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Review

Microbiological Safety of Chicken Litter or Chicken Litter-Based Organic Fertilizers: A Review

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Abstract: Chicken litter or chicken litter-based organic fertilizers are usually recycled into the soil to improve the structure and fertility of agricultural land. As an important source of nutrients for crop production, chicken litter may also contain a variety of human pathogens that can threaten humans who consume the contaminated food or water. Composting can inactivate pathogens while creating a soil amendment beneficial for application to arable agricultural land. Some foodborne pathogens may have the potential to survive for long periods of time in raw chicken litter or its composted products after land application, and a small population of pathogenic cells may even regrow to high levels when the conditions are favorable for growth. Thermal processing is a good choice for inactivating pathogens in chicken litter or chicken litter-based organic fertilizers prior to land application. However, some populations may become acclimatized to a hostile environment during build-up or composting and develop heat resistance through cross-protection during subsequent high temperature treatment. Therefore, this paper reviews currently available information on the microbiological safety of chicken litter or chicken litter-based organic fertilizers, and discusses about further research on developing novel and effective disinfection techniques, including physical, chemical, and biological treatments, as an alternative to current methods.

Keywords: chicken litter; compost; organic fertilizer; poultry; pathogen; inactivation

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1. Introduction

Chicken litter is a mixture of feces, wasted feeds, bedding materials, and feathers [1,2]. Over 14 million tons of chicken litter is produced every year in the US, most of which is usually recycled and spread on arable land as a low cost organic fertilizer [3,4]. Poultry manure contains significant amounts of nitrogen because of the presence of high levels of protein and amino acids. Owing to its high nutrient content, chicken litter has been considered to be one of the most valuable animal wastes as organic fertilizer [5]. Chicken litter is also the source of human pathogens, such as *Salmonella*, *Campylobacter jejuni*, and *Listeria monocytogenes*, that can potentially contaminate fresh produce or the environment and are frequently associated with foodborne outbreaks [1,6]. Composting of poultry waste prior to the application to agricultural land as an organic fertilizer is usually recommended to control pathogens in the end products. Nonetheless, several studies have demonstrated that some pathogenic cells have the potential to persist in the finished compost and also compost-amended soil [7–9]. Another major concern for composting is the possibility of pathogen regrowth [10], indicating that a small population of pathogen that either survives the composting process or gets transferred from the environment may multiply to high populations under favorable conditions.

Currently, physical heat treatment (heat-drying after composting or without composting) is one of the most commonly applied techniques to reduce or eliminate potential pathogens in animal wastes [1,2]. The physically heat-treated chicken litter is recommended and used by produce growers. However, some pathogenic cells may have the potential to become acclimatized to the hostile environment during build-up or composting, cross-protecting them against subsequent high temperature treatment [11,12]. Therefore, some current guidelines for heat-treated animal wastes may not be sufficient to eliminate pathogens from the physically heat-treated chicken litter as organic fertilizer. Land spreading of contaminated chicken litter or chicken litter-based organic fertilizers (fertilizers derived from chicken litter sources) can also potentially lead to the introduction of foodborne pathogens into the food chain. Contamination of fresh produce with fecal pathogenic bacteria in the agricultural environment has been implicated as the main cause of numerous food poisoning outbreaks [13]. Therefore, to ensure the absence of pathogens in the fresh chicken litter, poultry compost, or the physically heat-treated chicken litter, additional approaches such as physical, chemical, and biological treatments, should be considered as another means for pathogen control. Moreover, nutrient retention, fuel cost, efficiency, capital cost, and environmental and regulatory policies will be the principle factors when it comes to making decisions on selected processing techniques [14].

Although the application of poultry litter for commercial farming has rarely been associated with foodborne outbreaks, enhanced consumer awareness of food safety issues has increased the scrutiny of agricultural practices. This review thus focuses on the microbiological safety of chicken litter or chicken litter-based organic fertilizers.

