The control of poultry salmonellosis using organic agents: an updated overview

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ABSTRACT *Salmonellosis* is a severe problem that threatens the poultry sector worldwide right now. *Salmonella gallinarium* and *Salmonella pullorum* (Fowl typhoid) are the most pathogenic serovars in avian species leading to systemic infection resulting in severe economic losses in the poultry industry. Nontyphoidal serotypes of *Salmonella* (Paratyphoid disease) constitute a public health hazard for their involvement in food poisoning problems in addition to their zoonotic importance. Also, *Salmonella* species distribution is particularly extensive. They resisted environmental conditions that made it difficult to control their spread for a long time. Therefore, the current review aimed to through light on *Salmonellosis* in poultry with particular references to its pathogenesis, economic importance, immune response to *Salmonella, Salmonella* antibiotics resistance, possible methods for prevention and control of such problems using promising antibiotics alternatives including probiotics, prebiotics, symbiotics, organic acids, essential oils, cinnamaldehyde, chitosan, nanoparticles, and vaccines.

Key words: antibiotic resistance, organic feed additives, poultry, *Salmonella*, vaccine

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INTRODUCTION

In 1885, an American scientist called Daniel E. Salmon isolated the enteric pathogen from the pig's intestine. In 1900 to honor Daniel Salmon, a French bacteriologist called Joseph Léon Marcel Lignières proposed that the swine cholera be named "*Salmonella*" (White, 1926) then *Salmonella* has been isolated from water, soil, and sewage (Li et al., 2013). *Salmonella enterica* serotype *enteritidis* is one of *Salmonella* most

List of abbreviation: S. Salmonella; *S. enteritidis*, *Salmonella enteritidis*; *S. heidelberg*, *Salmonella heidelberg*; *S. typhimurium*, *Salmonella typhimurium*; *S. infantis*, *Salmonella infantis*; *S. lille*, *Salmonella lille*; *S. kentucky*, *Salmonella kentucky*; *S. senftenberg*, *Salmonella senftenberg*; *S. mbandaka*, *Salmonella mbandaka*; *S. montevideo*, *Salmonella montevideo*; *S. schwarzengrund*, *Salmonella schwarzengrund*; *S. anatum*, *Salmonella anatum*; *S. berta*, *Salmonella berta*; *S. newport*, *Salmonella newport*; *S. javiana*, *Salmonella javiana*; *S. pullorum*, *Salmonella pullorum*; *S. gallinarum*, *Salmonella gallinarum*; subsp., Sub-species; *E. coli*, *Escherichia coli*; *C. butyricum*, *Clostridium butyricum*; *C. perfringens*, *Clostridium perfringens*; *L. salivaris*, *Lactobacillus salivaris*; *C. jejuni*, *Campylobacter jejuni*; *L. acidophilus*, *Lactobacillus acidophilus*; *L. reuteri*, *Lactobacillus reuteri*; *B. bifidum*, *Bifidobacterium bifidum*; *E. faecium*, *Enterococcus faecium*; WHO, World Health Organization; CDC, Center for Disease Control and Prevention; USDA, U.S. Department of Agriculture; LITAF, Lipopolysaccharide Induced TNF Factor; IL-1ß, Interleukin -1ß; IFN- γ, Interferon gamma; TNF-α, Tumor Necrosis Factor Alpha; IL-10, Interleukin 10; Th2, T helper 2; IgA, Immunoglobulin A; IgG, Immunoglobulin G; IgM, Immunoglobulin M; NARMS, The National Antimicrobial Resistance Monitoring System; SCFAs, Short-chain fatty acids; RBCs, Red blood cells; °C, Celsius; RNA, Ribonucleic acid; DNA, Deoxyribonucleic acid

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isolated nontyphoidal serotypes. It can lead to a Salmonellosis infection, with variable symptoms including diarrhea, vomiting, and fever when infecting humans. In the United States, over 40,000 cases of Salmonellosis and 400 deaths due to acute Salmonellosis have been reported every year (Fabrega and Vila, 2013; Abd El-Hack et al., 2021a).

Bacteriologically, *Salmonella* is one of the Enterobacteriaceae family, which are known intracellular, Gram-negative motile bacilli with peritrichous flagella all over the entire cell body diameters, ranging from 0.7 to 1.5 μm, lengths from 2 to 5 μm, nonspore-forming, facultative anaerobe with optimal growth at 37°C in a pH range of 4 to 9 and biochemically, characterized by hydrogen sulfide and catalase production, and lack of oxidase enzyme as well as lactose fermentation property (Coburn et al., 2007; Li et al., 2013; Figure 1). They are heat-labile and killed at 70°C; however, they can survive in dust and harsh environmental conditions for 2 yr and more (Davies and Wray, 1996). *Salmonella* can be classified into 2 species, *Salmonella bongori* and *Salmonella enterica*. Further, *Salmonella enterica* is subdivided into 6 subspecies: *S. enterica* subsp. *enterica, S. enterica* subsp. *salamae, S. enterica* subsp. *arizonae, S. enterica* subsp. *diarizonae, S. enterica* subsp. *houtenae*, and *S. enterica* subsp. *indica* (Lan et al., 2009).

Salmonella isolates are most usually identified according to their serotype. *Salmonella* serotyping is based on identifying somatic antigen (oligosaccharide component of the lipopolysaccharide located on the outer membrane) and H flagellar antigen found in the bacteria's flagella (Brenner et al., 2000; Eng et al., 2015). *Salmonella enterica* serotype *enteritidis* is the most common serovar of *Salmonella,* which has less pathogenic effects in avian species but can lead to severe clinical Salmonellosis when transmitted to humans (Tarabees et al., 2017). *Salmonella* has a wide host range with public health concerns and a short incubation period from 8 h to 3 d. The disease severity increased in immune-suppressed, younger, and elderly hosts in the shape of variable clinical signs as nausea, abdominal pain, diarrhea, dehydration, and death (D'Aoust, 1991; WHO, 2015).

Salmonella-contaminated animal products resulted in 3% of the bacterial foodborne disease worldwide, with about 80 million infections and 155,000 deaths (Bell and Kyriakides, 2002; Majowicz et al., 2010; Abd El-Hack et al., 2021a). Salmonellosis is closely related to poultry production, and many efforts are being made to control this pathogen and reduce its spread during the different stages of poultry production (CDC, 2013c, d). Birds can consume contaminated rations with *Salmonella* and not exhibit any clinical illness, but later, these birds can contaminate processing facilities during evisceration and contaminate poultry carcasses with *Salmonella* causing human health hazards (Wibisono et al., 2020). To reduce *Salmonella* loads during poultry processing, it is essential to maintain *Salmonella*-free chickens and biosecurity practices in different poultry operations such as ration formulation, poultry farming, transportation, poultry processing should be adopted (Bailey, 1993) (Table 1. This review article highlights the hazards of poultry Salmonellosis and the use of organic control agents to cope with it.

