

Review

Salmonella spp. in Chicken: Prevalence, Antimicrobial Resistance, and Detection Methods

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Abstract: Multidrug-resistant *Salmonella* spp. is one of the leading causes of worldwide foodborne disease outbreaks. Animal-derived foods, particularly chicken and poultry products, are the most likely source of *Salmonella* transmission to humans. The increasing demand for chicken meat has raised a global food safety issue. This review aims to determine the prevalence and antimicrobial resistance of *Salmonella* spp. in chickens from various countries in Asia. The methods for detecting *Salmonella* will also be discussed in this review. The prevalence of *Salmonella* spp. in chicken and poultry products is lower in developed countries than in developing countries. In addition, the incidence of *Salmonella* spp. in chicken and poultry products from fresh markets is higher than those from supermarkets. Furthermore, this review also reported the presence of multidrug-resistant *Salmonella* strains in various Asian countries. Rapid *Salmonella* detection based on immunological assays, molecular-based assays, and biosensors can provide more accurate results with high sensitivity and specificity. These methods also require a shorter time than the cultural-based *Salmonella* detection method. The use of suitable detection methods to determine the presence of *Salmonella* spp. in chicken and poultry products is important to ensure food safety.

Keywords: food safety; *Salmonella* spp.; antibiotic resistance; chicken; poultry



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1. Introduction

Foodborne diseases, transmitted through the consumption of microbial-contaminated food, are a significant worldwide public health concern. A foodborne outbreak occurs when at least two people have the same illness after consuming similar contaminated food or beverages [1]. It has been identified as one of the primary causes of death and morbidity in humans. Every country devotes significant time and resources to treating foodborne diseases. It has become a threat for most countries and an obstacle to global economic progress [1,2].

Salmonella is one of the most common foodborne pathogens that has been widely linked to foodborne disease outbreaks. Globally, approximately 1.3 billion cases of salmonellosis and 155,500 fatalities are attributed to *Salmonella* each year [3]. It has been listed as the second leading cause of foodborne illness in the European Union, accounting for 91,856 foodborne infections in 2018 [4]. Furthermore, around 70–80% of foodborne diseases in China have been reported to be associated with *Salmonella* infections [3]. Today, more than 2500 *Salmonella* serovars are recognized worldwide.

Salmonellosis is usually associated with the ingestion of *Salmonella*-contaminated food products [1,2]. Animal-derived foods, particularly chicken and poultry products, are the most common transmission source of *Salmonella* to humans [3]. When a significant quantity of *Salmonella* has been ingested, it will colonize the infected human's intestinal tract, triggering a range of clinical manifestations. *Salmonella* infections are often accompanied by various symptoms, including gastroenteritis, bacteremia, and typhoid fever. Multiple salmonellosis outbreaks related to chicken and poultry product consumption have been reported in recent years, implying that these products constitute the primary vehicle for *Salmonella* transmission [5,6].

The increasing number of infectious diseases related to *Salmonella* has become a burden for most developing countries due to the high expenses incurred by treatment, prevention, and campaigns to control the diseases [7]. Moreover, the wide variation in *Salmonella* serovars and the high frequency of changing trends in salmonellosis due to the development of novel serotypes and antimicrobial resistance has raised awareness among researchers and the public [5]. Its resistance to single antibiotics, such as ampicillin and chloramphenicol, has been recorded. In addition, multiple drug resistance (MDR) of *Salmonella* spp. has been reported worldwide [8,9]. This antimicrobial resistance in *Salmonella* is mainly due to antibiotic misuse in the poultry industry and medical treatment.

Salmonella detection is critical for food safety monitoring in the supply chain of chicken and poultry products. *Salmonella* detection methods have evolved from conventional culture-based techniques to rapid detection, such as polymerase chain reaction (PCR), enzyme-linked immunosorbent assay (ELISA), loop-mediated isothermal amplification (LAMP), typhi-dot, and others [7,10]. This advancement is driven by the necessity for high specificity and rapid reactions in diagnosing *Salmonella*, particularly in food emergency-response laboratories [7,11]. Besides, the drawbacks of traditional *Salmonella* detection, which is time-consuming, labour-intensive, and with a high possibility of contamination, hamper detection efficacy, thereby delaying the “golden healing time” for salmonellosis patients [11].

Chicken is also known as *Gallus domesticus*, which represents medium-sized poultry. Concurrently, the chicken is bred and raised for two purposes, these being for meat consumption (broilers) and egg production (layers) [12]. The term “poultry” refers to a range of domesticated fowls, such as chickens, ducks, ostrich, turkey, and related species, while “poultry products” refers to commercially processed items [13].

In the last few decades, poultry meat has been widely consumed worldwide [14]. Global meat consumption has slowly shifted toward poultry, a healthier low-cost protein source that is suitable for both low- and high-income countries. It is believed that poultry meat will occupy 41% of the global meat protein sources by 2030. This value is strongly related to various determinants, such as the high nutritional value of poultry, low production costs, product consistency, culture, and religious issues. The development of commercial technology and the usage of robotic equipment in the poultry supply chain also boost its growth in the respective industries. Artificial intelligence, sensors, robots, and transportation systems play important roles in the future broiler industry and breeding management [15].

This review will focus on the prevalence and antimicrobial resistance of *Salmonella* spp. in chicken from various Asian countries and evaluate the traditional and current detection methods for *Salmonella* spp.

