAT])

These queries were employed across multiple databases, including Embase, PubMed, Scopus, ensuring coverage of literature sources. The aim was to extract literature that specifically sheds light on the evolution, challenges, interventions, and predictive models surrounding salmonellosis and its transmission pathways in the EU context.

The following eligibility criteria were considered:

#### Inclusion criteria:

- Full research articles, including primary research and reviews
- Availability of full-text online
- Published between 2018-2023
- Studies conducted in or focused on countries within the EU



- Published in English
- Examines Salmonella transmission from pigs to humans
- Content relevant to the research questions

**Exclusion criteria:**

- Editorials, commentaries, and letters
- Economic analyses
- Studies originating from non-EU countries
- Studies that predominantly focus on wild boars rather than domesticated pigs

Articles that did not conform to the eligibility criteria were excluded.

## **4.2. PRISMA Flow Chart**

Thirty-five articles (n=35) were included in this scoping review. PRISMA-ScR checklist was used to guide reporting of articles inclusion and exclusion for this scoping review (**Figure 1**). Initially, a total of 204 studies were identified. However, 90 duplicate records were promptly removed before the screening process commenced. This left 114 studies, which were then screened based on their respective titles and abstracts. From this group, 61 were unsuitable and excluded for various reasons (e.g., irrelevance to the research questions, insufficient data). The 54 remaining studies underwent a thorough assessment for eligibility against the predetermined criteria, and of these, 19 reports were further omitted due to several reasons: eight originated from non-EU countries, four were editorials, three were centered on economic analyses, and four primarily focused on wild boars rather than domestic pigs. After this meticulous selection process, **35 studies** were found to be apt for inclusion in the final review.

## **4.3. Data Extraction and Synthesis**

Following the identification of eligible articles, data extraction was the next step. Each article underwent an in-depth review, ensuring that all information relating to the research questions was accurately extracted systematically and organized. An extraction table, tailored in Excel, captured details such as the author's name(s), year of publication, country/region of study, study design, sample size, methodologies or genomic insights employed, main findings, limitations of the study, and recommendations. Particular emphasis was placed on studies delving into the evolution and understanding of the monophasic variant of *Salmonella* Typhimurium (MST) over the past five years, the broader challenges of controlling *Salmonella* in pigs, and any existing evidence of pre-harvest interventions that could aid in the successful control of this pathogen. Moreover, studies shedding light on the accuracy of current models portraying the real-world epidemiology of *Salmonella* transmission from pigs to humans, and the contribution of genomic studies in enhancing these models, were of particular interest.



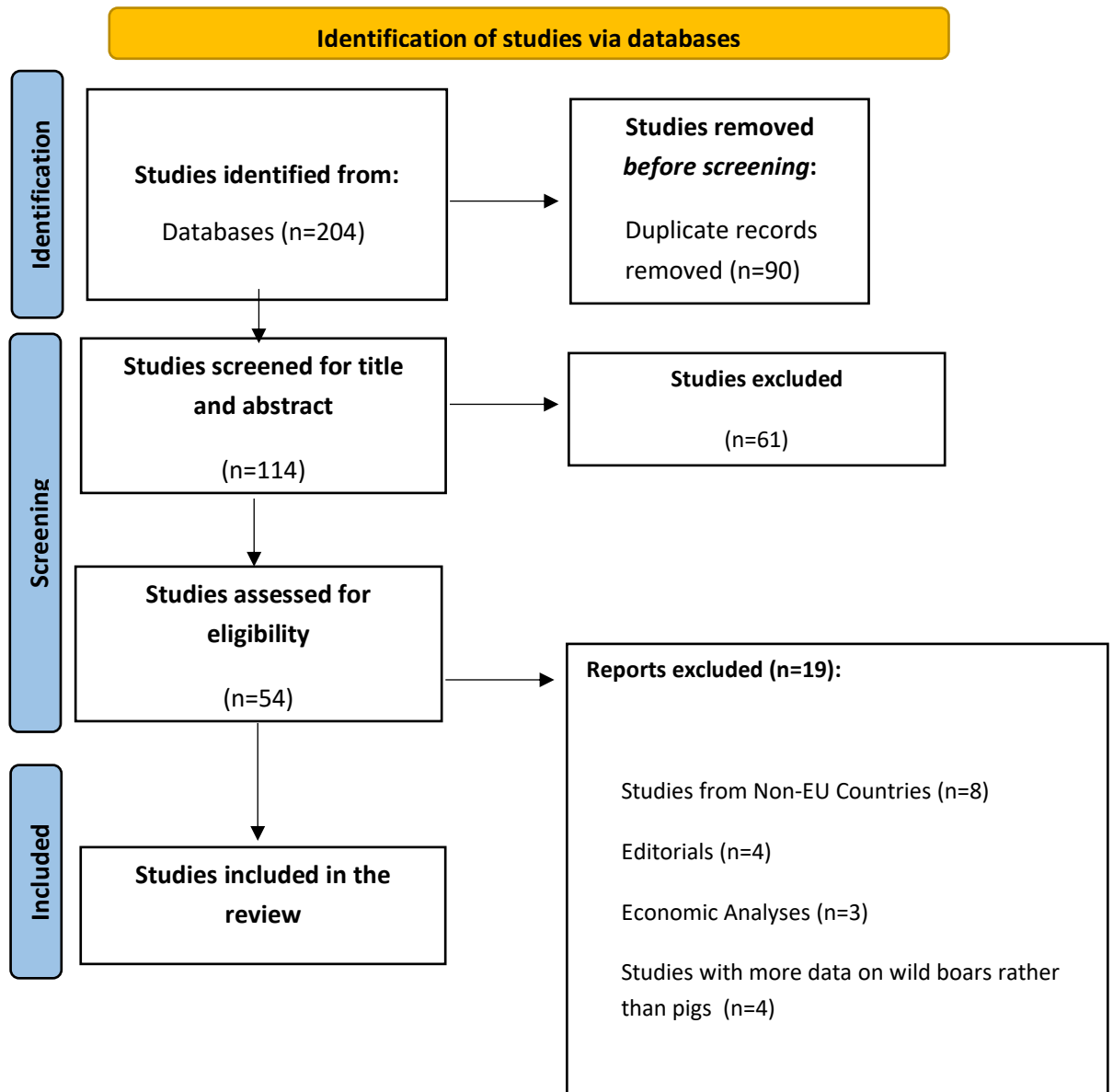
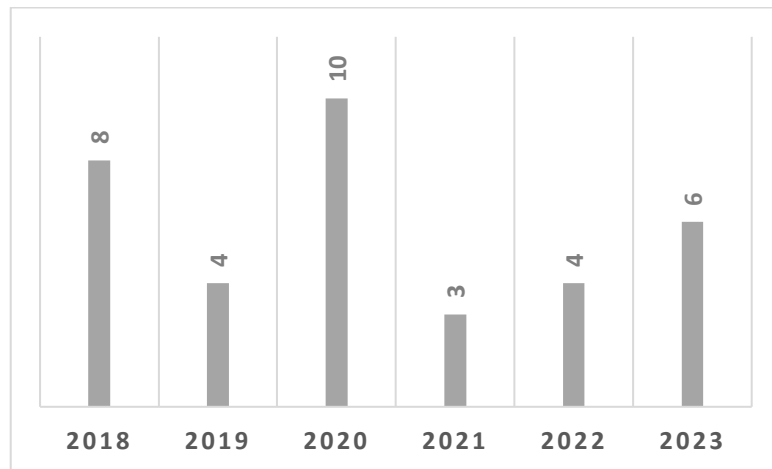


Figure 1: PRISMA-ScR flow chart

## 5. Results

### 5.1. Analysis of Selected Study

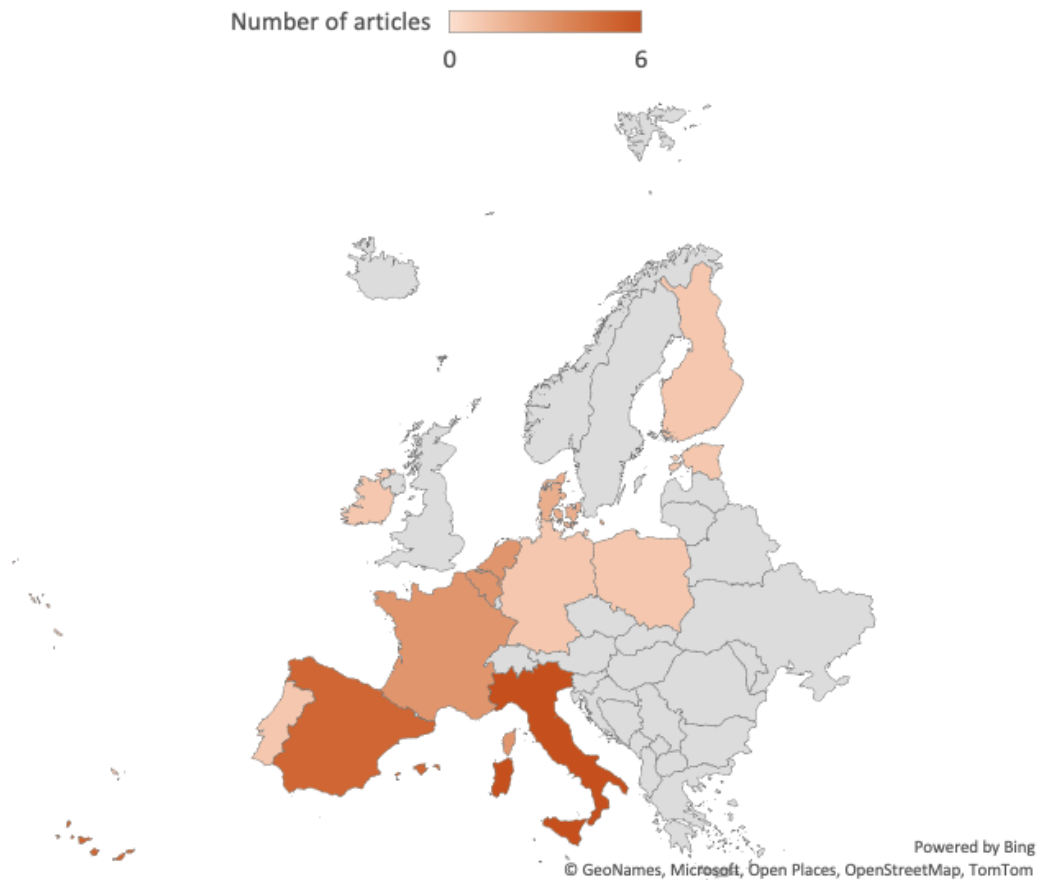
Thirty-five articles addressing the research questions were included in this scoping review. **Appendix A** provides a comprehensive summary table for a detailed overview of each article. The detailed summary table of reviewed literature includes the author(s), year, country/region, title, study's aim, primary findings, recognized gaps, and the specific research question to which each study contributed. As mentioned before, articles were published between 2018-2023.