Salmonella *Infection and Poultry Industry*

The poultry industry has been threatened by different pathogenic infections due to viral, bacterial, parasitic, or fungal origin (Setta et al. 2018; Attia and Salem, 2022; Salem et al. 2021, 2022; Soliman et al. 2021). Salmonellosis is a disease condition due to *Salmonella* infection, a severe problem facing the poultry industry worldwide

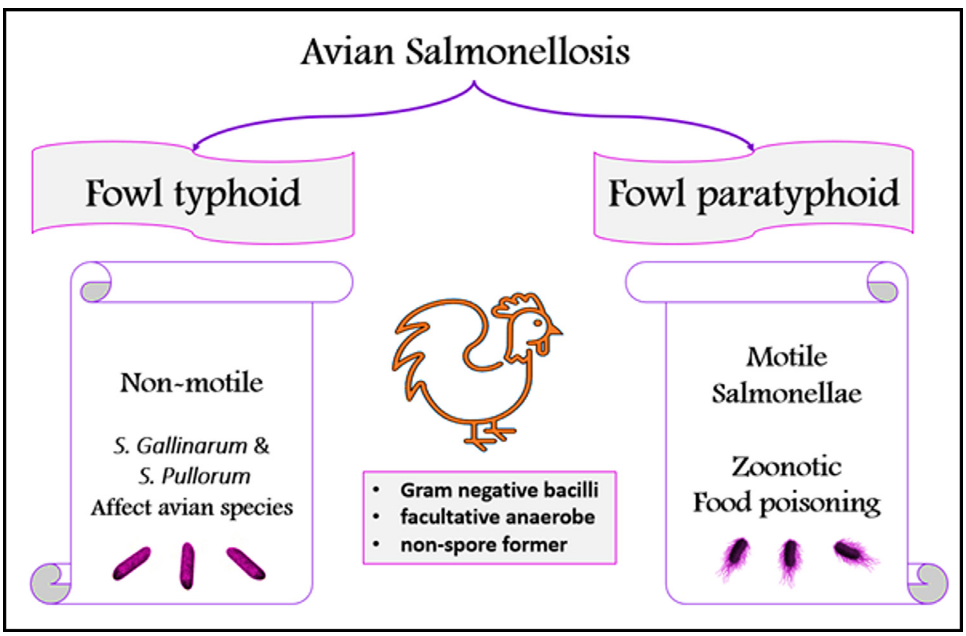

Figure 1. Avian *Salmonellosis* and its bacteriological characters.

(USDA, 2015). *Salmonella* is usually disseminated through chicken meat and eggshell contamination by chicken intestinal contents (Braden, 2006; Pires et al., 2014). Because eggs are more likely to contact the chickens' droppings, the number of laying hens kept in cage-free systems increases the danger of *Salmonella* contamination in eggs feces (Whiley and Ross, 2015). There are 2 main routes of egg contamination by *Salmonella enteritidis*, horizontal transmission; eggs can be contaminated by penetration through eggshell from the colonized chicken intestine or contaminated droppings during or after egg laying (de Reu et al., 2006) vertical transmissions, such as direct infection of the yolk, albumen, eggshell membranes, or eggshells before egg-laying, originating from the infection of reproductive organs with *Salmonella enteritidis* (Wibisono et al., 2020).

There are many types of *Salmonella* species infecting avian species. *Salmonella pullorum* and *Salmonella gallinarum* were found to be avian specific species. However, infections with other low avian-specific serovars such as *S. typhimurium, S. heidelberg, S. enteritidis, S. infantis, S. lille, S. kentucky, S. senftenberg, S. mbandaka, S. montevideo, S. schwarzengrund, S. anatum, S. berta, S. newport, S. javiana,* and another *Salmonella* serovars have been isolated from clinical poultry cases (Erdman et al., 2009; Center for Disease Control and Prevention CDC 2011; CDC, 2013e; CDC, 2013c). *Salmonella* infection is transmitted vertically and horizontally in poultry, and its incidence is high in one-day-old chicks (Shivaprasada et al., 2013; Figure 2). The severity of *Salmonella* infection is varied according to many factors, including hostage, host immunity, presence of coinfections, environmental, stress, managerial factors, and infective dose, with older birds less susceptible to Salmonellosis even with concentrations of 10^6 CFU/mL *S. typhimurium* (Wibisono et al., 2020).

Pathogenicity and Immune Response to Salmonella

Salmonella enters the host via the oral route and colonizes within the alimentary tract. Then *S. enteritidis* is attached to the intestinal villi and colonized in the presence of a group of proteins known as adhesins (Beachey, 1981). Therefore, the most effective technique for preventing bacterial infection may prevent *S. enteritidis* from attaching to intestinal epithelial cell receptors (Wizemann et al., 1999). In chickens, the innate immunity cellular component, which comprises macrophages and heterophils, is critical in preventing intestinal infection (Van Immerseel et al., 2005; Fasina et al., 2010).

When enteric pathogens like *Salmonella* enter the intestinal epithelial barrier, innate immune cells are drawn to the infection site and use processes like phagocytosis and oxidative burst to kill these pathogens (Brisbin et al., 2008). The 2 blind ceci, have the highest population of *Salmonella* in the bird intestine (Sivula et al., 2008). Immunohistochemical examination of the cecal lamina propria showed heterophils infiltration the cecal lamina propria 12 h after *Salmonella* infection. It increased T lymphocyte infiltration in the cecal lamina propria 20 after *Salmonella* infection (Van Immerseel et al. (2005)). *Salmonella* infection in chickens includes an influx of inflammatory cytokines involving LITAF, IL-1ß, and IFN-γ (Sheela et al., 2003; Matulova et al., 2013). *Salmonella* overcomes the host defense by increasing the suppressive cytokine, IL-10 (Ghebremicael et al., 2008).

The adaptive immune response to *Salmonella* infection involves both humoral and cell-mediated responses. The first adaptive immune defense against *S. enteritidis* infection is the mucosal immune system, including mucosal immunoglobulin A (IgA) and mucosa-associated lymphocytes and leukocytes (Wigley, 2014).