2. Prevalence of *Salmonella* spp. in Chicken

Salmonella spp. is one of the leading causes of global diarrheal diseases, known as salmonellosis, and is associated with consuming contaminated chicken or poultry products. Various studies from different countries have shown that food products with poultry origins are the most common vehicles in the transmission of *Salmonella* [16]. *Salmonella* can be classified into typhoidal (TS) and non-typhoidal *Salmonella* (NTS) [17,18]. The TS serovars have a high degree of host adaption in humans, whereas the NTS serovars’ host adaption is usually associated with animal hosts. The most common vehicle for NTS transmission is from animal-based products such as poultry, pork, and raw eggs [17,18]. The NTS usually causes salmonellosis via a clinical syndrome, such as abdominal pain, non-bloody vomiting, diarrhea, myalgia, and fever [5,19]. The most commonly associated *Salmonella* serotypes include *S. Typhimurium* and *S. Enteritidis*. Most of the recorded NTS salmonellosis is a self-limiting illness with a short incubation period (6–12 h) after ingesting contaminated products. The onset and duration can last for ten days [19].

Over the years, the prevalence of *Salmonella* in food has become a threat to public health. It is known as one of the leading causes of diarrheal disease, the second most common

contributing agent of food-transmitted disease in the European Union and the United States, and the third leading cause of death among foodborne illnesses worldwide [4,20]. Every year, there are an estimated 93.8 million NTS salmonellosis cases globally, with 155,000 deaths [5]. It has also been identified as the second most common zoonosis, with 82,694 confirmed cases in 2013 [5]. Besides NTS salmonellosis, enteric fever is one of the clinical manifestations caused by typhoidal *Salmonella*. The recognized *Salmonella* serotypes are *S. Typhi* and *S. Paratyphi*. Enteric fever usually has a longer incubation period than NTS salmonellosis, accompanied by symptoms such as abdominal pain, constipation, headache, and the onset of fever [19].

Salmonella-contaminated food products may raise concerns because they can be consumed directly by customers. Although most foods are heat-treated with cooking, boiling, steaming, and roasting, biological cross-contamination and undercooked food remain the biggest challenge [21]. Furthermore, salmonellosis can be caused by poor food preservation, environmental contamination, and poor sanitary and hygiene practices among food handlers. Various studies have been carried out worldwide to investigate the prevalence of *Salmonella* in food across countries.

Research on the prevalence of *Salmonella* and *Salmonella* serovars in chickens and poultry products is essential to ensuring food safety as they are widely consumed worldwide. In this review, the prevalence of *Salmonella* serovars in the respective products from various Asian countries, including Cambodia, China, Iran, Japan, Malaysia, Myanmar, Singapore, South Korea, Thailand, and Vietnam, are presented. It is believed that the rapid economic growth in Asia, especially in China, will increase Asia's chicken consumption in the future [16]. Various *Salmonella* serovars can infect poultry through vertical, horizontal, and cross-contamination transmission [17]. Table 1 shows the prevalence of *Salmonella* found in the selected samples from various Asian countries.

Table 1. Prevalence of *Salmonella* spp. in chicken and poultry products in selected Asian countries.

Region	Number of Samples	Number of <i>Salmonella</i> -Positive Samples	Percentage of <i>Salmonella</i> -Positive Samples	References
Cambodia	187	78	41.7	[22]
China	1152	601	52.2	[23]
Iran	452	111	24.6	[24]
Japan	821	164	20.0	[25]
Malaysia	191	79	41.4	[26,27]
Myanmar	141	138	97.9	[28]
Singapore	270	52	18.1	[29]
South Korea	330	65	19.7	[30,31]
Thailand	195	79	40.5	[22]
Vietnam	1000	459	45.9	[32]

Based on the studies, a high prevalence of *Salmonella* is observed in developing countries such as Myanmar, Vietnam, Cambodia, Thailand, and Malaysia. Different prevalence in each country could be attributed to the differences in hygiene administration, government effort, laboratory techniques, regulation administration, sample collection, etc. [25].

Researchers also pointed out that retailed chicken meat sold in Yangon, Myanmar recorded 97.9% of *Salmonella*-positive infections [28]. Poor hygiene practices and conditions on the market stalls were the main contributors to this high contamination rate. In addition, a slow chicken-meat turnover rate, unsuitable storage temperatures, and unsterilized utensils may contribute to pathogenic *Salmonella* growth in chicken meat. Singapore has the lowest *Salmonella* prevalence among the countries studied, owing to their government's efforts to ensure food safety and hygiene in the region [29].

Whole chicken carcasses are more sensitive to *Salmonella* infection than single pieces of chicken meat because the whole carcass is covered by more skin, which is prone to *Salmonella*

infection [28]. 52.2% of chicken carcasses in China tested positive for *Salmonella* [23]. In contrast, researchers reported 41.7% and 40.5% *Salmonella* prevalence in broiler carcasses in Cambodia and Thailand [22]. Furthermore, Zdragas and Ta revealed a greater prevalence of *Salmonella* contamination in chicken carcasses at 39.5% and 40.5% [32,33], respectively, in Greece and Vietnam. However, there may be some exceptional circumstances. For example, *Salmonella* recovery was lower in the chicken carcasses than in the drumstick and chicken-meat samples in South Korea and Egypt [30,31,34]. Cross-contamination could be the reason for these unusual cases throughout the production line [28]. Besides, according to Soodagari, significant *Salmonella* frequency in chicken flesh relative to the liver, heart, and gizzards samples was caused by an inappropriate defeathering process, resulting in *Salmonella* spread in the chicken meat [24].