**Figure 2:** Publication years of the articles included in this scoping review

Notably, 2020 saw the most significant number of publications, with ten articles ( $n=10$ ). It was closely followed by 2018, contributing eight articles ( $n=8$ ) to the pool. The subsequent years, 2023 and 2019, contributed six ( $n=6$ ) and four ( $n=4$ ) articles, respectively, with 2022 and 2021 accounting for only four ( $n=4$ ) and three ( $n=3$ ) articles successively.

Most studies were undertaken in Italy ( $n=6$ ), followed closely by Spain ( $n=5$ ). Belgium, France, and the Netherlands each contributed with three articles ( $n=3$ ), representing a consistent research interest in these regions. On the other hand, there was less identified literature for the scoping review from Denmark ( $n=2$ ), Estonia ( $n=1$ ), Finland ( $n=1$ ), Germany ( $n=1$ ), Ireland ( $n=1$ ), Poland ( $n=1$ ) and Portugal ( $n=1$ ) (**Figure 3**). The “Europe” category, which is not directly pinpointed on the map (**Figure 3**), accounted for five articles ( $n=5$ ). This category represents studies that covered the whole European Union region. One distinctive article within this grouping covered multiple EU countries, specifically Austria, Bulgaria, the Czech Republic, Germany, Estonia, Italy, the Netherlands, and Poland. On the provided map, it is worth noting that only individual studies linked to a distinct country are visually represented. No country-specific articles were identified from the following EU countries: Austria, Bulgaria, Croatia, Cyprus, Czech Republic, Greece, Hungary, Latvia, Lithuania, Luxembourg, Malta, Romania, Slovakia, Slovenia, and Sweden. This observation underscores potential areas of limited research activity or gaps in the available literature for these nations.



**Figure 3:** Distribution of studies across the EU

**NOTE:** The "Europe" category, which is not explicitly marked on the map, comprises five articles. These articles vary in scope: some exclusively cover the EU, others have a broader global perspective that includes European data, and one explicitly encompasses several EU countries: Austria, Bulgaria, Czech Republic, Germany, Estonia, Italy, the Netherlands, and Poland. Only individual studies tied to a specific country are visually represented on the map.

To elucidate the selection process and show how the relevance of each article was determined, criteria were set for each research question. These criteria were baselines for evaluating each article's alignment with the research objectives. A comprehensive breakdown of these criteria, organized by research question, is provided in **Table 1**. Readers are directed to **Appendix A** for an in-depth mapping of the article's relevance to specific questions.



**Table 1:** Criteria for Article Relevance by Research Question

| <b>Research Question</b>   | <b>Relevance Criteria</b>  |
|--|--|
| <b>RQ1:</b> Evolution of the perception and understanding of MST and its public health implications        | <ul style="list-style-type: none"> <li>• Emphasis on MST's potential risk to public health</li> <li>• Highlighting the significance of MST to public health in the EU</li> <li>• Details regarding the prevalence of MST</li> <li>• Comprehensive understanding or novel insights into MST</li> </ul>  |
| <b>RQ2A:</b> Challenges and limitations in controlling Salmonella in pigs                                  | <ul style="list-style-type: none"> <li>• Understanding and highlighting challenges in controlling Salmonella</li> <li>• Elucidating limitations in controlling Salmonella</li> <li>• Emphasizing the significance of early detection</li> <li>• Describing barriers in implementing prevention measures</li> <li>• Evaluating available tools and their efficacy in Salmonella control</li> </ul>                              |
| <b>RQ2B:</b> Pre-harvest interventions for successful control of Salmonella                                | <ul style="list-style-type: none"> <li>• Evidence for pre-harvest interventions leading to successful (or unsuccessful) control</li> <li>• Details of pre-harvest strategies</li> <li>• Outcomes and effectiveness of these strategies</li> </ul>  |
| <b>RQ3:</b> Accuracy of current models and the role of genomics in refining Salmonella transmission models | <ul style="list-style-type: none"> <li>• Insight into the current modeling approaches</li> <li>• Evaluation of their potential limitations due to data gaps</li> <li>• The transformative potential of genomic tools in refining epidemiological models</li> <li>• Case studies or examples where genomic data refined modeling</li> <li>• Evaluation of how these refined models influence intervention strategies</li> </ul> |

## 5.2. Evolution of MST Understanding

There was constant evidence that the monophasic variant of Salmonella Typhimurium (MST) plays a significant role in public health concerns across the EU, as highlighted by recent studies. Highly virulent strains of Salmonella Typhimurium, including its monophasic variant, have been found to be prevalent in specific regions. These strains often display multi-drug resistance and a marked presence of virulence genes, indicating potential public health threats (Barilli et al., 2018). Further investigations have underscored the robust association between specific Salmonella serovars, notably MST, and the pig chain. This association again suggests that pigs are a primary contributor to human salmonellosis. A study from Northern Italy



revealed that both *Salmonella* Typhimurium (ST) and its monophasic variant were present in pig farms, and over time, MST isolations surpassed ST (D’Incau et al., 2021). Most of these strains were isolated from animals during their weaning and post-weaning periods, implying a higher susceptibility in younger pigs. The study also revealed a potential competitive advantage of MST over ST, possibly due to its differing antigenicity and pathogenicity, making it more elusive and challenging to detect and control (D’Incau et al., 2021). Furthermore, the relevance of *Salmonella*-contaminated pigs and pork products to human salmonellosis could be exacerbated due to heightened control measures in poultry (Mancin et al., 2018). Shifting the lens to a broader landscape, Marin et al. (2020) pinpointed that contamination of pig carcasses with MST primarily originates from animals before slaughter. As the production chain progresses, starting from the farm level to transportation and culminating at the slaughterhouse, the risk of *Salmonella* contamination aggravates (Marin et al., 2020). The study demonstrated a significant *Salmonella* contamination rate at both the commencement and conclusion of the slaughtering process, with MST being the predominant serovar isolated (Marin et al., 2020).

During 2018-2019, Denmark witnessed an outbreak of MST, primarily linked to raw pork sausage and various other pork products (Helmuth et al., 2019). The univariable analysis denoted a substantial association of the outbreak with raw Danish pork sausage, known as medister sausage, and the multivariable analysis affirmed only medister sausage as the significant variable (Helmuth et al., 2019). A prior study conducted in Denmark between 2013-2014, which analyzed both human and food/animal isolates revealed a consistent association of MST with pigs, emphasizing the longstanding notion that pigs serve as a crucial reservoir for human *Salmonella* infections, particularly with regard to the prominence of MST (Merlotti et al., 2020). Outbreaks involving this serovar were frequently linked with pork products across various regions. The European Union, in particular, witnessed a substantial number of human salmonellosis cases attributable to monophasic *S.* 1,4,[5],12:i:-, underscoring its influence on both human and animal health (Sun et al., 2020) This serotype's distinct epidemiological, genomic, and phenotypic features, relative to *S.* Typhimurium, further accentuate the need for precise identification techniques, considering the challenges PCR methods pose in differentiating between the two (Sun et al., 2020).

Teng et al. (2020), while offering a focused exploration of pig farms in Spain, mentioned that 37.8% of the farms in their study tested positive for *Salmonella*. Considering the higher *Salmonella* infection rates in Spanish pig farms relative to other EU countries, it becomes evident that these farms could substantially influence the incidence of human salmonellosis within Spain (Teng et al., 2020). More significantly, the study emphasized that the upward trend in the number of *Salmonella*-positive farms may correlate directly with the rising prominence and concern related to MST in the region (Teng et al., 2020). Kuus et al. (2021) detailed the landscape of *Salmonella* prevalence within the Estonian meat production chain.



At subsequent stages of meat processing, particularly at the slaughterhouse and meat-cutting levels, *S. Derby*, monophasic *S. Typhimurium*, and *S. Typhimurium* emerged as the leading serotypes, with prevalence rates of 64.7%, 9.4%, and 7.0%, respectively (Kuus et al., 2021). The growing epidemiological significance of monophasic *S. Typhimurium* in Estonia, as a cause of human cases and its prevalence in the meat chain, cannot be understated. Moreover, the study highlighted the analogous prominence of *S. Typhimurium* and its monophasic variant in neighboring Latvia's meat and meat products, underscoring a regional trend (Kuus et al., 2021).

Another study explored the nuances of outbreaks instigated by various *Salmonella* serotypes across Europe and drew attention to the profound impact of the MST sourced from pork, attributing 34% of the outbreaks to this serotype (34 %, 95 % UI: 27–42%) (Chanamé Pinedo et al., 2022). Smith et al. (2023) undertook a comprehensive risk factor analysis encompassing 250 pig farms across nine European countries to elucidate the efficacy of biosecurity measures in curtailing *Salmonella* transmission. The findings underscored the presence of *Salmonella* on more than one-third of the studied farms, with 40% of the positive samples indicating strains that are of significant concern to human health, notably *S. Typhimurium*, its monophasic variants, and *S. Enteritidis* (Smith et al., 2023). An alarming discovery was the resistance displayed by *S. Typhimurium* and its monophasic variant against various antibiotics, including ampicillin, sulfamethoxazole, streptomycin, and tetracycline. This resistance trend underscores the potential public health risk emanating from multi-drug resistance along the pork production chain (Roasto et al., 2023). Additionally, Zajac et al. (2023) presented a pivotal study detailing the sequence characteristics of a multidrug-resistant (MDR) MST strain procured from a pig fecal sample in Poland. The strain was particularly notable due to the confirmed presence of the *mcr-1.1* gene (Zajac et al., 2023).