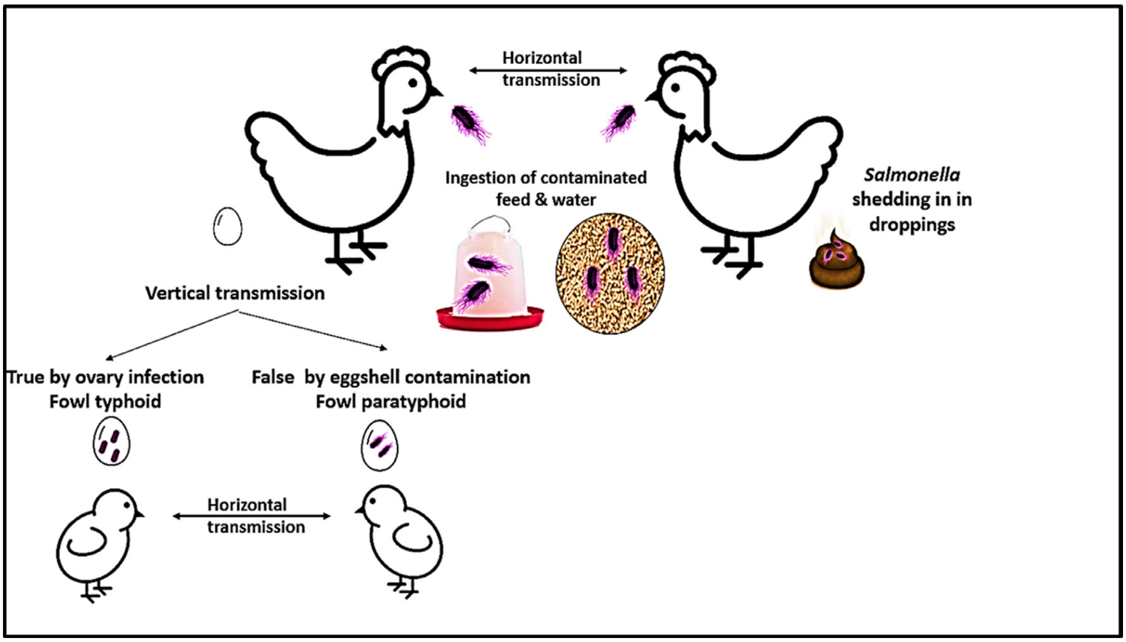

Figure 2. Transmission of avian *Salmonellosis.*

Secretory IgA prevents *S. enteritidis* from adhering to intestinal epithelial cells, limiting its mucosal colonization (Shroff et al., 1995; Wigley, 2014). The shape of the intestine is a crucial indicator of intestinal health. Because of the greater surface area and lower tissue turnover rate, increased villus height and decreased crypt depth correspond with increased nutrient absorption within the small intestine (Munyaka et al., 2012). Chicks infected with *S. typhimurium* revealed decreased jejunal villus height, crypt depth, and height: crypt depth ratio resulting in severe enteritis (Fasina et al., 2010; Borsoi et al., 2011).

Control and Prevention of Avian Salmonellosis

Antibiotic Growth Promoters Dietary antibiotics have been used at subtherapeutic levels (antibiotic growth promoters) to promote feed efficiency and sustain animal health for more than 50 yr (Danzeisen et al., 2011; Abd El-Hack et al., 2021b) and to overcome diarrhea and respiratory affections, which are devastating problems facing the poultry industry (Abd El Hamid et al., 2019; Marouf et al., 2020,Marouf et al., 2022; Salem and Attia, 2021). Antibiotics' effects on feed efficiency are thought to be due to their manipulation of the gut microbiota. In the digestive tract of chickens, pathogenic bacteria such as *Escherichia coli, Salmonella* species, and *Clostridium perfringens* compete for nutrients with the host (Swelum et al., 2021; Abd El-Hack et al., 2022a).

They may damage the intestinal epithelium, resulting in impaired digestion and absorption inside the host (Adil et al., 2010). Subtherapeutic antibiotic doses suppress pathogenic intestinal bacteria, lowering disease incidence and, as a result, promoting avian growth (Danzeisen et al., 2011). On the other hand, antibiotic growth promoters are becoming less popular due to worries about the development of microbial antibiotic-resistant (Danzeisen et al., 2011).

Salmonella Antimicrobial Resistant *Salmonella Enteritidis (S. Enteritidis)* is the most abundant serovar in poultry production, particularly chicken (Ray, 2004). Because of the massive use of antimicrobials in poultry, antimicrobial resistance of *Salmonella* has been developed to different antimicrobials as chloramphenicol, tetracyclines, streptomycin, sulfonamides, and Ampicillin (Ray, 2004). According to The National Antimicrobial Resistance Monitoring System (NARMS) laboratory from the Centers for Disease Control and Prevention (CDC), *Salmonella heidelberg* (*S. heidelberg*) linked with chicken was responsible for many human infections and these *Salmonella* serovars exhibited resistance to sulfisoxazole, chloramphenicol, ampicillin, kanamycin, gentamicin, tetracycline, and streptomycin. Also, nontyphoidal *Salmonella* showed multiple antimicrobial resistances, especially for ceftriaxone and ciprofloxacin. About 5% of *Salmonella* tested by the CDC has been shown to have resistance to at least one 5 different types of antimicrobials (CDC, 2015a). Recently, the world directed to limit the usage of antibiotics and encourage the use of organic antibiotic alternatives as prebiotics, probiotics, synbiotics, essential amino acids (Abou-Kassem et al., 2021a; Arif et al., 2021; Alagawany et al., 2021a), biologically synthesized nanoparticles, organic acids, essential oils, polyphenols (Saad et al., 2021a,b), bioactive peptides (El-Saadony et al., 2021a,b; Saad et al., 2021c), herbal extracts (Abou-Kassem et al., 2021b; El-Saadony et al., 2021c), enzymes (Llamas-Moya et al., 2019), bioactive plant compounds (El-Saadony et al., 2021d; Reda et al., 2021a), and phytogenic compounds (Abdelnour et al., 2020a,b; Ashour et al., 2020, 2021; Abd Elkader et al., 2021; Abdel-Moneim et al., 2021; Abd El−Hack et al., 2021c,d) to improve poultry performance and human health using safe and natural products.

Probiotics The World Health Organization defines probiotics as "live microorganisms that benefit the host when administered in suitable concentrations" (Mack, 2005). Probiotics are numerous and have different mechanisms of action as; competitive exclusion phenomena, the release of bacteriostatic and bactericidal agents, immune modulation, increased intestinal healthiness, lower intestinal pH, and improvement of intestinal mucosal barrier (Van Der Wielen et al., 2000, 2002; Yang et al., 2012; Alagawany et al., 2021b; El-Saadony et al., 2021e).

It was found that daily incorporation of poultry diet with probiotics such as *Lactobacillus reuteri* and *Lactobacillus salivarius,* can limit the population of *Salmonella* and *Campylobacter* in the intestine of 3 wk old chickens (Nakphaichit et al., 2011). Also, daily administration of *Bacillus subtilis,* can lower the *E. coli* population in the ileum of broiler chickens at 3 wk of age (Molnar et al., 2011). In addition, daily administration of *Clostridium butyricum* HJCB998 can limit the cecal *Salmonella* and *C. perfringens* population in the cecum of broilers from 3 to 4 wk of age (Yang et al., 2012). Multispecies probiotics have also been studied for their protective benefits, such as administering multispecies Probiotic, including "*Lactobacillus reuteri, Enterococcus faecium, Lactobacillus salivaris, Pediococcus acidilactici,* and *Bifidobacterium,*" can lower cecal coliform count (Mountzouris et al., 2010).