The prevalence of *Salmonella* in chicken and poultry products also varies depending on the types of poultry stores (supermarkets and fresh food markets). The fresh food market is a traditional open-air market that offers its products at ambient temperature, whereas the supermarket is a well-established indoor market with better cleanliness and a temperature control system [23,35]. Overall, the prevalence of *Salmonella* in selected fresh food market samples was higher than in samples from supermarkets, ranging from 25.0 to 53.9%.

Meanwhile, *Salmonella* prevalence in chicken from supermarkets ranged from 12.7 to 52.3% [23,27,29,32]. This disparity could be attributed to the wet market's weaker cleanliness standards and hygiene practices than the supermarket. Because pest control at the traditional open-air market is challenging, there will be a greater risk of *Salmonella* cross-contamination [35]. Furthermore, other factors, such as the use of unclean utensils (chopping board and knife) when handling products, improper storage methods, cross-contamination from contaminated ice or water, and improper temperature display conditions could all contribute to the higher rate of *Salmonella* prevalence in the wet market samples [29]. Cross-contamination could occur between the earlier batches as well as in subsequent batches.

Salmonella is very contagious in chicken and poultry products and can spread the disease throughout the production chain. Identifying *Salmonella* serovars has become a significant public health problem since they can be linked to various diseases [21]. As a result, identifying *Salmonella* serovars in chicken and poultry samples is critical for disease assessment and epidemiological surveillance. In various research works, the distribution of *Salmonella* serovars was heterogeneous. As demonstrated in Table 2, each country has its prevalent *Salmonella* serovars, which differ in quantity and variety. Iwabuchi found that *S. Infantis* was the most common *Salmonella* serotype in Japan (81, $n = 452$; 17.9%) [25], whereas Shafini reported that the most predominant *Salmonella* serotype in Malaysia was *S. Enteritidis* (25, $n = 62$, 39.7%) [27]. In contrast, another study by Abatcha from Malaysia found that *S. Corvallis* was the most prevalent serovar in chicken (7, $n = 17$; 41%) [26]. Lee also discovered that *S. Typhimurium* (12, $n = 18$, 66.7%) is the most widespread serotype in Korea. *S.* [32]. Thompson was the predominant serovar in Iran, and *S. Albany* in Myanmar [24,28]. In Japan, there were 27 serovars detected, 24 in Myanmar, 11 in Singapore, 8 in Malaysia, 5 in Iran and South Korea, 11 in Singapore, and 8 in Malaysia. *Salmonella* prevalence varies widely due to geography, sampling methodology, sample types, and bacteriological approaches [34].

Table 2. Distribution of *Salmonella* serovars identified from selected chicken and poultry products.

Region	Sample Type	<i>Salmonella</i> serotypes (No. of Samples)	References
Iran			
Mansoura	Chicken meat	<i>S. Thompson</i> (35), <i>S. Enteritidis</i> (12), <i>S. Typhimurium</i> (3), <i>S. Hadar</i> (6), UN (2)	[24]
	Liver	<i>S. Thompson</i> (8), <i>S. Enteritidis</i> (7), <i>S. Typhimurium</i> (7), <i>S. Newport</i> (3), UN (1)	
	Heart	<i>S. Thompson</i> (6), <i>S. Enteritidis</i> (4), <i>S. Typhimurium</i> (2), <i>S. Newport</i> (5)	
	Gizzards	<i>S. Thompson</i> (5), <i>S. Enteritidis</i> (2), <i>S. Typhimurium</i> (2), UN (1)	
Japan			
Hokkaido	Chicken meat	<i>S. Infantis</i> (39), <i>S. Nigeria</i> (26), <i>S. Wien</i> (24), <i>S. Limete</i> (10), <i>S. Uppsala</i> (10), <i>S. Canada</i> (9), <i>S. Adime</i> (5), <i>S. Abony</i> (4), <i>S. Brezany</i> (4), <i>S. Lomita</i> (4), <i>S. Rissen</i> (4), <i>S. Reading</i> (3), <i>S. Derby</i> (3), <i>S. Tripoli</i> (3), <i>S. Eko</i> (2), <i>S. Montevideo</i> (2), <i>S. Stanley</i> (2), UN (16)	[25]
		<i>S. Infantis</i> (42), <i>S. Kalamu</i> (35), <i>S. Manhattan</i> (25), <i>S. Uppsala</i> (24), <i>S. Canada</i> (13), <i>S. Schwarzengrund</i> (11), <i>S. Stanleyville</i> (11), <i>S. Eko</i> (6), <i>S. Finaghy</i> (5), <i>S. Brezany</i> (2) <i>S. Schwarzengrund</i> (32), <i>S. Stanleyville</i> (26), <i>S. Kalamu</i> (21), <i>S. Manhattan</i> (8), <i>S. Canada</i> (6), <i>S. Uppsala</i> (5), <i>S. Brezany</i> (4), <i>S. Eko</i> (4), <i>S. Finaghy</i> (2)	
Aichi, Gifu, Mie			
Miyazaki, Oita, Saga			
Malaysia			
Selangor, Negeri Sembilan	Raw chicken	<i>S. Enteritidis</i> (20), <i>S. Hadar</i> (14), <i>S. Gallinarum</i> (6), <i>S. Dublin</i> (5), <i>S. Stanley</i> (4), <i>S. Anatum</i> (1), <i>S. Choleraesuis</i> (1), <i>S. Typhimurium</i> (1)	[27]
	Minced chicken	<i>S. Enteritidis</i> (4), <i>S. Hadar</i> (1), <i>S. Dublin</i> (1), <i>S. Stanley</i> (1)	
	Processed products	<i>S. Enteritidis</i> (1), <i>S. Stanley</i> (1), <i>S. Anatum</i> (1)	
Myanmar			
Yangon	Chicken meat	<i>S. Albany</i> (53), <i>S. Kentucky</i> (15), <i>S. Braenderup</i> (14), <i>S. Indiana</i> (11), <i>S. Virchow</i> (5), <i>S. Brunei</i> (5), <i>S. Weltevreden</i> (4), <i>S. Derby</i> (3), <i>S. Typhimurium</i> (3), <i>S. Enteritidis</i> (3), <i>S. Wagenia</i> (3), <i>S. Diogoye</i> (2), <i>S. Bareilly</i> (2), <i>S. Lexington</i> (2), <i>S. Stanley</i> (2), <i>S. Agona</i> (2), <i>S. Hindmarsh</i> (2), <i>S. Cerro</i> (1), <i>S. Yoruba</i> (1), <i>S. Mbandaka</i> (1), <i>S. Newport</i> (1), <i>S. Stuttgart</i> (1), <i>S. Paris</i> (1), <i>S. Apeyeme</i> (1)	[28]