Complementing these findings, evidence suggests a shared pool of zoonotic *Salmonella* strains between Griffon vultures and pig farms. The most predominant serotype was MST detected in 49.3% of positive samples from vultures and pig faeces from farms and carcasses disposed of in supplementary feeding stations (SFS) (Marin et al., 2018).

### **5.3. Challenges in Controlling *Salmonella* in Pigs and the Efficacy of Pre-Harvest Interventions**

#### **5.3.1. Challenges in Controlling *Salmonella* in Pigs**

Controlling *Salmonella* in pigs presents a multifaceted challenge, which has been brought to light through various research findings. One of the primary challenges is the significant variability in *Salmonella* contamination levels in pig carcasses. Some instances have displayed extremely high levels of contamination, posing difficulties



in predicting and systematically controlling the spread (Biasino et al., 2018; Bonardi et al., 2018). Further complicating control measures is the diverse distribution of *Salmonella* serovars (Mancin et al., 2018; Teng et al., 2020). Diving deeper into the intricacies of on-site farm conditions, recent surveys encompassing pig farms across Europe revealed that *Salmonella* strains of particular concern for human health were present in more than a third of them (Smith et al., 2023). Although the study did identify some biosecurity measures, such as rodent control and isolating sick pigs, that could lower the risk, the consistent presence of *Salmonella* underscores the need for more robust strategies and perhaps a reevaluation of current practices (Smith et al., 2023). Another dimension of the challenge emerges with the zoonotic nature of *Salmonella*, and the presence of shared strains between species, such as Griffon vultures and pigs, indicates a potential for bidirectional transmission (Marin et al., 2018). This interconnectedness between species introduces complexities in breaking the transmission chain and shows the need for broader control measures (Marin et al., 2018). Highlighting the transmission mechanics, Supplementary Feeding Stations (SFS) emerge as significant facilitators, emphasizing the importance of control measures in such areas (Marin et al., 2018). Other challenges arise from the wide range of risk factors associated with biosecurity, including threats from other birds, rodents, insects, water, manure, and even humans entering the piggery (Rodrigues da Costa et al., 2021). Due to their non-mandatory data submission protocols and non-harmonized sampling schemes, some studies introduce a degree of uncertainty. These limitations highlight the necessity for more standardized and comprehensive research to inform effective control strategies (Marin et al., 2018).

Another concern centers on the contamination of pig feed. Specifically, feed ingredients high in protein, such as soy and rapeseed, have been identified as susceptible to *Salmonella* contamination (Rönnqvist et al., 2018). These ingredients can act as a gateway, introducing the pathogen to feed mills, which then find their way into pig farms (Rönnqvist et al., 2018).

Despite implementing various control strategies, some regions, such as southern Italy, have seen an escalating prevalence of *Salmonella*. This rise can be attributed to factors such as the changing distribution of *Salmonella* serovars. For instance, there has been an observable increase in strains like *S. Infantis* and monophasic *S. Typhimurium*, which are frequently found in the pig production chain (Peruzy et al., 2022). Porter et al. (2020) highlighted *S. Typhimurium* as the most isolated serovar in pigs, a major human salmonellosis cause. Another concern, as illustrated by Cota et al. (2019), is the route of contamination. It was observed that the primary sites of *Salmonella* contamination in abattoirs are through the skin of the pigs, particularly in the dirty areas, underscoring the importance of rigorous cleanliness and hygiene measures even before the slaughtering process begins (Cota et al., 2019). This research underscores the importance of comprehensive investigations encompassing all stages - from pre-harvest to post-harvest (Cota et al., 2019). Hdaifeh et al. (2020) placed emphasis on how stressors, especially during





transportation and lairage, can escalate the incidence of infected pigs and pointed out that contamination rates tend to be unique to each slaughterhouse, contingent on various factors, including protocol adherence. Further in-depth studies on the significant role transportation and stay in lairage pens played in contamination were also recommended (Marin et al., 2020).

A notable finding was the increased prevalence of antimicrobial resistance in *S. Typhimurium* within the pig population, contrasted with a concurrent decrease observed in poultry (Porter et al., 2020). This could signify an intensifying problem in the pig sector, necessitating reinforced surveillance and control measures (Cota et al., 2019; Galán-Relaño et al., 2022; Porter et al., 2020). Further complicating the landscape are the inconsistencies in the monitoring program. While the poultry sector benefits from harmonized *Salmonella* control efforts, the pig production domain faces a patchwork of national monitoring programs, particularly within the European Union (Campos et al., 2019). This observation is contextualized within the framework of an expanding globalized food supply. With pork exports witnessing a surge, the interconnectedness of food sources across nations adds layers of complexity to *Salmonella* control, paving the way for emergent challenges in outbreak management (Campos et al., 2019). France is another region that witnessed a high prevalence of *Salmonella* within the pig production sector. This higher prevalence necessitates rigorous and continuous monitoring coupled with innovative control strategies (Cevallos-Almeida et al., 2019). There is a clear need for broader research, especially longitudinal ones, to provide a comprehensive understanding of *Salmonella*'s dynamics because current studies, though invaluable, often have geographical limitations, emphasizing the necessity for more encompassing research initiatives (Cevallos-Almeida et al., 2019). The absence of a national control or monitoring initiative in Spain has created a significant void in understanding the geographical spread of *Salmonella* infection in pigs (Teng et al., 2020). While some research leans on secondary data, it often faces challenges such as obsolescence or inadequate sample representation. Additionally, the lack of farm-specific data in some research, such as farm categorization and operational methods, obstructs the crafting of specific intervention strategies (Kuus et al., 2021; Teng et al., 2020). Even though the prevalence of *Salmonella* across diverse meat production sequences is acknowledged, the lack of information on Estonia's *Salmonella* oversight and control strategies renders a holistic assessment difficult. The void in the study regarding potential sources of contamination, transmission pathways, and the efficacy of prevailing control strategies further blurs the understanding (Kuus et al., 2021).

### **5.3.2. Pre-harvest Interventions for Successful Control of *Salmonella*?**

Evidence from some studies underscores the importance of pre-harvest interventions in mitigating the spread of *Salmonella* contamination. Cevallos-Almeida et al. (2019) highlight the variable ages at which *Salmonella* seroconversion occurs in pigs across





different farms. This variability points out the potential existence of farm-specific factors at play even before the pigs reach the slaughterhouse, thus suggesting that intervening at the pre-harvest step might lead to better control of the pathogen. Similarly, Cota et al. (2019) emphasize the necessity for comprehensive studies across all stages, including pre-harvest. Their findings point towards a potential link between the persistence of specific *Salmonella* serotypes in slaughterhouses and pre-harvest factors, suggesting that measures taken before harvest could be pivotal in controlling contamination levels during subsequent stages (Cota et al., 2019). The extensive colonization of pig populations by Non-Typhoidal *Salmonella* (NTS) predominantly manifests through asymptomatic carriers in regions such as the tonsils, gut, and gut-associated lymphoid tissue, and it presents a potent point of intervention (Campos et al., 2019). It is worth noting that the presence of these carriers has been identified as a paramount risk factor, facilitating the spread of the bacteria across the extensive pig production chain, particularly during the pre-harvest phase, thereby accentuating the vital importance of timely interventions (Campos et al., 2019). Another study found a correlation between the *Salmonella* status of a batch of pigs arriving at the slaughterhouse and the subsequent contamination of pork carcasses (Marin et al., 2020). Remarkably, identical strains were frequently isolated from the carcasses and the respective batch of animals upon their arrival at the slaughterhouse, amplifying the significance of pre-harvest interventions, primarily at the farm level, mitigating the *Salmonella* burden in subsequent stages of the production chain (Marin et al., 2020).

Further emphasizing the significance of these interventions, Cargnel et al. (2020) discuss the potential inadequacy of relying solely on vaccination. They propose a blend of various control measures may be more fruitful, especially considering pork's role as a primary vector for *Salmonella* infections in humans. Their study further suggests that addressing socio-economic challenges at the pre-harvest level, such as the concerns regarding mandatory vaccination programs, is essential to realize the full benefits of such interventions (Cargnel et al., 2020). Rodrigues da Costa et al. (2021) conducted a systematic review that presented key interventions such as in-feed and water treatments and vaccination as effective strategies for controlling *Salmonella*. The review highlighted that 72% of trials examining interventions like feed or water acidification, fermentation, and herbal extracts yielded positive results at the pre-harvest level, and vaccination trials boasted an 88% success rate (Rodrigues da Costa et al., 2021). Despite these promising results, the enduring endemic nature of *Salmonella* infections in global pig herds underscores the challenges in the pathogen control (Rodrigues da Costa et al., 2021). However, countries like Finland, Norway, and Sweden have documented the viability of pre-harvest interventions in successfully controlling *Salmonella* across various livestock (Rodrigues da Costa et al., 2021). The findings underscore that a preventive approach, focusing on high herd health, effective management, and stringent biosecurity, is more promising than reactive control measures in combating this



pathogen at the pre-harvest level (Rodrigues da Costa et al., 2021). In a recent study, Mu et al. (2023) focused on resilience strategies addressing Salmonella risks within the pork supply chain. The research emphasized that for supply chains with reduced risk profiles, enhancing the resilience of pigs to Salmonella infection during the farm phase is essential (Mu et al., 2023). Implementing these interventions during the pre-harvest stages serves as vital safeguards against potential challenges to food safety in subsequent stages of the supply chain.