Another model supplying broiler chickens with multispecies probiotics, including *L. reuteri, Bifidobacterium animalis, L. salivaris, Pediococcus acidilactici,* and *Enterococcus faecium,* significantly limited cecal *Campylobacter jejuni* population in broiler chickens at 2 wk postinoculation (Ghareeb et al., 2012). When comparing probiotics supplemented in water to probiotics in feed, some studies have found that probiotics supplied in the water had a higher efficacy (Karimi Torshizi et al., 2010; Ritzi et al., 2014). The most used probiotics in the poultry field are described in the below sections.

Probiotics Mode of Action The "competitive exclusion" phenomenon occurs within the digestive system when microbes compete for resources such as nutrition and attachment sites (Nurmi et al., 1992). Pathogenic bacteria such as *E. coli* and *Salmonella* must first connect to the intestinal epithelial barrier to infect birds (Lan et al., 2005). To healthy birds, commensal bacteria populate the intestinal mucosa, generating a complex layer of beneficial bacteria that successfully blocks harmful bacteria from attaching to the mucosal surface and blocking the intestinal receptors (Lan et al., 2005).

Additionally, certain beneficial bacteria acquire a competitive advantage in the intestine by creating bacteriostatic or bactericidal chemicals that are toxic to pathogenic competitors. Also, lactic acid bacteria, as *Lactobacillus* species, ferment carbohydrates and liberate lactic acid, which lower intestinal pH and inhibit pathogens like *E. coli, C. perfringens*, and *S. typhimurium* in vitro (Murry et al. 2004; Abd El−Hack et al., 2020a) and this phenomenon was confirmed by in vivo studies as Van Der Wielen et al. (2000) found that when the concentrations of SCFAs (acetate, propionate, and butyrate) increased, the abundance of *Enterobacteriaceae* including *Salmonella* and *E. coli* in broilers cecum decreased. In addition, SCFAs in nondissociated form can penetrate pathogenic bacterial cell membrane into the cell where they dissociate, resulting in bactericidal and bacteriostatic effects on the pathogen (Van Der Wielen et al., 2000). The SCFAs are considered as an energy source of intestinal villi (den Besten et al., 2013), stimulate enterocyte growth and proliferation (Blottiere et al., 2003), regulate mucin secretion (Willemsen et al. 2003), and modulate intestinal immunity (Correa-Oliveira et al., 2016).

The SCFA production in broilers is undetectable in one-day-old chicks, whereas at 2 wk of age, the cecal microflora stabilizes, and the SCFAs reach high amounts and remain stable (Van der Wielen et al., 2002). Also, some probiotics can secrete bacteriocins, which are considered antimicrobials that can inhibit pathogenic bacteria (Dobson et al., 2012). *Lactobacillus salivarius* strains isolated from the chicken intestine produce bacteriocins that inhibit *S. enteritidis* and *C. jejuni* growth (Svetoch et al., 2011). *Enterococcus faecium, Pediococcus pentosaceus* and *Bacillus subtilis,* isolated from broiler chickens, secrete bacteriocins that inhibit *C. perfringens* (Shin et al., 2008). Additionally, *E. faecium* produces bacteriocins against the oocysts of *Eimeria* species (Strompfova et al., 2010). Probiotics stimulate the bird's immune response to prime the host immune system by interacting with immune cells within the bird's gut (Kamada and Nunez, 2014).

Many researchers found that the intestinal microbiota can influence the antibody-mediated immune response. For example, chickens receiving probiotics including *Streptococcus faecalis*, *Bifidobacterium bifidum*, and *L. acidophilus* had higher systemic antibody response to sheep red blood cells (RBCs) than unsupplemented chickens (Haghighi et al., 2006). It was expected that probiotics enhance the production of Th2 cytokines (e.g., IL-4 and IL-10), which may subsequently stimulate the immune response mediated by antibodies as chickens supplied with *Streptococcus faecalis, Lactobacillus acidophilus*, and *Bifidobacterium bifidum* had higher levels of serum IgG and IgM at 2 wk of age (Haghighi et al., 2006). *Lactobacillus* species could stimulate differential cytokine expression in T cells of chicken cecal tonsils (Brisbin et al., 2012). It was noticed that broiler chickens supplied with probiotics including *Bifidobacterium bifidum, L. acidophilus*, and *Streptococcus faecalis* after *Salmonella* experimental infection had a marked decrease in gene expression IL-12 and IFN- γ (Haghighi et al., 2006). The most used probiotics in the poultry sector were as follows:

Lactobacillus reuteri *L. reuteri* is a heterofermentative species of bacteria that can produce lactic acid, ethanol, acetate, hydrogen peroxide, carbon dioxide, reuterin, and retericylin (Yu et al., 2007). They resist heat, bile salts and low pH (Yu et al., 2007) and it has mucus binding proteins on their surface, which facilitates adherence to intestinal villi (Mackenzie et al., 2010). *L. reuteri* inhibits the growth *Staphylococcus epidermidis, Staphylococcus aureus, E. coli, S. Typhimurium, Helicobacter pylori*, and *rotavirus* (Seo et al., 2010). Additionally, chickens supplemented with 10^8 CFU/mL *L. reuteri* revealed reduced lesions score in birds infected with *C. perfringens*, suggesting that *L. reuteri* can modulate the innate immune response by regulating inflammatory cytokines and inhibiting *C. perfringens* proliferation (Cao et al., 2012). Furthermore, *L. reuteri* inactivated TNF-α production of lipopolysaccharide-activated monocytes and macrophages in vitro (Lin et al., 2008).

Pediococcus acidilactici *P. acidilactici* is considered a facultative anaerobe with optimal growth at pH 6.2, overnight at 37°C up to 65°C and less sensitive to acidic pH (Lin et al., 2006). *Pediococci* suppresses enteric pathogens' growth via the production of lactic acid and bacteriocins as pediocins (Daeschel and Klaenhammer, 1985). Dietary supplementation with *Pediococcus acidilactici*, mannan-oligosaccharide and butyric acid reduces colonization of S. *Typhimurium* and improves broiler chickens' growth performance (Jazi et al., 2018).

Enterococcus faecium *E. faecium* is a Gram-positive, facultative anaerobe, grows 10°C to 45°C, survives in both basic and acidic circumstances. *E. faecium* secretes bacteriocins (Kang and Lee, 2005). After vaccination, *E. faecium* improves layer chickens' immune and health status with live attenuated *S. Enteritidis* vaccine by improving gut microbiota (Beirão et al., 2018).

Bifidobacterium animalis *B. animalis* is anaerobic, Gram-positive, rod-shaped bacterium, optimum growth 42°C to 49°C, highly resistant to bile salts, tolerate both oxygen and heat during manufacturing of probiotic feed additives, found naturally in the intestines of rabbits, chickens, and humans (Scardovi and Zani, 1974; Simpson et al., 2005; Sanchez et al., 2007). Incorporating *Bifidobacteria* and *Lactobacillus* with broiler feed reduce the colonization of experimentally infected *Salmonella enterica* and improves broilers' body condition (El-Sharkawy et al., 2020).