Table 2. Cont.

Region	Sample Type	<i>Salmonella</i> serotypes (No. of Samples)	References
Singapore	Fresh, chilled chicken meat	<i>S. Saintpaul</i> (17), <i>S. Brancaster</i> (11), <i>S. Albany</i> (6), <i>S. Stanley</i> (5), <i>S. Agona</i> (4), <i>S. Typhimurium</i> (3), <i>S. Gaminara</i> (2), <i>S. Bovismorbificans</i> (1), <i>S. Give</i> (1), <i>S. Newport</i> (1), <i>S. Weltevreden</i> (1)	[29]
South Korea Jeonla, Chungcheong, Gyeongsang, Gyeonggi	Whole chicken carcasses	<i>S. Typhimurium</i> (12), <i>S. Hadar</i> (2), <i>S. Rissen</i> (2), <i>S. Virchow</i> (1), <i>S. Bareilly</i> (1)	[31]

Shafini revealed the predominance of *S. Enteritidis* in Malaysia [27]. The relevant serovar is the essential factor in spreading foodborne illness. The previous studies by Maka and Ramya conducted in Malaysia, demonstrated that *S. Enteritidis* was the predominant serovar in chicken. *S.* [36,37]. *Enteritidis* can infect chickens by colonizing the reproductive organs in layers and eggs [38]. As a result, *S. Enteritidis* is frequently found in poultry products. Interestingly, the respective serovars were also exist in Iran, and Myanmar, despite their low percentage [24,28]. This low prevalence may be identified as the result of implementing *Salmonella* control measures, such as pest control, biosecurity, vaccination, monitoring, and cleaning management [34]. The absence of *S. Enteritidis* in Japan, Singapore, and South Korea can be explained by changes in epidemiology in the respective serovars in the different nations, due to the globalization of the food trade [25,29,31]. According to WHO, *S. Enteritidis* is one of the most widely spread *Salmonella* serotypes from animal to human, which can cause various diseases such as gastroenteritis and fever.

Furthermore, poultry is one of the dominant vectors for spreading *S. Typhimurium* serovar to people, resulting in a *Salmonella* outbreak [4,17]. Only Japan was free of *S. Typhimurium* among the countries studied in this review. Meanwhile, according to Moe [28], the isolation of *S. Infantis* in chicken meat samples in Japan is compatible with the prior study conducted in Japan by Kusunoki [39]. In addition, Lee and Zdragas also reported that *S. Typhimurium* has a higher human salmonellosis rate in the summer due to cross-contamination occurring in production plants [31,33]. The predominance of *Salmonella* serovars in Africa is of *S. Kentucky* [40]. This is in agreement with the study by Amajoud, which found that *S. Kentucky* is abundant in chicken (54.5%) and turkey (18.2%) in Morocco [41]. Another study by Abd-Alghany also stated that 10.8% of *S. Kentucky* was isolated from chicken from Egypt [34]. Concerning *S. Hadar*, one of the five most commonly isolated *Salmonella* serovars in the European Union, Zdragas reported that the serovar is usually isolated from broiler flocks [33]. Besides this, it is also widely presented in chicken and poultry products, including raw chicken and poultry products in Malaysia, Iran, and South Korea [24,27,31].

Besides *S. Typhimurium* and *S. Enteritidis*, *Salmonella* serovars can be varied and also specific to each geographical region. For example, *S. Infantis* is the predominant non-typhoidal *Salmonella* serovar in Japan. According to a previous study by Murakami, the high prevalence of this *Salmonella* strain can be explained by the high colonization rate among chicken flocks in Japan. *S.* [42]. *Albany* is ranked as one of the most commonly isolated serovars in Asian countries [43], which aligns with the findings discussed in this review paper. It has been detected at high levels in Myanmar and Singapore [28,29]. It is undeniable that identifying the serovars of *Salmonella* can help us understand the illness better so that proper treatments can be given to the patients.