#### **5.4. Evaluation of Model Accuracy in Reflecting Salmonella Transmission from Pigs to Humans in Europe: The Role of Genomic Studies in Model Refinement**

This section describes the accuracy of models, with emphasis on the influence of genomic studies in refining these models, showcasing their critical role in aligning with real-world transmission dynamics. Researchers have faced challenges in attempting to unravel the complexities surrounding MST, mainly due to its monomorphic nature. Such challenges obscure the precise identification of its sources and evolutionary dynamics when relying on traditional typing methods. Nevertheless, the advent of genomic methods including cgMLST (core genome multilocus sequence typing) holds promise, allowing for in-depth insights into the pathogen's population structure and transmission chains (Palma et al., 2018).

As Mancin et al. (2018) observed, while *S. Typhimurium* exhibited a prevalent distribution across various animal species, specific serovars, notably *S. 4,[5],12,i:-* (MST) and *S. Derby*, demonstrated a stronger association with pigs. This distinct distribution of serovars underscores the importance of a nuanced approach to epidemiological studies, suggesting that current models may only partially capture the complexity of Salmonella epidemiology in real-world scenarios. Nevertheless, it is not merely the distribution of serovars that presents challenges but the nature of the bacteria, particularly MST, which adds another layer of complexity (Sun et al., 2020). Palma et al. (2018) emphasize that the inherent challenges posed by the monomorphic nature of the pathogen render traditional typing methods ineffective. Such methods often need to be more exact in accurately tracing the source and understanding the intricate transmission dynamics of these serovars, further casting doubt on the efficacy of prevailing models.

However, amidst these challenges, there is a positive aspect: the growing importance of genomic studies in refining these models. The exponential growth of public databases encompassing Salmonella genomes is signaling a significant transition, leading to epidemiological models that are more grounded in extensive genomic data. Genomic methods offer practical value beyond theory. A retrospective study by Simon et al. (2018) on the *S. Derby* outbreak in Germany during 2013-2014 highlighted the strengths of Whole Genome Sequencing (WGS). Compared to traditional methods (e.g., serotyping, pulsed-field gel electrophoresis), WGS offers more precise results and has an exceptional role in tracing intricate clusters in



extensive sequence datasets, underscoring its potential for broader use (Campos et al., 2019). Despite the challenges that genomic methods face, including the exigencies of international standardization and the financial implications of sequencing, it is undeniable that these tools are revolutionizing our understanding (Simon et al., 2018). They promise to refine epidemiological models, making them more reflective of real-world scenarios and better equipped to guide interventions. Understanding the complexities of Salmonella transmission from pigs to humans within the EU presents a significant challenge. The research underscores that while current models, grounded in traditional typing methods, have their merits, they often need to catch up in capturing the full breadth and depth of real-world epidemiology. As noted by Field Palma et al. (2018) and Field Simon et al. (2018), the integration of genomic tools suggests the potential for improved and practical models. These tools offer enhanced accuracy and are becoming pivotal in Salmonella transmission research, highlighting a shift in the current approach to Salmonella control within the EU. An exemplary use of WGS in national surveillance was observed during the 2018 MST outbreak in Denmark. The analysis revealed the outbreak's source as raw pork products, emphasizing the clear transmission from pigs to humans (Helmuth et al., 2019). Looking ahead, the potential for refining source attribution models to WGS data could be a sign of transformative change, providing pathways for ultra-precise backtracking and facilitating targeted interventions (Cevallos-Almeida et al., 2019). Another research by Merlotti et al. (2020) honed in on a network-based approach, aiming to source attribute Salmonella Typhimurium and its monophasic variant via whole-genome data. Focusing on Danish human and food/animal isolates from 2013-2014, the study assessed the cohesiveness of source clustering based on varying genetic distances, from SNP (Single Nucleotide Polymorphism) and cgMLST to wgMLST (whole genome multilocus sequence typing)(Merlotti et al., 2020). The weighted network approach was pivotal, especially when considering animal species, country of origin, and collection year as potential clustering drivers. When human isolates entered the network, most were attributed to pigs, in line with historical data associating the serovar *S. Typhimurium* and its variant predominantly with pigs (Merlotti et al., 2020). Mughini-Gras et al. (2020) embarked on a comprehensive exploration of invasive Non-Typhoidal Salmonella (iNTS) infections in the Netherlands, cataloging dominant serotypes, trends, and risk factors. Their findings have the potential to refine existing epidemiological models in the context of the EU. By spotlighting surveillance data dependencies and the increasing need for molecular screening methods, the study stressed genomic studies' transformative potential. Sévellec et al. (2020) underscored the prevalence of *S. Derby* in France, particularly pinpointing pigs and poultry as its primary animal hosts. The research delineated that two pork-associated genetic lineages were accountable for 94% of human contaminations, specifically, ST40 was linked to 71% of human cases (Sévellec et al., 2020). This research identified four genomic clusters from pork and poultry in France were not uniformly present in human clinical strains, a pattern replicated in other European nations. Another case in France, which encountered a multi-serotype



outbreak of *Salmonella Bovismorbificans* ST142 and monophasic *Salmonella* Typhimurium ST34 linked to dried pork sausages, underscored the challenges posed by multi-serotype detection (Pardos de la Gandara et al., 2023). A concerning discovery in Poland was the emergence of a multidrug-resistant *Salmonella* strain, emphasizing the importance of genomic data in understanding transmission patterns (Zajac et al., 2023).

While WGS offers promising insights, it poses challenges, especially for smaller institutions, Gand et al. (2020) underscoring the cost-efficiency of the genosertotyping system. While some EU member states are leveraging WGS for insights, it is noteworthy that Estonia has only recently integrated it systematically, and other states have yet to adopt it all (Kuus et al., 2021). Interestingly, some argue that the uptick in *Salmonella* outbreaks may not necessarily indicate an actual increase in exposure but could reflect improved detection methods (Chanamé Pinedo et al., 2022).

## **6. Discussion**

This scoping review summarized studies that offer valuable insights into the complex dynamics of the monophasic variant of *Salmonella* Typhimurium, the emerging challenges in controlling *Salmonella*, and the potential of pre-harvest interventions in the pig sector. Furthermore, the review showed the indispensable contribution of genomic studies in refining the current models. In assessing the scope of this literature review, a striking observation emerges: a notable absence of country-specific articles from 15 EU Member States. This gap not only suggests potential research voids in these countries but also impacts the comprehensiveness of the EU-wide understanding. In essence, a more holistic representation is needed for effective decision-making and strategizing across the entire EU.

Current literature revealed a pronounced shift in the perception of MST, particularly in light of findings that elucidate its high virulence and frequent multi-drug resistance (Barilli et al., 2018). The consistent association of MST with the pig supply chain underscores the evolution in understanding its epidemiological significance. D’Incau et al. (2021) underscore that MST, once deemed a sporadic variant, has now asserted its prevalence, often surpassing other *Salmonella* strains in pig farm environments. The recurrent MST-related outbreaks, particularly those reported in Denmark, Italy, and Spain have refocused attention on the consumption risks affiliated with pork, underscoring the imperative to revise and reinforce public health advisories about raw pork products (Helmuth et al., 2019; Marin et al., 2020; Palma et al., 2018; Teng et al., 2020). Furthermore, from a regional perspective, the nuanced variation of MST prevalence between specific geographies - most notably the pronounced disparities observed between Spanish pig farms and their counterparts in other EU territories - demands a recalibrated, region-tailored approach to intervention. Dedicated interdisciplinary teams equipped with specialized training and resources could offer timely interventions during outbreaks and maintain routine oversight through



consistent inspections and audits. The rising prominence of MST in areas like Estonia and Latvia accentuates the fluidity of regional dynamics and underscores the need for tailored interventions (Kuus et al., 2021). The discovery of antibiotic-resistant MST strains, like the one in Poland with the *mcr-1.1* gene, demands not only recognition but also a thorough reassessment of the risk posed by the monophasic variant, along with adjusted response strategies (Zajac et al., 2023). Smith et al. (2023) detailed risk factor analysis revealed that current biosecurity measures may not be as effective against MST as previously assumed. Consequently, there is a need to investigate alternative interventions, such as phage therapy, to strengthen the approach against MST's proliferation. The shared *Salmonella* strains between Griffon vultures and pig farms underscore the need for an integrated One Health approach. This holistic strategy, encapsulating human, animal, and environmental health paradigms, could provide a comprehensive framework for monitoring, controlling, and preventing interspecies transmission of infectious diseases.

The endeavor to control *Salmonella* in pigs within the EU has encountered multifaceted challenges. Some of them were the marked variability in *Salmonella* contamination levels on pig carcasses, the diverse and changing distribution of *Salmonella* serovars, the contamination of pig feed, the need for enhanced surveillance and control efforts (Biasino et al., 2018; Bonardi et al., 2018; Galán-Relaño et al., 2022; Peruzy et al., 2022; Porter et al., 2020). Given the persistent presence of *Salmonella* strains on European pig farms, it is vital to revisit and strengthen current biosecurity measures. This may involve training programs for farm workers, stricter quarantine measures for infected farms, and rigorous checks on farm infrastructure. Through periodic consultations, training sessions, and collaborative research projects, we can engage with the pork industry more actively to ensure that they are aligned with the latest research findings and are implementing recommended best practices. Finally, while standardized monitoring protocols exist within the EU, it is essential to continually review and update these based on emerging data and challenges. Regular assessment will ensure they remain effective in capturing the dynamics of *Salmonella* distribution and prevalence across different regions and contexts.

Research also illustrated a potential link between specific *Salmonella* serotypes persisting in slaughterhouses and pre-harvest factors, emphasizing the crucial role pre-harvest measures might play in controlling contamination during subsequent production stages (Cota et al., 2019). A direct correlation was observed between the *Salmonella* status of a pig batch upon arrival at the slaughterhouse and the subsequent contamination of pork carcasses, reiterating the significance of interventions, primarily at the farm level (Marin et al., 2020). While vaccination could be an option, combining various control measures (feed or water acidification, fermentation, and herbal extracts) might offer more promising outcomes (Cargnel et al., 2020; Rodrigues da Costa et al., 2021). Countries such as Finland, Norway, and Sweden provided empirical evidence of the effectiveness of a preventive approach,



emphasizing herd health, effective management, and robust biosecurity over-reactive measures (Rodrigues da Costa et al., 2021). Resilience strategies, especially those enhancing pig resistance to *Salmonella* infection during the farm phase, have also been highlighted as pivotal for safeguarding food safety in subsequent supply chain stages (Mu et al., 2023). Despite the evident surge in interest surrounding pre-harvest interventions in the context of *Salmonella* control in pigs, it remains apparent that there is a substantial gap in research directed explicitly at this phase. Placing more emphasis on these stages is instrumental, as it promises to provide a comprehensive insight into the *Salmonella* life cycle within pig production. Such an understanding might empower stakeholders with the necessary knowledge, facilitating the development of more precisely tailored interventions and helping to manage this persistent challenge.