Prebiotics Prebiotics are indigestible substances utilized explicitly by beneficial microbiota in the intestine (Cummings and Macfarlane, 2002; Yaqoob et al., 2021). Mannan-oligosaccharides or fructo-oligosaccharides are commonly used prebiotics in poultry ration and daily corporation of these prebiotics in broiler feed improves their performance and increases boy weight gain (Ao and Choct, 2013). Fructooligosaccharide daily incorporation with broiler feed limited the colonization of experimentally infected *Salmonella enteritidis* by increasing ileal mucosal thickness and elevating the transcription of ileal IL-1ß, IL-10, and interferon (IFN)-γ mRNA, increasing leukocyte numbers and serum IgY levels in response to LPS challenge (Shang et al., 2015).

In broiler chickens, fructooligosaccharide supplementation has been demonstrated to improve growth performance, improve innate and adaptive immunological response, increase small intestinal villi length, and boost beneficial intestinal bacteria colonization (Xu et al., 2003). Dietary supplementation with fructooligosaccharides alters the gut microbiota toward more beneficial bacteria, enhancing the production of short-chain fatty acids and the immune response to *Salmonella* (Shang et al., 2015). Chickens supplied with 0.25% to 0.5% fructooligosaccharidess showed improved weight gain (Xu et al., 2003; Shang et al., 2015). On the other hand, chickens supplied with 0.5% fructooligosaccharide did not differ in feed conversion of body weight gain compared to untreated ones (Kim et al., 2011) and these variations may be attributed to many factors as age, sex, breed, general health of the birds, environmental condition, hygienic measures, and inclusion level of the fructooligosaccharides (Shang et al., 2015).

Symbiotics Symbiotics incorporate probiotics and prebiotics to act in synergy in improving bird health (Pandey et al., 2015; Figure 3). The prebiotic serves as a nutrient source for the probiotic, increasing the probiotic's persistence in the bird gut and symbiotics are more effective than probiotics and prebiotics administered separately (Pandey et al., 2015). The combination between probiotics and 0.1% fructooligosaccharide decreased intestinal *Salmonella enteritidis* colonization in chicks than when used independently (Fukata et al., 1999). Also, supplementation of laying chickens with symbiotic products including *E. Faecium* and fructooligosaccharides resulted in higher egg production and eggshell quality than control ones at 57 wk of age (Radu-Rusu et al., 2010).

Organic Acids Organic acids are short-chain fatty acids (C1-C7) produced in the intestine by microbial fermentation of carbohydrates and considered weak acids that dissociate in water in a pH-dependent manner (Lueck, 1981). The acid dissociation constant is defined by the pKa, enhanced protonated acid, which decreases the polarity of the acid molecule, results in increased acid diffusion against the bacterial barrier and into the cytoplasm when the pH of its surroundings is reduced (Davidson and Taylor, 2007).

Microbial activity is influenced by organic acids in 2 ways, by lowering the pH of the bacterial cytoplasm, causing energy generation and control to become uncoupled. Furthermore, the dissociated acid anions accumulate in hazardous quantities (Davidson and Taylor, 2007). Also, organic acids lower proventriculus pH, resulting in increased proteolytic enzyme activity, increased protein digestibility, and suppressing pathogenic bacterial growth. Acetic acid, lactic acid, benzoic acid, formic acid, and propionic acid are the most common organic acids incorporated in poultry ration.

Many researchers confirmed that organic acids supplementation enhances the bird's growth and performance. For example, broilers supplemented with a mixture of benzoic, acetic, and formic acids showed improved feed intake, weight gain, and feed conversion ratio (Fascina et al., 2012). Also, the addition of organic acid in salt form (0.5% of sodium propionate or calcium propionate) improved egg production, egg weight, and FCR in layer chickens (Dahiya et al., 2016). Furthermore, the researchers found that adding 0.5% acetic, lactic, or formic acid in drinking water limits *S. Typhimurium* propagation in the chicken crop (Byrd et al., 2001).

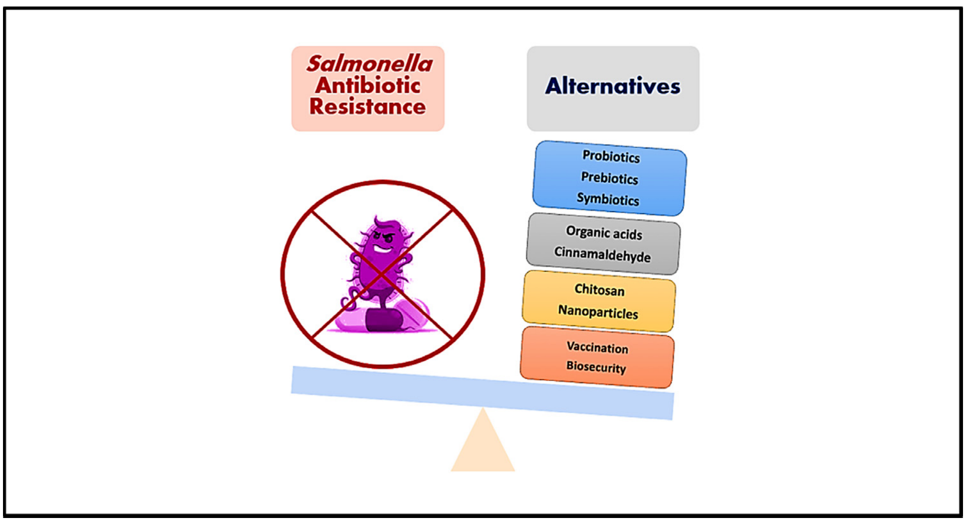

Figure 3. Impact of probiotics, prebiotics, and symbiotics on *Salmonella* infection and bird status.

The effects of organic acids on the immune system remain unclear. However, there is evidence that organic acids can modulate intestinal microbiota, required for immune system stimulation. Also, organic acids limit pathogen propagation (Van der Wielen et al., 2002; Khan and Iqbal, 2016). Lee et al. (2017) found that broiler chickens vaccinated with H9N2 vaccine and supplemented with organic acid blend, including lactic, citric, and formic acids showed increased regulatory T cells and decreased H9N2-specific antibodies.

Acetic Acid Acetic acid is a monocarboxylic acid and is considered as second simplest carboxylic acid (after formic acid). Vinegar is no less than 4% acetic acid by volume, making acetic acid the main component of vinegar apart from water and other trace elements. In this concentration, acetic acid is considered safe for usage. However, acetic acid has a pungent odor and taste, which often limits its use in foods restricting its use. Alvarez-Ordonez et al. (2010) compared growing *Salmonella* in a culture medium with a pH of 4.25 with acetic, citric, lactic, and hydrochloric acids and found that acetic acid had the highest antimicrobial activity (followed by lactic, citric, and hydrochloric acid). Zhou et al. (2007) reported a synergistic effect of acetic acid (0.10%) and essential oils thymol and carvacrol (100 uL/L each) for in vivo inhibition of *S. typhimurium.*

Lactic Acid Lactic acid (2-hydroypropanoic acid) is a monocarboxylic acid with a pKa of about 3.8. It is produced during anaerobic respiration by fermentation of many bacteria, mainly lactic acid-producing bacteria as; *Lactobacillus, Bifidobacterium, Streptococcus*, and *Pediococcus* (Kim et al., 2005). Lactic acid occurs in 2 isomeric forms (D-, L-). The L isomer is more effective in pathogens suppression (Leitch and Steward, 2002).