The implementation of Hazard Analysis Critical Control Points (HACCP) and Good Manufacturing Practice (GMP) throughout the supply chain will improve the quality and safety of food products for either export or domestic supply [44]. In the poultry industry,

these certifications may help reduce the risk of *Salmonella* contamination. The prevalence of *Salmonella* in chicken and poultry products from GMP and HACCP-certified companies is expected to be lower than in those companies that do not implement such certifications. Furthermore, the good implementation of HACCP and GMP systems in local enterprises is believed to reduce the risk of foodborne illnesses [44,45]. However, its implementation will not be successful without good coordination from the food industry and regulatory bodies to address this issue, especially in big or developing countries. According to Lam, implementing food safety certifications is very difficult, especially in a big country like China, due to the broad administrative structure and regulatory control among national, provincial, and local government authorities [45].

3. Antimicrobial Resistance in *Salmonella* spp.

Antimicrobial resistance is a bacteria's capacity to interfere with antimicrobial agents, hence reducing their fatal effect [9,46]. Different *Salmonella* serovars demonstrate varying levels of antimicrobial resistance. Antibiotic misuse in farm animals has dramatically contributed to the emergence and persistence of resistant strains. The global scenario has revealed a crisis in *Salmonella* resistance since *Salmonella* resistance to single antibiotics has been recorded, and multiple drug resistance (MDR) has been reported worldwide [8,9]. According to the Department of Veterinary Services in Malaysia (2013/2014), *Salmonella* isolates from chicken and poultry samples demonstrate a wide range of antibiotic resistance profiles [47]. Moreover, 26% of *Salmonella* strains recovered from human infections in European Union countries have shown MDR characteristics toward ciprofloxacin and cefotaxime [4].

There is a wide variety in terms of the classes of antimicrobial medications in the current market. Aminoglycosides, β -lactams, chloramphenicol, quinolones, tetracyclines, sulfonamides, and trimethoprim are the most common antibiotics against which *Salmonella* has developed resistance [21]. In general, the energy-dependent removal of antimicrobial via membrane-bound efflux pumps, changes in bacterial cell permeability, modifications to the target site by drug action, acquisitions of replacements for the target protein, and inactivation of antimicrobial agents by the secretion of enzymes are all part of *Salmonella*'s antimicrobial resistance mechanism. *Salmonella*'s resistance to aminoglycosides results from antimicrobial enzymatic modification, which is produced by aminoglycoside-modifying enzymes. Genes carried on plasmids are usually responsible for this process. Streptomycin and kanamycin are the best-known members of this group [9,46].

Besides this, *Salmonella*'s resistance to β -lactams families, including penicillin, cephalosporins, and carbapenems has also been widely publicized in various studies. The mechanism can be described by the secretion of β -lactamases into the periplasmic of *Salmonella*, hydrolyzing the β -lactams ring into β -amino acids, which has no antimicrobial effects [9,46]. In addition, chloramphenicol resistance is defined as a combination of the enzymatic inactivation of antibiotics by chloramphenicol O-acetyl-transferase and drug elimination by an efflux pump [9,46]. Furthermore, *Salmonella*'s strong resistance to fluoroquinolones is due to the combination of multiple target-gene mutations and an active efflux mediated by AcrAB-TolC [9]. *Salmonella* resistance to tetracyclines is acquired by creating energy-dependent efflux pumps that excrete the antibiotic from *Salmonella*.

4. Antimicrobial Resistance of *Salmonella* spp. in Chicken and Poultry Products

In recent years, the antimicrobial resistance of *Salmonella* spp. has increased due to the widespread use of antimicrobial agents in the food industry as veterinary medicine, growth-promoting substances, prophylactics, and therapeutics [24,34]. However, this undesirable trend has sparked a public concern since it is boosting not only the difficulty of treating salmonellosis but also the spread of antimicrobial resistance across the food chain supply, particularly among chicken and poultry products [9,24,31]. Besides this, it has raised further concerns about the multidrug-resistant (MRD) strain. The MDR strains are those strains that are resistant to at least one microbial agent. For example, *S. Heidelberg*

isolates demonstrated resistance to ampicillin, amoxicillin-clavulanic acid, ceftiofur, and cephalothin. Over time, the public's focus shifted to establishing antimicrobial resistance rather than on the favorable influence on human health and agricultural produce.

Meanwhile, a variety of research into *Salmonella* spp. antimicrobial resistance in chicken and poultry products has been conducted in various countries. *Salmonella* spp. antimicrobial resistance was isolated from chicken and poultry products in Egypt, Iran, Japan, Malaysia, Myanmar, Singapore, and South Korea, as shown in Table 3. Except for the study conducted in Japan, all the studies used the disk diffusion test to assess the antimicrobial resistance of *Salmonella* spp. that were isolated from chicken and poultry products. Overall, each country's *Salmonella* strains demonstrated varying resistance levels to antimicrobial medicines.

Table 3. Antimicrobial resistance of *Salmonella* spp. isolated from chicken and poultry products in selected countries.