Traditional epidemiological models, while pivotal, may only partially embrace the nuanced transmission dynamics of *Salmonella*. Genomic studies, with techniques like WGS, cgMLST, and wgMLST are reshaping the comprehension of *Salmonella*'s dynamics. The promise of these techniques lies not just in their theoretical relevance but also in their practical implications. Retrospective examinations of outbreaks, as seen in Germany with *S. Derby* or the MST outbreak in Denmark, showed how WGS can help identify transmission sources and how to effectively address them (González-Santamarina et al., 2021; Merlotti et al., 2020). However, the advancement of genomic techniques introduces some challenges. The integration of such tools is uneven across the EU, with disparities in accessibility and adoption evident across member states. Financial constraints, differing priorities, infrastructure limitations, awareness gaps, and concerns about cost-efficiency, especially for smaller institutions, pose barriers to the universal embrace of these methods. As explained by Chanamé Pinedo et al. (2022), improved detection methods might show increased *Salmonella* outbreaks. However, he asks: is this truly reflective of heightened exposure, or merely an artifact of refined detection? As the field advances, fostering research and innovation in both traditional methods and genomic-informed strategies remains crucial to sculpt effective, evidence-based interventions.





## 7. Conclusion

In conclusion, this scoping review aimed to clarify the public health implications of the monophasic variant of *Salmonella* Typhimurium (MST) within the European Union. Additionally, it delved into the challenges and strategies pertinent to the control of *Salmonella* in the pig industry, emphasizing both the contributions and challenges posed by genomic studies. The review highlights the necessity for focused future research on areas requiring immediate intervention. Embracing a **One Health** approach, which acknowledges the intertwined nature of human, animal, and environmental health, emerges as a promising strategy for addressing these challenges in the forthcoming years.



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**Appendix A: Detailed Summary Table of Reviewed Literature**

| <b>Author(s)</b>      | <b>Year</b> | <b>Country/<br/>Region</b> | <b>Title</b>   | <b>Aim of the Study</b>  | <b>Main Findings/Contributions</b>   | <b>Identified gaps</b>  | <b>Relevance to Research Questions</b> |
|-----------------------|-------------|----------------------------|--|--|--|---|--|
| <b>Barilli et al.</b> | 2018        | Italy                      | <i>Antimicrobial resistance, biofilm synthesis and virulence genes in salmonella isolated from pigs bred on intensive farms</i>                    | to evaluate the virulence patterns of 11 Salmonella isolates from pigs bred in intensive farms located in Northern Italy   | Presence of highly virulent Salmonella Typhimurium and its monophasic variant in that region, many with multi-drug resistance and a high presence of virulence-genes   | A need of continuous monitoring and further evaluation of circulating <i>Salmonella strains, especially concerning their virulence patterns</i> | <b>RQ1</b>                             |
| <b>Biasino et al.</b> | 2018        | Belgium                    | <i>Correlation between slaughter practices and the distribution of Salmonella and hygiene indicator bacteria on pig carcasses during slaughter</i> | to map the distribution of the microbiological contamination (hygiene indicators and Salmonella) on pig carcasses and to investigate the potential use of aerobic bacteria and Enterobacteriaceae as indicators for the presence of Salmonella | Positive correlations were found between hygiene indicators and Salmonella presence at several carcass areas. Some slaughter practices, such as splitting the head and incising tonsils, were linked to higher bacterial contamination | A need for more studies providing detailed mapping of bacterial contamination on pig carcasses in various contexts                              | <b>RQ2A</b>                            |



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| <p><b>Bonardi et al.</b></p> | <p>2018</p> | <p>Italy</p> | <p><i>Salmonella Detection and Counting on Pig Carcasses and Cutting Lines in Italian Slaughterhouses</i></p> | <p>To detect Salmonella on pig carcasses and food contact surfaces in three Italian slaughterhouses</p>   | <p>A) Prevalence of Salmonella contamination on carcasses is 12.3% in slaughterhouses A and B, and 11.2% in C. B) Contamination is critically dependent on the day of sampling, with high intraday clustering. C) There's a non-negligible risk of occasional downstream food-chain contaminations, implying potential consumer infections</p>  | <p>A need for consistent sampling methods across slaughterhouses and an exploration into the causes of intraday clustering</p>  | <p><b>RQ2A</b></p>           |
| <p><b>Mancin et al.</b></p>  | <p>2018</p> | <p>Italy</p> | <p><i>Salmonella serovar distribution from non-human sources in Italy</i></p>                                 | <p>to describe the distribution frequency of different Salmonella serovars from pig origin, and the link between those serovars and human infections in Italy</p> | <p>The study demonstrates a significant association between certain Salmonella serovars (e.g., S. Typhimurium, S. 4,[5]:i:-) and the pig chain. There's a clear indication that pigs are a primary source of Salmonella contributing to human salmonellosis in Italy. The importance of Salmonella-contaminated pigs and pork products in human salmonellosis has possibly increased due to control measures in poultry</p> | <p>A) Non-mandatory data transmission from participating laboratories<br/>B) Non-structured and non-harmonized sampling schemes, leading to uncertainty in the findings<br/>C) Absence of data for some species and sources not covered by structured surveillance programs</p> | <p><b>RQ1, RQ2A, RQ3</b></p> |



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| Palma et al. | 2018 | Italy | <i>Genome-wide identification of geographical segregated genetic markers in Salmonella enterica serovar Typhimurium variant 4,[5],12:i:-</i> | Evolution dynamics, source attribution, and epidemiology of the monophasic variant of S. Typhimurium | The study underscores the challenges posed by MST due to its monomorphic nature, making it difficult to identify its source and its evolutionary dynamics using traditional typing methods. The importance of genomic methods, like cgMLST and SNP-based phylogenies, is highlighted. These methods, especially when coupled with the growing databases of Salmonella genomes, can provide deeper insights into the population structure and transmission chains of this pathogen | The technical challenges and potential misclassifications associated with traditional serotyping methods. The need for a more advanced understanding using genomic tools | RQ1, RQ3 |
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| Simon et al. | 2018 | Germany | <i>Evaluation of WGS based approaches for investigating a food-borne outbreak caused by Salmonella enterica serovar Derby in Germany</i> | Centers on the evaluation of Whole Genome Sequencing (WGS) and its associated bioinformatics approaches in investigating a food-borne outbreak caused by Salmonella Derby in Germany, and contrasting these with conventional typing methods | A) S. Derby linked to a major outbreak via raw pork sausage.<br>B) WGS confirmed and surpassed conventional methods in precision.<br>C) Both SNP and cgMLST were suitable for cluster definition | A) The cost of WGS, especially library preparation and sequencing reagents, is still high. B) The need for extensive computing and storage capacities due to the large volume of data generated. C) Lack of international standards and protocols for WGS in public health contexts, making inter-laboratory collaborations and communications challenging. D) The study had a limited scope, including only a few sporadic cases and animal isolates, potentially leading to a selection bias. E) Ambiguity remains around how many SNPs or allelic differences are necessary to define clusters, with no consensus reached yet | RQ3 |
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| Marin et al. | 2018 | Spain | <i>Supplementary feeding stations for conservation of vultures could be an important source of monophasic Salmonella typhimurium 1,4,[5],12:i</i> | To determine whether free-living griffon vultures and pig farms share zoonotic Salmonella strains, particularly monophasic S. typhimurium 1,4,[5],12:i-, and to assess the potential transmission and cross-infection of Salmonella between these two populations | <p>A) Griffon vultures and pig farms share several zoonotic Salmonella strains, including MST.</p> <p>B) MST occurrence suggests potential bidirectional transmission between vultures and pig farms.</p> <p>C) Genetic analysis shows MST strains from both sources have high genetic homogeneity, indicating a shared infection source.</p> <p>D) Supplementary feeding stations (SFS) play a key role in Salmonella transmission, emphasizing the need for control measures.</p> <p>E) Identification of MST transmission between these sources raises public health concerns and potential for foodborne illness</p> | Absence of detailed exploration of Salmonella transmission mechanisms between pig farms and vultures at SFS. No specific recommendations provided for measures to minimize Salmonella transmission at SFS | RQ1, RQ2A |
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| Rönnqvist<br>et al. | 2018 | Finland | <i>Salmonella risk to consumers via pork is related to the Salmonella prevalence in pig feed</i> | Understanding the risk of Salmonella contamination in pork in relation to the prevalence of Salmonella in pig feed | A) Pigs are a crucial source of Salmonella infections in humans. Feed can be a potential Salmonella source in countries with low prevalence. B) Feed ingredients like soy & rapeseed, often contaminated with Salmonella, can introduce it to feed mills and pig farms. C) Using low-risk and controlled feed ingredients reduces pig contamination and aids in tracing and controlling Salmonella sources. D) Bayesian methods can assess Salmonella risk throughout the pork chain, even with data uncertainties | A) Gaps in prevalence data throughout the pork production chain. B) Limited time-series data due to scarce findings. C) The study's data is from 2013 and 2014, suggesting a gap in more recent data. D) The within-herd spread of Salmonella in pigs was not studied | RQ2A |
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| <p><b>Campos et al.</b></p>           | <p>2019</p> | <p>global, includes Europe</p> | <p><i>Non-typhoidal Salmonella in the Pig Production Chain: A Comprehensive Analysis of Its Impact on Human Health</i></p> | <p>Analysis of Non-typhoidal Salmonella (NTS) in pig production &amp; its impact on human health; exploration of WGS as a tool for investigation</p>  | <p>A) Whole Genome Sequencing (WGS) is emerging as a powerful tool for real-time outbreak investigations and surveillance. B) Salmonella control programs differ between poultry (harmonized) and pigs (each EU member state has its own specific program). C) Globalization of food supply, especially pork, might cause challenges in controlling salmonellosis. D) NTS colonization of pigs is frequent, usually resulting in asymptomatic carriers, with both horizontal and vertical transmission routes in the pig production chain</p> | <p>The study suggests a need to review and compare the situation in subsequent years (2021/2022/2023) regarding the globalization of food supply and its implications</p>  | <p><b>RQ2A, RQ2B, RQ3</b></p> |
| <p><b>Cevallos-Almeida et al.</b></p> | <p>2019</p> | <p>France</p>                  | <p><i>Longitudinal study describing time to Salmonella seroconversion in piglets on three farrow-to-finish farms</i></p>   | <p>Longitudinal study of Salmonella seroconversion timing in piglets across three farrow-to-finish farms. Monitoring of pigs from one week of age to slaughter, with serological tests like ELISA to detect Salmonella antibodies</p> | <p>A) Age of Salmonella seroconversion ranged from 4 to 10 weeks across farms. B) Pigs in the French industry have a high prevalence of Salmonella and are a major reservoir. C) Farm practices, biosecurity, and presence of other animals are influencing factors for seroconversion</p>  | <p>A) Further research is required for larger sample sizes and inclusive of fecal sample bacteriological analysis. B) A noticeable gap exists in longitudinal studies on Salmonella infection dynamics in pig herds. C) The study's scope, being limited to three farms in Brittany, suggests a need for broader geographical coverage</p> | <p><b>RQ2B, RQ3</b></p>       |