Propionic Acid Propionic acid and its salts, calcium, and sodium propionate are considered safe substances for usage. Propionic acid has a pKa of 4.88 (Serjeant and Dempsey, 1979). Chung and Goepfert (1970) found that propionic acid at pH 5.5 has a higher antibacterial effect against *Salmonella* than acetic, succinic, lactic, fumaric, and citric acids. Cherrington et al. (1990) observed that at a concentration of 5 mM, propionic acid decreased the rate of RNA, DNA, protein, lipid, and cell-wall synthesis of *Salmonella.*

Salmonella and Organic Acids Resistance The bactericidal effects of weak organic acids rely on their concentration, surrounding pH, and the dissociation constant of the acids. Nonoptimal organic acid handling risks the development of adapted or resistant strains of pathogens (Foster and Hall, 1991). *Salmonella* can accommodate acidic conditions and survive in pH extremes (Foster and Hall, 1991). *Salmonella's* acid tolerance reaction can protect it against the effect of the organic acids, especially at low pH values (Foster and Spector, 1995). *Salmonella* has many inducible amino acid decarboxylases, including (lysine decarboxylase, lysine cadaverine antiporter, and regulatory proteins) in which the *Salmonella* organic acid-induced tolerance contributed to them (Foster and Spector, 1995).

Essential Oils Essential oils are natural, aromatic, safe, volatile compounds, and oily fluids extracted from different plant parts (Bakkali et al., 2008; El-Tarabily et al., 2021; Abd El-Hack et al., 2022b). Essential oils are efficient growth promoters, hypolipidemic agents, digestive stimulants, immune-stimulants, antioxidants, antimicrobial, antifungal, and antiparasitic substances and they have a good impact on broiler performance and egg production (Jamroz et al., 2005; Alagawany et al., 2021c; El- Shall et al., 2022). Lemongrass (*Cymbopogon citratus*) extract shows potent antibacterial activity against different pathogenic bacteria as *S. typhimurium, S. enterica* (Singh and Ebibeni, 2016; Alagawany et al., 2021c). Also, the essential oils obtained from oregano and thyme effectively reduced *Salmonella* species colonization in the chicken gastrointestinal tract (Koščová et al., 2006). In addition to, trans-cinnamaldehyde, eugenol, thymol, and carvacrol have antibacterial effects against *Salmonella* and *Campylobacter* in both broiler and layer chickens (Kollanoor-Johny et al., 2010).

Cinnamaldehyde Cinnamaldehyde is an aldehyde found in the bark of cinnamon trees that gives cinnamon its odor and it has anti-inflammatory, antibacterial and antifungal characters (de Cássia da Silveira e Sá et al., 2014). Cinnamaldehyde showed broad-spectrum antimicrobial activity via different pathways, including inhibition of glucose utilization and disruption of membranes permeability (Gill and Holley, 2004). Cinnamaldehyde has antibacterial effects against *S. Enteritidis* and found that 10 mM cinnamaldehyde lowered *S. Enteritidis* propagation in chicken cecal content (Kollanoor-Johny et al., 2010). Cinnamaldehyde has also been used in the diet to protect chickens from gastrointestinal infections and it was found that daily supplementation of 0.5% or 0.75% of cinnamaldehyde to broiler chicks showed a marked decrease in cecal *S. Enteritidis* count (Kollanoor-Johny et al., 2012).

Chitosan Chitosan is a sugar found in the hard outer skeletons of shellfish such as crabs, lobsters, and shrimp. It is applied widely in the medical field (Abd El-Hack et al., 2020b; Attia et al., 2021)**.** Chitosan mode of action was described in a prevalent theory shows and chitosan molecules (amino group at second carbon atom) and the negative charge of bacterial cell membranes alters bacterial cell permeability (Friedman and Juneja, 2010). Also, the chelation of metals and vital nutrients for bacteria by chitosan molecules enable it to suppress bacterial growth (Rabea et al., 2003). Chitosan is described as an immune-modulating agent that enhances host immune response against pathogens (Lee et al., 2008; Lee et al., 2009). In vitro studies showed that chitosan has an antibacterial effect against *S. paratyphi Staphylococcus aureus* (Isalm et al., 2011). Chitosan 0.2% showed both in vitro and in vivo antibacterial activity against *Salmonella enterica* serovar *typhimurium* infection in broiler chicks and could be applied to reduce crop, cecal, and consequently *S. typhimurium* carcass contamination as well as decreasing its shedding in the surrounding environment (Menconi et al., 2014).

Nanotechnology and* Salmonella *Control Nanomaterials have attracted much attention for developing new biotechnology approaches because of their unique physical and chemical features (Sheiha et al., 2020; Reda et al., 2020; Yousry et al., 2020; El-Saadony et al., 2021e,f,g; Salem et al., 2021a). Fe_3O_4 magnetic nanoparticles (Fe_3O_4-NPs) could effectively reduce the viability of intracellular *S. Enteritidis* in chicken cells (Shen et al., 2020). Silver nanoparticles produced by Rosemary aqueous extracts as a cheap, eco-friendly technique showed antibacterial effects against *S. typhimurium, S. enteritidis,* and *E. coli* O78 (Mohamed et al., 2017).

Using zinc oxide nanoparticles with a concentration of 3% (mg g^{-1}) has a bactericidal effect against *S. typhimurium* and *Staphylococcus aureus.* It could be incorporated with poultry ration to protect them from serious pathogens (Silva et al., 2021). Gold nanoparticles revealed antibacterial effects against *S. typhimuruim* (El Sabry et al., 2018; Reda et al., 2021b; Abd El−Hack et al., 2021d). Also, Boatemaa et al. (2019) confirmed the in-vitro inhibitory effect of gold nanoparticles against *S. typhii* and *S. paratyphi.* Recently, thymol nanoemulsion provoked a good antibacterial activity against *S. typhimurium* infection in broilers and succeeded in decreasing *S. typhimurium* count. At the same time, *Lactobacilli* numbers increased in the chicken intestine and consequently increased weight gain and general health (Ibrahim et al., 2021). Furthermore, garlic and onion extract chitosan nanoparticles showed limited colonization of pathogenic enteric bacteria, *E. coli, S. typhi*, and *C. jejuni,* while the number of beneficial intestinal microbiota was increased in rainbow rooster chicken (Enoka et al., 2021).