Region	No. of Isolates (Animal Hosts)	Testing Methods	Antimicrobial Resistance (n, %)	Reference
Iran	111 (retailed chicken meat and giblets)	Mueller-Hinton agar disk diffusion method	AMC (6, 5.4%); AMP (13, 11.7%); CHL (4, 3.6%); KAN (41, 36.9%); NA (103, 92.8%); STR (63, 56.8%); TET (90, 81.1%); TMP (76, 68.5%); SXT (68, 61.3%) * Multiple antimicrobial resistance pattern present	[24]
Japan	452 (chicken meat)	Agar dilution method	AMP (81, 17.9%); BCM (222, 49.1%); CFZ (26, 5.8%); CTF (9, 2.0%); CST (13, 2.9%); DSM (313, 69.2%); GEN (2, 0.4%); KAN (180, 39.8%); NA (72, 15.9%); OXY (72.6%); TMP (217, 48.0%) * Multiple antimicrobial resistance pattern present	[25]
Malaysia	11 (chicken meat)	Disk diffusion method	AMX (3, 27.3%); AMP (8, 72.7%); CFP (3, 27.3%); CIP (3, 27.3%); ERY (11, 100.0%); NA (1, 9.1%), PEN (11, 100.0%); STR (1, 9.1%), VAN (1, 9.1%) * Multiple antimicrobial resistance pattern present	[48]
Myanmar	138 (raw chicken carcasses)	Disk diffusion method	AMC (24, 17.4%); AMP (65, 47.1%); CRO (5, 9.6%); CHL (32, 61.5%); CIP (13, 9.4%); GEN (11, 8.0%); LIS (8, 5.8%); NOR (1, 0.7%); STR (68, 49.3%); TET (75, 54.3%); TOB (12, 8.7%); SXT (97, 70.3) * Multiple antimicrobial resistance pattern present	[28]
Singapore	52 (chicken meat sample)	Disk diffusion method	AMC (8, 15.4%); AMP (41, 78.8%); CRO (5, 9.6%); CHL (32, 61.5%); CIP (2, 3.8%), GEN (12, 23.1%); NA (16, 30.8%); TET (32, 61.5%); SXT (29, 55.8%) * Multiple antimicrobial resistance pattern present	[29]
South Korea	18 (chicken carcasses)	Disk diffusion method	AMP (1, 5.6%); CFZ (1, 5.6%), CTX (1, 5.6%), CAZ (1, 5.6%); NA (7, 38.9%), STR (32, 61.5%), SXT (29, 55.8%) * Multiple antimicrobial resistance pattern present	[31]

* AMX, amoxicillin; AMC, amoxicillin-clavulanic acid; AMP, ampicillin; BCM, bicozamycin; CFZ, cefazolin; CFP, cefoperazone; CTX, cefotaxime; CAZ, ceftazidime; CTF, ceftiofur sodium; CRO, ceftriaxone; CEF, cephalothin; CHL, chloramphenicol; CIP, ciprofloxacin; CST, colistin; DSM, dihydrostreptomycin; ENR, enrofloxacin; ERY, erythromycin; GEN, gentamicin; KAN, kanamycin; LIS, lincomycin-spectinomycin; NA, nalidixic acid; NEO, neomycin; NOR, norfloxacin; OXY, oxytetracycline; PEN, penicillin; RMP, rifampicin; STR, streptomycin; SMZ, sulphamethoxazole; TET, tetracycline; TOB, tobramycin; TMP, trimethoprim; SXT, trimethoprim-sulfamethoxazole; VAN, vancomycin.

According to the studies listed in Table 3, *Salmonella* spp. resistance to ampicillin (AMP) was observed in all the countries studied (Iran, 11.7%; Japan, 17.9%; Malaysia, 72.7%; Myanmar, 47.1; Singapore, 78.8%; South Korea, 5.6%). This suggested that AMP was commonly utilized as a main antibiotic in the poultry sector in these countries [29,48]. AMP belongs to the β -lactams family, and it works by interfering with the penicillin-binding proteins involved in synthesizing peptidoglycan formation [9,46]. The secretion of β -lactamases (*bla*_{TEM-1} and *bla*_{PSE-1}) into the periplasmic fluid, hydrolyzing the beta-lactam ring into the non-antimicrobial-ability beta-amino acid, representing the resistance mechanism *Salmonella* spp., a Gram-negative bacterium, toward AMP [46].

Aside from AMP, nalidixic acid (NA) resistance was found in all studied countries except Myanmar. It was reported that the high *Salmonella* resistance rate to NA in Iran was due to the overuse of NA for growth promotion and salmonellosis treatment [24].

The capacity of ERY to induce the transposition of the erythromycin-resistant gene from a non-conjugative to a conjugative plasmid contributed to its high resistance level. As a result, it can be transferred from one bacterium to another [34,49]. Thung found that *Salmonella* was utterly resistant to ERY. This can be explained as the result of the misuse of ERY, causing resistance to happen [48]. Therefore, ERY is no longer encouraged for use in livestock, especially in the chicken industry.

Streptomycin (STR) has the highest resistance rate of the 12 antimicrobial medicines, at 64.5%. STR resistance is classified as a global issue. Previous studies conducted in Myanmar and Iran revealed a similar resistance rate ranging from 49.3 to 67.9% [24,28,34]. STR is an aminoglycoside antimicrobial drug that was first discovered in early 1940 and has been used as a therapeutic in animal *Salmonella* infections [9,50,51]. As time has passed, this usage has increased the resistance level of *Salmonella* spp. to STR [51].

Moreover, *Salmonella* strains also showed an MDR pattern. Among MDR isolates in Singapore, the most common phenotypic resistance pattern was AMP-CHL-SXT-TET. This might be due to the main antibiotics used in treating bacterial disease in poultry: penicillin, sulfonamides, and tetracyclines [29]. Therefore, the strains showed a high resistance rate to these antimicrobial drugs. Besides this, Sodagari claimed a high percentage of MRD *Salmonella* strains in Iran (62.2%) [24], with multidrug resistance to NA and TET. SXT-TET-STR-AMP-CHL-AMC was the most common antimicrobial resistance pattern in Myanmar [28]. The difference in MDR patterns was most likely due to the various antimicrobial agents in the poultry industry at approved doses in each country [48].