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| Cota et al.    | 2019 | Portugal | <i>Pheno and genotyping of Salmonella from slaughtered pigs in a Portuguese abattoir reveal differential persistence ability</i>  | To understand the persistence ability of Salmonella in the slaughterhouse environment and its impact on the dissemination of resistant and virulent strains | A) Main contamination via pig's skin. B ) Common serotypes: Salmonella 4,[5],12:i:-, S. Rissen, S. Derby. C) 79.5% of isolates multidrug-resistant. D) All isolates had key virulence genes. E). Some isolates from different farms were genetically identical. F) S. Rissen might be more persistent in abattoirs   | A) Need for broader research on Salmonella in Portugal. B) No detailed corrective measures provided. C) No exploration of pre-harvest contamination sources or impact of varied hygiene practices. D) Unexamined factors: temperature, humidity, and broader public health implications | RQ2A, RQ2B, RQ3 |
| Helmuth et al. | 2019 | Denmark  | <i>An outbreak of monophasic Salmonella Typhimurium associated with raw pork sausage and other pork products, Denmark 2018–19</i> | to identify the source and control an outbreak of monophasic Salmonella Typhimurium in Denmark in 2018-19   | A) Outbreak of monophasic Salmonella Typhimurium in 2018-19 linked to raw pork products. B) Medister sausage remained significant in multivariable analysis as contamination source. C) Consumption of raw or undercooked sausage identified. D) Despite efforts, Denmark still at risk of major salmonella outbreaks. 5. Salmonella Typhimurium still exists in Danish pork with potential outbreak risks | A) Unclear contamination mechanism. B) No breaches in procedures identified to explain prolonged contamination. C) No link between outbreak and specific supermarket chain. D) Time gaps in stool collection, WGS, and case linking not discussed                                       | RQ1, RQ2A, RQ3  |



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| <b>Cargnel et al.</b> | 2020 | Belgium | <i>Combining quantitative and qualitative approaches to determine viability of a potential Salmonella Typhimurium vaccination program in pigs in Belgium</i> | to determine the viability of a potential Salmonella Typhimurium (ST) vaccination program in pigs in Belgium | A) Vaccination alone insufficient for reducing human salmonellosis from ST. B) Pork meat main salmonellosis source in Belgium. C) Concerns in pig sector about mandatory ST vaccination. D) Holistic approach with harmonized communication recommended | A) Need for comprehensive food chain approach. B) Lack of solutions for pig sector's concerns. C) Applicability of findings outside Belgium | <b>RQ2A,<br/>RQ2B</b> |
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| Gand et al. | 2020 | Belgium | <i>A genosertyping system for a fast and objective identification of Salmonella serotypes commonly isolated from poultry and pork food sectors in Belgium</i> | to develop a genosertyping system for the rapid and objective identification of Salmonella serotypes commonly isolated from the poultry and pork food sectors in Belgium | A) Genosertyping system using MOL-PCR and Luminex technology was developed for rapid identification of common Salmonella serotypes in Belgium's poultry and pork sectors. B) Three additional assays were developed for detecting 13 invasive serotypes. C) A web-based DSS was created for the automatic and objective interpretation of the Luminex data. C) Genosertyping system aims to reduce human food poisoning and respond quickly to outbreaks caused by invasive serotypes. D) Genosertyping system offers a rapid, cost-effective, and accurate method for Salmonella serotype identification in Belgium's poultry and pork sectors. E) WGS is time-consuming and expensive for smaller institutions | A) The study doesn't explicitly state the identified gaps but does imply a need for a faster, more cost-effective method for Salmonella serotype identification. B) The study indicates WGS's limitations as time-consuming and potentially cost-prohibitive for some institutions | RQ2A, RQ2B, RQ3 |
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| <p>Hdaifeh et al.</p> | <p>2020</p> | <p>EU</p>    | <p><i>Critical Analysis of Pork QMRA Focusing on Slaughterhouses: Lessons from the Past and Future Trends</i></p>   | <p>to conduct a critical analysis of pork quantitative microbial risk assessment (QMRA) with a specific focus on slaughterhouses, to review the existing literature, identify gaps, and guide future research activities</p> | <p>A) 29 studies focused on Salmonella spp. in pork QMRA, especially impacts at the slaughterhouse. B) Importance of good hygiene practices in slaughterhouses emphasized. C) Potential risk mitigation pathways identified for each slaughterhouse step. D) Emphasis on a comprehensive farm-to-fork approach for better bacteria control. E) Transportation and lairage as critical stages where prevalence of infection might increase due to stress, animal mixing, and other factors</p>  | <p>A) Need for further research on factors influencing contamination events in pork meat. B) Necessity for QMRAs covering the entire farm-to-fork chain for a holistic view on mitigation possibilities</p>  | <p>RQ2A, RQ2B</p>      |
| <p>Marin et al.</p>   | <p>2020</p> | <p>Spain</p> | <p><i>Contamination of pig carcass with Salmonella enterica serovar Typhimurium monophasic variant 1,4[5], 12:i:- originates mainly in live animals</i></p> | <p>to investigate the contamination of pig carcasses with Salmonella enterica serovar Typhimurium monophasic variant 1,4 [5], 12: i:- and determine its origin</p>   | <p>A) Contamination of pig carcasses with MST mainly originates from alive animals. B) Risk of contamination increases across the production chain - from farm, through transportation, to slaughterhouse &amp; further processing. C) High contamination level at slaughterhouse arrival &amp; post-slaughter (71.4% &amp; 66.7% respectively) with mST as the predominant serovar. D) Carcasses had reduced Salmonella positives, but a similar MST rate compared to faeces &amp; caeca samples. Whips had a high proportion of positive samples but lower MST frequency</p> | <p>A) Need to study the impact of transport &amp; stay in lairage pens more deeply due to their dual role. B) Study's geographical limitation (Valencian Region) might limit generalizability. C) Lack of information on sample size or representativeness from various slaughterhouse points. D) Absence of discussion on farm-level control measures &amp; their effectiveness. E) No consideration of other factors (e.g., hygiene practices, transportation) impacting carcass contamination</p> | <p>RQ1, RQ2A, RQ2B</p> |



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| <p><b>Merlotti et al.</b></p>     | <p>2020</p> | <p>Denmark</p>         | <p><i>Network Approach to Source Attribution of Salmonella enterica Serovar Typhimurium and Its Monophasic Variant</i></p>     | <p>to evaluate a network-based approach for source attribution of Salmonella enterica serovar Typhimurium and its monophasic variant using whole-genome data</p>   | <p>A) Application of a weighted network approach to source attribution with whole-genome data. B) Animal source was primary driver of clustering. C) Majority of human isolates were attributed to pigs, then broilers, ducks, cattle, and layers. D) Promising outcomes for source attribution studies using this approach. E) Pigs were a major source of human Salmonella infections</p>  | <p>A) Specific dataset and timeframe limit generalizability. B) Study focused only on Salmonella enterica serovar Typhimurium and its monophasic variant. C) Exclusion of outbreak data. D) Short timeframe (two years). E) Further studies on different datasets needed to confirm the reliability of this approach for Salmonella source attribution</p>  | <p><b>RQ1, RQ2A, RQ3</b></p> |
| <p><b>Mughini-Gras et al.</b></p> | <p>2020</p> | <p>the Netherlands</p> | <p><i>Changing epidemiology of invasive non-typhoid Salmonella infection: a nationwide population-based registry study</i></p> | <p>to investigate the changing epidemiology of invasive non-typhoid Salmonella (iNTS) infection in a high-income setting, specifically in the Netherlands. The study aims to determine the trends, risk factors, serotype distribution, antimicrobial resistance (AMR), and attributable sources of iNTS infection</p> | <p>A) Increase in iNTS infections since 2012, with a 4.6% annual average. B) Identified risk factors: wintertime, male sex, older age, living in rural areas. C) Serotypes Dublin, Panama, Poona showed highest invasiveness relative to their occurrence. D) Cattle were identified as a larger source for iNTS than for non-iNTS. E) AMR rates were lower for iNTS isolates than non-iNTS isolates. F) The reasons for the increase in iNTS are multifactorial</p> | <p>A) Reliance on surveillance data might lead to underreporting. B) Lack of information on potential factors influencing iNTS risk like travel, ethnicity, immunocompetence, comorbidities. C) Definitions used may not capture all clinical outcomes of iNTS. D) Findings may not be generalizable to other high-income countries. E) Study did not incorporate molecular screening methods</p> | <p><b>RQ2A, RQ3</b></p>      |