Salmonella *Vaccines* *Salmonella* vaccines are routinely used in hens to reduce *Salmonella* infection (Figure 4). The efficacy of vaccines is evaluated by the level of pathogen propagation, morbidity, and mortality rates of the vaccinated bird's subsequent experimental infection (Barrow, 2007). Many factors influence vaccine efficacy, such as challenge bacteria, route of administration, dose, age of birds, species, breed, environmental condition, hygienic measures, and vaccine handling and storage (Barrow, 2007). Thus, it is hard to compare the efficacy of currently available vaccines (Barrow, 2007). Many types of vaccines protect birds against *Salmonella* infection in the form of bacterins, attenuated, subunit vaccines, and nanoparticles-based vaccines.

Bacterins Poultry vaccination is an effective tool for controlling and preventing diseases (El-Naggar et al., 2022). Bacterins are entire bacteria that have been killed and have had varying degrees of protection against *Salmonella* (Davison et al., 1999). Vaccinating hens with an oil-emulsion bacterin of *S. enteritidis* at 38 wk (booster one mo later) showed lower *S. enteritidis* shedding and colonization in the ovary and spleen (Miyamoto et al.,1999). Also, vaccinated chickens at 2 wk of age with formalin-inactivated *S. Enteritidis* encapsulated in biodegradable microspheres lowered *S. enteritidis* fecal shedding and organ colonization (Liu et al., 2001).

However, other studies using bacterins only without adjuvants have shown no effects in lowering *Salmonella* count. Davison et al. (1999) found that layer flocks vaccinated with *S. enteritidis* bacterins between 14 and 20 wk of age revealed no *Salmonella* colonization compared to the control flock. In another study, commercial breeding chicken farms vaccinated with the killed *Salmonella* vaccine showed higher antibody titers, but vaccination did not reduce *Salmonella* shedding (Berghaus et al., 2011).

Live Attenuated Vaccines Live attenuated Salmonella vaccines undergo attenuation through negative mutations of essential enzymes, resulting in a prolonged generation period and lowered pathogenicity (Linde et al., 1998). They are frequently preferred over bacterins because they are easy to administer and generate mucosal, cellular, and humoral responses (Lalsiamthara et al., 2016). Attenuated *S. enteritidis* vaccine orally inoculated in chickens showed decreased *Salmonella* colonization in the ceca, liver, and spleen (Cerquetti and Gherardi, 2000).

In contrast, Groves et al. (2016) administered commercial oral live *Salmonella* vaccine to layer chickens and reported that the vaccine failed to decrease *Salmonella* colonization in the ceca. Also, concerns about the safety of live vaccinations exist for the consumers' health (Zhang-Barber et al., 1999, Lauring et al., 2010).

The Subunit or Cellular Vaccines They include immunogenic parts or antigens of the bacteria, which may offer a more effective alternative to killed and live attenuated vaccines (Sharma and Hinds, 2012). *Salmonella* immunogenic parts were cleared using electrophoresis after evaluating antigens that stimulated strong B and T lymphocyte responses in immune-blot and western blot techniques (Vordermeier and Kotlarski, 1990). These immunogenic antigens include outer-membrane proteins, lipopolysaccharides, flagellate epitopes, and fimbriae (Ochoa et al., 2007). Subunit vaccines have a variable level of protection, although low resistance levels against *Salmonellosis* have been found (Tennant and Levine, 2015). However, subunit vaccines, including the outer membrane proteins, have successfully been used to limit *S. enteritidis* infection in poultry.

Vaccinated 9-wk-old chickens with subunit vaccine including 2 outer membrane proteins, followed by 2 boost doses with time intervals of 15 d, showed decreased *Salmonella* colonization in the ceca (Khan et al., 2003). Vaccine adjuvants and delivery systems stimulate humoral, cellular, and mucosal immunological responses, strengthening the immune system. Successful delivery systems are biodegradable, cost-effective, and easy to process, with few adverse effects on the bird (Tiwari, 2012).

Figure 4. Different types of *Salmonella* vaccines.

Nanoparticles-Based Salmonella Vaccines They are a promising technology that helps eliminate *Salmonella* infection in the poultry sector (Abd El-Ghany et al., 2021). Nanoparticles are very small-sized copolymer delivery systems that preserve antigens against chemical, enzymatic or immunological destruction, thus facilitating targeting and presentation of antigens to particular locations of the mucosal immune system (Tiwari, 2012; Yehia et al., 2021; Alagawany et al., 2021d).

The vaccine antigen encapsulated with the nanoparticles and encapsulated with the nanoparticles prevents the antigen from rapidly destruction after administration (Delgado et al., 1999). Also, conjugation of the antigens onto the nanoparticle surface can derive the vaccine to specific immune sites within the gut (Salman et al., 2005). For successful nanoparticle vaccine preparation, it is essential to characterize the size and composition of the nanoparticle to avoid any variation within the batches, which could arise from contamination, different nanoparticles size distribution, the accumulation of toxic elements, or incomplete particle formation; thus, nanoparticle's uniformity is a serious point to ensure homogenous antigen loading efficiency between each nanoparticle (Gregory et al., 2013).

Salman et al. (2005) designed "*Salmonella*-like" nanoparticles vaccine by conjugating *Salmonella enteritidis* flagellin to the nanoparticle's surface to mimic the natural colonization of *S. enteritidis* in the gut. The flagellin ligands conjugated with nanoparticles successfully stimulated specific intestinal mucosa uptake, including Peyer's patches (Salman et al., 2005). Orally applied *Salmonella* subunit vaccine including immunogenic *Salmonella* outer membrane proteins (OMPs) and flagellar (F) protein-entrapped and surface F-protein-coated with polyanhydride nanoparticle in layer chickens revealed specific immune response and limited *Salmonella* colonization in the intestines (Renu et al., 2018). Oral administration of *Salmonella* subunit vaccine involving immunogenic outer membrane proteins and flagellin protein loaded and F-protein surface coated chitosan nanoparticles in chickens stimulate specific systemic IgY and mucosal IgA antibodies responses (Renua et al., 2020).

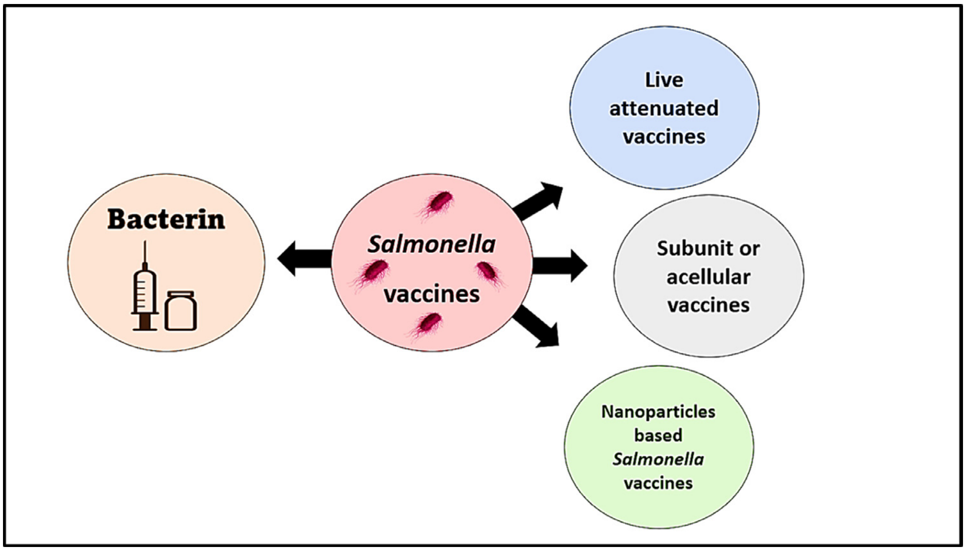

Figure 5. Different antibiotics alternative that used for *Salmonella* control in poultry.