5. Detection Methods for *Salmonella* spp. in Chicken and Poultry Products

Table 4 shows the advantages, disadvantages, and limits of detection for traditional and rapid *Salmonella* detection methods. The culture-based *Salmonella* detection method is the foundation of various detection methods in food safety analysis and public health laboratories. In general, this traditional method is designed based on *Salmonella*'s ability to grow on differential agar media; thus, we can rapidly identify the presence of *Salmonella* by calculating the colonies isolated from the agar [7,52,53]. For example, *S. Arizona* on XLD agar may appear in light pink-red halo colonies, whereas *S. Typhi* will produce colourless colonies with a black centre on *Salmonella* Shigella (SS) agar [7]. The appearance of the settlements is highly sensitive, as it depends on the biochemical reaction of the sugar and the use of nutrients on the agar [52]. This specialism in colour formation will ease the process for the researchers to identify the *Salmonella* serotypes in their investigation. Besides, compared to other methods, it only requires a lower level of investment [7,12,52,53].

However, these cultural-based methods take a long time to interpret, starting from agar preparation until the final confirmation test. Both the global standards, ISO 6579-1:2017 and BAM Chapter 5: *Salmonella*, require approximately one week. Furthermore, due to the competitive presence of *Proteus* in the samples, there is a considerable risk of false-positive results [7]. Other drawbacks of the conventional *Salmonella* detection method include the labour-intensiveness of the laboratory techniques, the risk of microbial contamination, and the presence of viable but non-cultural bacteria (VBNC) [11]. The consequences of VBNC will result in failure to isolate *Salmonella* from chicken samples or underestimation of the viable cell. The requirement of labor intensiveness is to produce a more reliable and highly sensitive result. Therefore, rapid *Salmonella* detection, based on an immunological assay, molecular-based assay, and biosensors, has been developed to provide a faster and more humane detection method in the food industry.

Table 4. Advantages and drawbacks of the traditional detection of *Salmonella* method and rapid *Salmonella* detection method.

Detection Methods	* Advantages	* Disadvantages	Limit of Detection
Cultural-based <i>Salmonella</i> detection method (ISO 6579:2017, BAM)	Sensitive and selective with chromogenic media Low cost Ease of use	Laborious Time-consuming Results confirmation requires a minimum of 4–6 days Requires a sterile environment to avoid microbial contamination Requires pre-enrichment and selective enrichment Low sensitivity Presence of viable but non-culturable bacteria (VBNC)	10^2 – 10^3 CFU/g [54]
Immunological-based assays	Real-time detection Shorter time compared to cultural methods Results within 48 h	Low affinity and sensitivity Stability issue of the antibody Prone to cross-reactivity issues among <i>Salmonella</i> serovars Potential interference from contaminants	ELISA 10^4 – 10^5 CFU/mL [11]
Molecular-based assays	High sensitivity and specificity Detection time within a few hours Detection of small amounts of target nucleic acid Can be applied in situ Real-time monitoring for detection in food	High operational cost for PCR Prone to cross-reactivity issues among <i>Salmonella</i> serovar Unable to differentiate between live and dead cells may cause false-positive or false-negative results Presence of food material may inhibit the amplification process May require enrichment step Requires expensive machines and trained personnel Complicated primer design	10 CFU/g Real-time PCR [55] 10 CFU/mL Loop-mediated isothermal amplification (LAMP) [56]
Biosensor	High affinity and specificity Detection within a few hours Sensitive and specific detection response Can be integrated into a biosensor device Possible on-site testing User-friendly	High early instrument cost Non-standardized sample preparation Lack of multiplex detection	25 CFU/mL Electrochemical-based biosensor [57] 10^3 CFU/mL DNA aptamer-based calorimetric detection [58] 10^3 CFU/mL Microfluidic nano-biosensor [59] 15 CFU/mL Surface-enhanced Raman scattering-based aptasensor [60]

* Adapted from [11,61].

An ELISA, or enzyme-linked immunosorbent assay, is the most widely used immunological approach to detect antigens in *Salmonella* by utilizing antibody-conjugated enzymes [7,62]. This is one of the most versatile ways of detecting *Salmonella* in chicken and poultry products [63]. The main advantage of ELISA is that it can provide a shorter time duration (less than two days) for *Salmonella* detection, compared to the cultural-based method (approximately one week) [7,11,63]. Besides this, Park claimed that ELISA is more specific than the conventional cultural method [63]. This is because the unique binding site of the antibody can only bind with the complementary epitope of the antigen, which

is known as the antibody–antigen coupling approach [64]. Therefore, ELISA has been developed into the commercial kit form available on the market, such as the ELISA test SELECTA/OPTIMA in Denmark, and TECRA *Salmonella* in Australia [7]. ELISA is suitable for use when handling large samples. There are several advantages to the ELISA methods that are still encountered, as shown in Table 4.

However, every coin has two sides. While ELISA is widely reproducible, it has some flaws, including a restricted sensitivity limit, low antibody affinity for the pathogen, extended enrichment time for cell cultivation, and the possibility of impurities interfering with the results [7,11,63]. Besides, this quick detection method necessitates a large *Salmonella* population. The enzyme amplification process enhances the sensitivity of this technique. A collaboration with immunomagnetic separation (IMS) techniques can help overcome this low-sensitivity restriction [7]. A more specific protocol, such as competitive, double-sandwich, and fluid-phase ELISA can be used to overcome the false signal problem presented in ELISA assays [64].