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| Porter et al.   | 2020 | Ireland | <i>Trends in Salmonella serovars and antimicrobial resistance in pigs and poultry in Northern Ireland between 1997 and 2016</i> | to assess the effectiveness of control measures and surveillance systems in addressing public health concerns related to salmonellosis   | A) S. Typhimurium was the most isolated serovar in pigs and is a significant cause of human salmonellosis. B) A decrease in antimicrobial resistance was noted in poultry, but an increase was observed in pigs. C) The study emphasizes the role of ongoing surveillance in evaluating control strategies   | The study had several limitations, including a lack of control over samples submitted, absence of denominator data, and potential non-representativeness due to sampling from symptomatic pigs | RQ2A |
| Sévellec et al. | 2020 | France  | <i>(!)Source Attribution Study of Sporadic Salmonella Derby Cases in France</i>   | to explain the spread and sporadic pattern of human salmonellosis cases that occurred during the studied period by analyzing epidemiologic data and the structure of the pork sector in France | A) Identified S. Derby as a frequent serovar in humans in France, primarily sourced from pigs and poultry. B) Two genetic lineages from pork strains were responsible for 94% of human contaminations. C) The ST40 profile was the main contributor, responsible for 71% of human cases. D) The study shed light on the epidemiological pattern and the structure of the pork sector in France in relation to S. Derby contamination | The study didn't explicitly mention any identified limitations or gaps concerning S. Derby source attribution in France  | RQ3  |





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| <p><b>Sun et al.</b></p>  | <p>2020</p> | <p>global,<br/>includes<br/>Europe</p> | <p><i>The Epidemiology of Monophasic Salmonella Typhimurium</i></p>   | <p>to investigate the epidemiological and genetic characteristics, mechanisms of antibiotic resistance, and the evolution path and bacterial adaptation of the monophasic variant of Salmonella Typhimurium (S. 1,4,[5],12:i:-)</p> | <p>A) S. 1,4,[5],12:i:- has a strong link to swine, contributing to its global spread and impact on public health. B) WGS has revealed its phylogenetic associations and AMR genes, indicating the zoonotic pathogen's role in AMR spread. C) The variant has been linked to pork products outbreaks across different regions in Europe. D)The serotype has distinct features from S. Typhimurium and there's a need for accurate identification methods. Heavy metal tolerance might play a role in its prevalence</p> | <p>A) Comprehensive and accurate identification method for S. 1,4,[5],12:i:- is needed. B) More understanding of the serotype's epidemiology, transmission dynamics, reservoirs, and sources. CO Effectiveness of control measures and interventions. D) Further study on the association among different S. 1,4,[5],12:i:- clones, and its evolution and transmission path</p>   | <p><b>RQ1, RQ3</b></p>       |
| <p><b>Teng et al.</b></p> | <p>2020</p> | <p>Spain</p>                           | <p><i>Spatial trends in salmonella infection in pigs in Spain</i></p> | <p>to report the spatial distribution of Salmonella-positive pig farms in Spain and investigate the presence of potential spatial trends over a 17-year period</p>  | <p>A) Spain has a high proportion (37.8%) of Salmonella-positive pig farms, particularly in the East and Northeast. The country has a rising trend of Salmonella-positive farms, correlating with increasing presence of S. 1,4,[5],12:i:-. B) Pigs could play a significant role in human salmonellosis. C) Prevalence and diversity of Salmonella serotypes could increase from farm to abattoir due to various factors including stress and exposure to contaminated environments</p>                                | <p>A) Lack of a national Salmonella monitoring program for pigs. B) Limited data on spatial-temporal distribution and evolution of Salmonella in Spanish pigs. C) Limitations of the study include using secondary data, potential non-representative sampling from large abattoirs, unavailability of within-farm prevalence and farm-specific data, and potential data quality issues related to pig distribution averaging and sampling procedures</p> | <p><b>RQ1, RQ2A, RQ3</b></p> |



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| <p>D'Incau et al.</p> | <p>2021</p> | <p>Italy</p>   | <p><i>Occurrence of Salmonella Typhimurium and its monophasic variant (4, [5], 12:i:-) in healthy and clinically ill pigs in northern Italy</i></p> | <p>to describe and evaluate the occurrence of Salmonella Typhimurium (ST) and its monophasic variant Salmonella enterica subsp. enterica 4, [5], 12:i:- (MST) in pigs and its association with clinical conditions</p> | <p>A) Salmonella Typhimurium (ST) and its monophasic variant (MST) were identified in pig farms, with a higher presence during the weaning and growing period, suggesting younger pigs are more susceptible. B) Over time, MST surpassed ST in prevalence. MST may have a competitive advantage over ST, possibly due to differences in antigenicity and pathogenicity. C) Clinical cases were mainly seen in young pigs post-weaning. Multiple phage types were identified, with DT193 most frequent for MST and U302 for ST</p> | <p>Gaps include lack of data on specific reasons for MST's rising prevalence over ST, unexplored potential contamination sources and transmission routes, no genetic analysis or virulence factor assessment of MST and ST strains, absence of impact evaluation on public health and no investigation on control measure effectiveness. The study also noted it is not directly comparable to others due to its clinical sampling method</p> | <p>RQ1, RQ2A, RQ3</p> |
| <p>Kuus et al.</p>    | <p>2021</p> | <p>Estonia</p> | <p><i>Prevalence and Serotype Diversity of Salmonella enterica in the Estonian Meat Production Chain in 2016–2020</i></p>                           | <p>to determine the prevalence and serotype diversity of Salmonella enterica in the Estonian meat production chain from 2016 to 2020, as well as to provide data on human salmonellosis during the same period</p>     | <p>A) The prevalence of Salmonella varies across animal species in Estonia with fattening pigs showing the highest prevalence. B) At the slaughter and meat cutting levels, the top serotypes are S. Derby, monophasic S. Typhimurium, and S. Typhimurium. C) These serotypes also correlate with the top serotypes causing human infections, with S. Enteritidis being the leading cause. However, monophasic S. Typhimurium's significance is rising both in the meat chain and human cases</p>                                 | <p>Lack of information on: control measures for Salmonella, factors influencing Salmonella prevalence in animals, potential contamination sources or transmission routes, effectiveness of current control measures, antimicrobial resistance patterns, public health implications of Salmonella contamination, economic implications, and specific interventions or recommendations for Salmonella control</p>                               | <p>RQ1, RQ2A, RQ3</p> |



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| <p><b>Da Costa et al.</b></p>       | <p>2021</p> | <p>EU</p>     | <p><i>A Systematic Review on the Effectiveness of Pre-Harvest Meat Safety Interventions in Pig Herds to Control Salmonella and Other Foodborne Pathogens</i></p> | <p>to assess the effectiveness of pre-harvest interventions in pig herds to control foodborne pathogens, specifically focusing on Salmonella and other relevant pathogens in pork in the European Union</p> | <p>The review analyzed 52 studies on pre-harvest interventions for controlling foodborne pathogens in pigs, with a focus on Salmonella.</p> <p>The most effective interventions included in-feed/water treatments and vaccination, with 72% and 88% positive results, respectively. Despite available literature, Salmonella infections remain endemic in pig herds worldwide, although some countries like Finland, Norway, and Sweden show success in controlling it. High herd health, good management, and biosecurity are highlighted as effective measures</p> | <p>Few studies declare the magnitude of the effect of positive interventions. No comprehensive mapping and summarization of risk factors for each foodborne pathogen and their respective pre-harvest interventions</p>   | <p><b>RQ2A, RQ2B</b></p>     |
| <p><b>Chanamé Pinedo et al.</b></p> | <p>2022</p> | <p>Europe</p> | <p><i>Sources and trends of human salmonellosis in Europe, 2015–2019: An analysis of outbreak data</i></p>   | <p>to determine the main food sources and recent trends of Salmonella outbreaks in Europe, specifically focusing on human salmonellosis</p>   | <p>The study found a significant increase in Salmonella outbreaks, particularly those caused by S. Enteritidis in Eastern Europe. Outbreaks by S. Typhimurium and its monophasic variant were mainly attributed to pork, while those caused by S. Enteritidis were mostly due to eggs. The use of whole-genome sequencing has led to an increase in identified outbreaks</p>   | <p>The recent increase in Salmonella outbreaks may be attributed to the broader application of whole-genome sequencing, but the actual cause (increased exposure or enhanced surveillance) remains unclear. There's a significant rise in outbreaks caused by unknown Salmonella serotypes, especially in Eastern and Southern Europe</p> | <p><b>RQ1, RQ2A, RQ3</b></p> |



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| <p><b>Galán-Relaño et al.</b></p> | <p>2022</p> | <p>Spain</p>           | <p><i>Phenotypic and genotypic antibiotic resistance patterns in Salmonella Typhimurium and its monophasic variant from pigs in southern Spain</i></p> | <p>to determine the Minimum Inhibitory Concentration (MIC) distribution of 12 antimicrobials and 12 resistance-associated genes in a collection of Salmonella Typhimurium (ST) and its monophasic variant (mST) isolates from slaughtered pigs reared in extensive systems in southern Spain</p>   | <p>98.6% of ST and MST isolates from slaughtered pigs were MDR. Results highlight a higher antimicrobial pressure in intensive farming systems, but also note the emergence of resistance in extensive systems</p>   | <p>A clear disconnect between AMR gene patterns and their phenotypic resistance. The research suggests the need to delve deeper into the actual expression of these genes in real-world conditions versus in vitro scenarios. The study also underscores a need for an improved understanding of the MDR prevalence and determinants in extensive farming systems</p>  | <p><b>RQ2A</b></p> |
| <p><b>Mughini-Gras et al.</b></p> | <p>2022</p> | <p>the Netherlands</p> | <p><i>A statistical modelling approach for source attribution meta-analysis of sporadic infection with foodborne pathogens</i></p>                     | <p>to develop a statistical model within a Bayesian estimation framework to integrate attribution estimates from expert elicitations with estimates from microbial subtyping and case-control studies for sporadic infections with four major bacterial zoonotic pathogens in the Netherlands (Campylobacter, Salmonella, Shiga toxin-producing E. coli [STEC] O157)</p> | <p>Pigs were identified as the most significant reservoir for Salmonella (41.6%). The primary attributable sources for Salmonella were pork (21.8%), eggs (15.4%), and poultry meat (13.2%). Notably, the dominance of Enteritidis serovar has decreased since 2011, with pigs emerging as the primary reservoir. The contribution of travel as a source has gained importance in Salmonella attribution, aligning with the observed decrease in travel-related cases during the COVID-19 pandemic</p> | <p>The study identifies limitations associated with relying on expert elicitations, which can introduce biases. It recognizes that the expert elicitation done in 2008 has become outdated and emphasizes the need for updated and empirical data to inform source attribution. It also acknowledges potential limitations in the data collection phase and points to the need for tailored approaches to address specific research questions. The precision set for experts' fractional estimates and the use of anonymized</p> | <p><b>RQ2A</b></p> |