Table 1. Most used antibiotic alternative treatment agents for *Salmonella* control.

Treatment agents	Bird	Impact	References
Lactobacillus reuteri	Broiler chicken	Suppress the growth of *Staphylococcus epidermidis, Staphylococcus aureus, E. coli, S. Typhimurium, Helicobacter pylori*, and rotavirus	Seo et al. (2010)
Lactobacillus reuteri	Broiler chicken	Inhibits *C. perfringens* propagation	Cao et al. (2012)
Pediococcus acidilactici, mannan-oligosaccharide, and butyric acid	Broiler chicken	Reduces the colonization of *S. Typhimurium* and improves bird weight gain.	Jazi et al. (2018)
Enterococcus faecium	Layer chickens	Stimulates antibody production following live attenuated *S. Enteritidis* vaccination by intestinal microbiota modulation.	Beirão et al. (2018)
Bifidobacteria and *Lactobacillus*	Broiler chicken	Limits colonization of *Salmonella enterica* and improve bird body condition	El-Sharkawy et al. (2020)
Fructo-oligosaccharide	Broiler chicken	Limits *Salmonella Enteritidis* colonization by increased ileal mucosa thickness and improving immune response	Shang et al. (2015) Xu et al. (2003)
E. Faecium and fructooligosaccharides	Layer chicken	Increase quality and quantity of eggs	Radu-Rusu et al. (2010)
Prebiotics	poultry	Increase body weight	Abd El-Hack et al. (2021a)
Acetic, lactic, and formic acid	Broiler chicken	Limits S. *Typhimurium* propagation in the chicken crop	Byrd et al. (2001)
Acetic acid 0.01, 0.1, and 1% concentration	*In-vitro*	Ineffective in reducing *S. Enteritidis* levels	Barnhart et al. (1999)
Sodium propionate or calcium propionate	Layer chicken	Improved egg production, egg weight, and FCR	Dahiya et al. (2016)
Cinnamaldehyde	Broiler chicken	Lowered *S. Enteritidis* propagation in chicken cecal content	Kollanoor-Johny et al. (2010)
Cinnamaldehyde and eugenol	Broiler chicken	Reduce *S. enteritidis* load	Kollanoor-Johny et al. (2012)
Cinnamaldehyde, eugenol, thymol, and carvacrol	In vitro Broiler chicken	Antibacterial effect against *S. enteritidis*	Kollanoor-Johny et al. (2010)
Trans-cinnamaldehyde and eugenol's	Broiler chicken	Decreased *S. enteritidis* colonization	Kollanoor-Johny et al. (2012a)
Oregano and thyme	Broiler chicken	Reduced *Salmonella* species colonization	Koščová et al. (2006)
Essential oils.	Eggs	Reduce shell egg contamination with *S. enteritidis*	Upadhyaya et al. (2013)
Lemongrass extract	In vitro	Inhibitory effect against *S. typhimurium S. enterica*	Singh and Ebibeni (2016)
Chitosan	Broiler chicken	Reduced *S. Typhimurium* colonization	Menconi et al. (2014)
Chitosan	In vitro	Antibacterial activity against *S. Paratyphi* and *Staphylococcus aureus*	Islam et al. (2011)
Chitosan	In vitro	Antibacterial activity against intracellular *S. Typhimurium*	Edson et al. (2021)
Insect chitosan	Food safety studies	Antibacterial effect against *S. Typhimurium, E. coli* O157:H7 and *Listeria monocytogenes*	Ibañez-Peinado et al. (2020)
Chitosan and lactic acid	Chilled chicken	Control of *Salmonella* and *E. coli* contamination	El-Khawasa et al. (2020)
Fe_3O_4 magnetic nanoparticles	Broiler chicken	Reduced the viability of intracellular *S. Enteritidis*	Shen et al. (2020)
Silver nanoparticles	In vitro	Antibacterial effect against *S.Typhimurium, S. Enteritidis* and *E. coli* O78	Mohamed et al. (2017)
Zinc oxide nanoparticles	Broiler chicken	Bactericidal effect against S. *Typhimurium* and *Staphylococcus aureus*	SILVA et al. (2021)
Gold nanoparticles	In vitro	Inhibitory effect against *S. Typhimuruim, S. typhi*, and *S. paratyphi*	El Sabry et al. (2018); Boatemaa et al. (2019)
Thymol nanoemulsion	Broiler chicken	Antibacterial activity against *S. Typhimurium*	Ibrahim et al. (2021)
Sunflower oil nanoemulsion	In vitro	Antibacterial activity against foodborne bacteria such as *Salmonella typhi*	Joe et al. (2012)
Garlic and onion extract chitosan nanoparticles	In vitro	Minimize the colonization of pathogenic enteric bacteria especially, *E. coli, S. typhi,* and *C. jejuni*	Enoka et al. (2021)

Salmonella chitosan-nanoparticle vaccine, based on *S. enteritidis* outer-membrane-proteins and flagellin proteins, induced an antigen-specific immune response against *S. enteritidis* in ovo and lower *S. enteritidis* cecal count in broilers (Acevedo-Villanueva et al., 2021). Also, Renu et al. (2020) designed *Salmonella* subunit chitosan nanoparticles-based vaccine containing immunogenic outer membrane proteins and -flagellin protein for oral administration in laying hens, the prepared vaccine increased the expression of toll-like receptor (TLR)-2, TLR-4, IFN-γ, TGF-ß, and IL-4 mRNA expression in chicken cecal tonsils and lower *Salmonella* load. Figure 5 shows various antibiotics alternative that used for *Salmonella* control in poultry.

CONCLUSIONS

Salmonella infection is considered one of the most dangerous and common diseases spread worldwide and threaten the poultry industry and public health. Antibiotics use is declining in the poultry sector due to the rise of antibiotic-resistant. To avoid its residual effect on poultry meat consequentially, many alternatives have been used to overcome these problems to obtain safe, cheap, and organic poultry meat. Probiotics, prebiotics, symbiotics, organic acids, essential oils, cinnamaldehyde, chitosan, nanoparticles and vaccines are available alternatives for antibiotics and revealed promising results in control of avian *Salmonellosis* that guarantee the strategies for control and prevention of avian *Salmonellosis* in either developing or developed countries and provide safe and liable poultry meat for human consumption and fulfill the meat gap and deficit as a source of protein.

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DISCLOSURES

The authors declare no conflict of interest.

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