Another immunological-based method for *Salmonella* detection is typhi-dot, which is a rapid diagnosis kit that has been launched on the market. Generally, it is widely used as an earlier detection test for typhoid fever, especially in developing countries [65]. It is an indirect solid-phase immunochromatographic assay that identifies the presence of *Salmonella* Typhi by detecting the specific antibodies against the outer membrane protein of *Salmonella*. The respective antibodies include immunoglobulin M (IgM) and immunoglobulin G (IgG). Once the reagent in the test kits detects the presence of IgM and IgG in the samples, colour changes will happen within minutes. These colour changes can indicate the presence of *Salmonella* [65,66]. Additionally, this method does not require specific training and equipment. However, the study published by Salama and Said also indicated that typhi-dot showed higher sensitivity and specificity in terms of diagnostic accuracy compared to the Widal test [67]. Unfortunately, this detection method can only be used within a limited range of samples, such as in human serum, plasma, and blood cultures. It will become a more compatible *Salmonella* diagnosis kit if it can be used with a wider range of detection samples.

The PCR assay used in *Salmonella* detection is a molecular approach for detecting *Salmonella* by targeting the specific DNA genes present within *Salmonella*, including *fimC*, the invasion gene (*invA*), *phoP*, and other genome markers [7,68]. This idea is used in most PCR-based methods, such as classic PCR, real-time PCR, multiplex PCR, and reverse-transcriptive PCR, which apply this principle for their operation. These techniques enable the possibility of getting the result for *Salmonella* detection after a shorter waiting period (less than three days), even with the presence of other competitive populations in the examined samples or in the absence of an enrichment medium [11,68]. The success in developing a multiplex fluorogenic PCR assay by Sharma and Carlson [69] has proved the possibility of detecting *Salmonella* and *E. coli* at once. Therefore, this can help in saving time to isolate *Salmonella* more accurately from the food sample. Due to the high capabilities in detecting *Salmonella*, PCR technology has also been introduced to the market in commercial kit form (BAX system, ABI Prism 7500, Probelia, TagMan) with different sensitivities and specificities to ease the screening process in the food industry [8]. The likelihood of obtaining false-positive or false-negative PCR results is an obstacle to the widespread use of PCR-based technologies [22]. They can be integrated by degrading target nucleic acid and inhibiting amplification reaction. Additionally, *Salmonella* detection is complicated by the same DNA fragment present in dead and living cells. Moreover, the requirements for pricey equipment and reagents are undeniably one of the most significant drawbacks of the PCR-based method.

LAMP, a novel nucleic acid amplification method, was invented by Notomi and colleagues in 2000 [10,70,71]. It is an advanced alternative to PCR that is widely used for the rapid detection of *Salmonella* within foods in isothermal conditions, especially in under-equipped laboratories [10,70]. The mechanism behind this assay is the auto-cycling ability of *Bst* DNA polymerase. A set of 4 to 6 primers bind with different regions on

the targeted gene; hence, it can amplify several copies of DNA, ensuring high-specificity results. Besides this, it takes a lesser amount of time in terms of amplification period as it can amplify the target DNA until 10^9 copies are created within an hour, which is less than a normal PCR assay that requires 1–2 h [10]. There are still some limitations to the LAMP assay as it requires a complicated primer design (4 to 6 primers, comparing 2 of the primers in a PCR assay) [10]. Moreover, it may result in a high chance of false-positive results as there is a high risk of carryover contamination [72].

In recent years, researchers have gained more interest in biosensor detection methods due to their rapidity, high sensitivity, portability as a small device, and real-time detection. A biosensor consists of biorecognition elements (enzymes, antibodies, aptamers, cells, antigens, etc.), transducer components (optical, electrochemical, mass-based, etc.), and the electronic systems needed to display the measurable signal. The detection of *Salmonella* in food usually employs an electrochemical biosensor since it is more rapid, easy to use, cost-effective, and offers easy miniaturization as a portable device [11]. Extensive reviews on the application of biosensors for *Salmonella* detection have been published by several researchers [11,61,73].

As stated above, both culture-based and more rapid *Salmonella* detection methods have their pros and cons. The researchers must determine the most appropriate detection method for each sample and situation to obtain the most accurate result in the quickest time possible.

6. Conclusions

Chicken has been reported to be *Salmonella*'s main reservoir. The prevalence and serovars of *Salmonella* spp. in chicken varies according to region. Compared to developing or underdeveloped countries, the prevalence of *Salmonella* in developed countries is lower. Contamination with *Salmonella* spp. in chicken and poultry products from the wet markets was higher than those from supermarkets. The difference in the occurrence of *Salmonella* spp. in each country could be related to hygiene practices, government efforts, regulation administration, cross-contamination in the handling process, and sample type. Meanwhile, antimicrobial resistance was abundant in *Salmonella* isolates from chicken and poultry products. *Salmonella* resistance toward ampicillin (AMP) was observed in all countries. Moreover, MDR was identified in numerous different countries. These quantitative results indicated that the emergence of antimicrobial resistance in chicken and poultry products is a severe problem for food safety due to antimicrobial abuse in the poultry food chain. The culture method, using selective media for *Salmonella* detection, has been recognized as the gold standard. However, this method has many drawbacks, such as being time-consuming, laborious, less sensitive, etc., which hampered the efficacy of cultural-based *Salmonella* detection, especially in foods. Therefore, current detection methods, which include immunological and molecular-based assays, have been developed to reduce the detection time and gain better sensitivity and accuracy. Recently, the biosensor method has been garnering more interest among researchers, due to its rapid detection ability and its potential to be used on site as a portable device.

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