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|                      |      |                 |  |  |   | aggregate data from other studies also posed challenges  |                  |
| <b>Peruzy et al.</b> | 2022 | Italy           | <i>Occurrence and distribution of Salmonella serovars in carcasses and foods in southern Italy: Eleven-year monitoring (2011–2021)</i> | to evaluate the occurrence and distribution of Salmonella serovars in carcasses and foods in southern Italy over an eleven-year period (2011–2021) | The study observed a high occurrence of Salmonella spp. in food and carcasses in southern Italy. The prevalence of Salmonella has been on the rise despite existing control measures. There was a noted shift in the distribution of Salmonella serovars: a rise in S. Infantis and monophasic S. Typhimurium while a decline in S. Typhimurium and S. Enteritidis. In the pig production chain in Italy, prevalent serovars in meat were S. Typhimurium and S. Derby, and for carcasses, they were monophasic S. Typhimurium, S. Derby, and S. Rissen. The study found a higher contamination level in broilers, buffalo, and pork, likely due to faecal contamination from improper evisceration procedures | The study does not explicitly discuss its limitations or potential biases  | <b>RQ2A, RQ3</b> |
| <b>Mu et al.</b>     | 2023 | the Netherlands | <i>Building a resilient pork supply chain to Salmonella spp.</i>   | to explore the resilience performance of the pork supply chain under different food safety shocks caused by Salmonella spp. and to investigate the | The study highlighted the inadequacy of conventional risk management approaches for handling potential Salmonella spp. shocks in the pork supply chain. It underscored the importance of embedding the resilience concept in quantitative models  | Lack of access to real monitoring data led to reliance on literature data and expert opinions. Some parameters, such as speed of recovery, were not addressed in the model due to data | <b>RQ2B</b>      |



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|                             |      |        |   | effectiveness of interventions in reducing the impact of these shocks on the resilience performance of the chain  | to manage such risks more effectively. A simulation model was developed to evaluate the pork supply chain's resilience under various Salmonella spp.-related food safety shocks. This model can gauge the efficacy of different interventions to diminish the shocks' impact. Scenario analyses revealed that intervention effectiveness relies on the pork supply chain's risk profile. The added value of food safety resilience assessment models over traditional food safety risk assessment models is highlighted  | constraints. Assumptions were used for simulating resilient pigs, hinting at a need for more research in this area to refine parameters   |            |
| <b>De La Gándara et al.</b> | 2023 | France | <i>Countrywide multi-serotype outbreak of Salmonella Bovismorbificans ST142 and monophasic Salmonella Typhimurium ST34 associated with dried pork sausages in France, September 2020* to January 2021</i> | to investigate a multi-serotype outbreak of Salmonella in France, specifically focusing on the serotypes Salmonella Bovismorbificans ST142 and monophasic Salmonella Typhimurium ST34, which were associated with dried pork sausages | A) The study highlighted the inadequacy of conventional risk management approaches for handling potential Salmonella spp. shocks in the pork supply chain. It underscored the importance of embedding the resilience concept in quantitative models to manage such risks more effectively. B) A simulation model was developed to evaluate the pork supply chain's resilience under various Salmonella spp.-related food safety shocks. This model can gauge the efficacy of different interventions to diminish the shocks' impact. C) Scenario analyses revealed that intervention effectiveness | A) Lack of access to real monitoring data led to reliance on literature data and expert opinions. B) Some parameters, such as speed of recovery, were not addressed in the model due to data constraints. C) Assumptions were used for simulating resilient pigs, hinting at a need for more research in this area to refine parameters | <b>RQ3</b> |



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|               |      |    |   |   | relies on the pork supply chain's risk profile. The added value of food safety resilience assessment models over traditional food safety risk assessment models is highlighted   |   |           |
| Roasto et al. | 2023 | EU | <i>Salmonella enterica prevalence, serotype diversity, antimicrobial resistance and control in the European pork production chain</i> | to provide an overview of <i>Salmonella enterica</i> in the European pork production chain, focusing on prevalence, serotype diversity, antimicrobial resistance, and epidemiological importance over the past 20 years. The study also aimed to introduce future trends and recommendations for the control of <i>Salmonella</i> in the European pork production chain | A) <i>Salmonella</i> is a significant hazard in the EU's pig meat inspection. It's the second most reported zoonosis and the top cause of foodborne outbreaks in the EU. B) The three dominant serotypes in the EU are <i>S. Enteritidis</i> , <i>S. Typhimurium</i> , and its monophasic variant. C) The highest prevalence in the pork chain is seen at the fattening pig farm level, with a lower rate on pig carcasses. D) The study found resistance to multiple drugs among significant serotypes. E) Suggestions for future control in the European pork chain include adopting a risk-based meat safety assurance system and tailoring control strategies to national epidemiological contexts | The study primarily offers an overview of <i>Salmonella</i> 's prevalence, serotype diversity, antimicrobial resistance, and control measures without explicitly identifying gaps in research or control measures | RQ1, RQ2B |





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| Rodríguez et al. | 2023 | Spain | <i>Salmonella assessment along the Spanish food chain: Likelihood of Salmonella occurrence in poultry and pig products is maintained across the food chain stages</i> | to assess the likelihood of Salmonella occurrence in pig (and poultry) products across different stages of the Spanish food chain | A) The most determinant product for Salmonella's probability was 'meat', especially in pig and poultry. The key stage was 'slaughterhouse'. B) The presence of Salmonella was significant at the beginning and end of the food chain but not in the middle. The final stages are concerning due to the potential for human infections. C) The study uses the random forest method to pinpoint key areas and assess control efforts. D) Pigs and pork products are a major source of Salmonella contamination in the EU. Salmonella prevalence in these products is rising, with positive results linked to pig meats in slaughterhouses and processing plants. There's a need for better control measures and an official control program for Salmonella in Spain | The study points out inconsistencies in data collection and reporting across different regions of Spain. Certain stages of the food chain lack proper data collection, such as certain communities not performing tests in medical facilities while others do. An official control program for Salmonella in Spain is also required, indicating a gap in current measures | RQ2A, RQ3 |
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| <p><b>Smith et al.</b></p> | <p>2023</p> | <p>Austria, Bulgaria, Czech Republic, Germany, Estonia, Italy, the Netherlands, Poland and the <i>United Kingdom (UK)</i></p> | <p><i>Assessing pig farm biosecurity measures for the control of Salmonella on European farms</i></p>   | <p>to conduct a comprehensive European-wide risk factor analysis to identify farm biosecurity measures that are relevant for limiting the probability of introduction and transmission of Salmonella within pig farms. The study aimed to determine which biosecurity measures should be prioritized for Salmonella control on-farm</p> | <p>A) Over a third of the farms studied had Salmonella presence, with 40% of positive samples being strains of concern to human health. B)The study highlights the importance of maintaining hygiene during slaughtering. C) The study analyzed the effectiveness of various biosecurity measures on 250 pig farms across nine European countries. Results indicated measures like rodent bait usage, downtime of at least 3 days after cleaning in farrowing areas, use of fully slatted flooring in fattening areas, and not mixing stay-behinds with healthy pigs as being effective in reducing Salmonella risk</p> | <p>The study recognized several limitations. A relatively small number of samples were used to determine farm Salmonella status, which might have caused bias. The inclusion of farms from the Netherlands (NL) and Estonia (EE) and their different sampling methods might have introduced classification bias. Differences in the type of samples used, especially serological samples in NL, might have influenced sensitivity in detecting Salmonella. There were variances in farm types across countries, hindering cross-country comparisons. The recruitment process of the farms could have introduced selection bias</p> | <p><b>RQ1, RQ2A, RQ3</b></p> |
| <p><b>Zajac et al.</b></p> | <p>2023</p> | <p>Poland</p>   | <p><i>The first description of the complete genome sequence of multidrug-resistant Salmonella enterica serovar monophasic Typhimurium (1,4,[5],12:i:-) isolate with the mcr-1.1 gene on IncHI2 found in pig in Poland</i></p> | <p>to characterize a multidrug-resistant (MDR) monophasic Salmonella Typhimurium strain isolated from a pig fecal sample in Poland, which carried the mcr-1.1 gene, and to analyze its genome content and antimicrobial resistance profile</p>  | <p>A) The study presents genomic details of a multidrug-resistant monophasic S. Typhimurium strain isolated from pig feces in Poland, particularly emphasizing the presence of the mcr-1.1 gene. Genome analysis showcased the strain had multiple antimicrobial resistance genes located on both plasmids and the chromosome. B)This is the first report of mcr-positive</p>   | <p>Further research is needed to assess the potential spread of mcr-positive Salmonella strains and the implication for antimicrobial resistance in both animal and human populations</p>  | <p><b>RQ1, RQ3</b></p>       |



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|  |  |  |  |  | Salmonella in Poland, raising concerns regarding resistance to colistin, a critical antimicrobial. C) The findings suggest the urgency of enhanced surveillance to prevent the spread of such strains and the potential threats to public health |  |  |
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