2. Pathogens and Antibiotic-Resistant Bacteria in Chicken Litter or Chicken Litter-Based Organic Fertilizers

Chicken litter contains a large and diverse population of microorganisms. Microbial concentrations in chicken litter can reach up to 10^{10} CFU/g, and Gram-positive bacteria, such as *Actinomycetes*, *Clostridia*/*Eubacteria*, and *Bacilli*/*Lactobacilli*, account for nearly 90% of the microbial diversity [15]. Pathogens in chicken litter represent the major group of bacteria of special interest to litter processors. A variety of pathogens can be found in chicken litter or chicken litter-based organic fertilizers, such as *Actinobacillus*, *Bordetalla*, *Campylobacter*, *Clostridium*, *Corynebacterium*, *Escherichia coli*, *Globicatella*, *Listeria*, *Mycobacterium*, *Salmonella*, *Staphylococcus*, and *Streptococcus* [15–20]. While different microbes display different metabolic activities within the litter environment, high levels of background microflora may interfere with the survival and growth of pathogens in chicken litter. Fully understanding the levels and prevalence of human pathogens in chicken litter or chicken litter based-organic fertilizers is essential for developing intervention strategies for controlling produce contamination on farms. As shown in Table 1, microbiological surveys have revealed the prevalence of some foodborne pathogens in chicken litter or chicken litter-based organic fertilizers, depending on pathogen species and serotype, chicken age, season, geographic area, farm handling practice, and so on [19,21–23]. For example, Li *et al*. [23] observed that fecal samples of 18-week-old layer birds had the highest prevalence of *Salmonella* (55.6%), followed by the 25- to 28-wk birds (41.7%), 75- to 78-wk birds (16.7%) and 66- to 74-wk birds (5.5%). Renwick *et al*. [22] surveyed randomly selected commercial broiler chicken flocks in Canada to determine flock and management factors associated with the prevalence of *Salmonella* contamination in the floor litter. They found that the prevalence of *Salmonella* in floor litter samples was significantly associated with the age of the flock and the region of Canada in which the flock was located.

Active surveillance data on foodborne diseases from the United States reveal that among pathogens associated with foodborne outbreaks, *Salmonella*, *E. coli* O157:H7, *Campylobacter*, and *L. monocytogenes* are responsible for the majority of outbreaks. *Salmonella* spp. is the most widely distributed pathogen in chicken litter with poultry and eggs remaining as the predominant reservoir. During 1998–2008, foodborne disease outbreaks caused by *Salmonella* were associated most commonly with poultry meat products (30%) and eggs (24%) [24]. Chicken eggs can be contaminated with *Salmonella* either horizontally or vertically. The contamination of egg shell can result from horizontal transmission, such as fecal contact [25]. And vertical transmission of *Salmonella* has been observed in infected ovaries, oviducts, or infected eggs [26]. Although only low numbers of *Salmonella* can contaminate eggs via the fecal route, these small populations cannot be ignored. Notably, *S*. Enteritidis, *S*. Typhimurium, or *S*. Heidelberg present in chicken feces may not only penetrate into the interior of eggs but also multiply during storage [27]. *Salmonella* is more frequently isolated from chicken litter or fecal samples as compared to other pathogens being investigated and its prevalence level can range widely from 0 to 100%. And the population of *Salmonella* in chicken litter can range from 4 to 1.1×10^5 MPN/g litter [6].

Table 1. Prevalence of foodborne pathogens in chicken litter or chicken litter based-organic fertilizers.

Pathogen	Year/Location	Sample source	Sample type	Sample size	Prevalence	References
Actinobacillus	N.A. [a]/Canada	Broiler, hen, and turkey	Litter samples	44	2%	[16]
Campylobacter	1995/US	19 broiler flocks	Fecal samples	948	86%–100%	[19]
	1996–1997/US	Poultry	Litter samples intended for dairy cattle feed from 13 dairy ranches	104	- [b]	[28]
	2001/US	9 broiler flocks	Fecal samples	450	80%–100%	[19]
	N.A./Australia	28 sheds of 28 broiler farms	Litter samples	60 sites/shed and three sets of 20 were combined	36%	[6]
Clostridium	N.A./Canada	Poultry	Litter samples	44	57%	[16]
	N.A./Nigeria	Layer	Litter samples	N.A.	+[c]	[20]
E. coli	1994–1995/US	Poultry	Litter samples	86 (64 composted, 18 not composted, 4 samples not analyzed)	- for *E. coli* O157:H7	[29]
	1996–1997/US	Poultry	Litter samples intended for dairy cattle feed from 13 dairy ranches	104	- for *E. coli* O157, 8%–15% for non-O157 *E. coli*	[28]
	N.A./Nigeria	Layer	Litter samples	N.A.	+	[20]
	N.A./Australia	28 sheds of 28 broiler farms	Litter samples	60 sites/shed and three sets of 20 were combined	100%	[6]
	2004–2007/US	Poultry	Samples of compost heaps with chicken litter or chicken carcasses	N.A.	26% surface and 6.1% internal samples (1st composting phase); absent in all samples (2nd composting phase)	[30]

Table 1. *Cont.*

Pathogen	Year/Location	Sample source	Sample type	Sample size	Prevalence	References
Listeria	N.A./Australia	28 sheds of 28 broiler farms	Litter samples	60 sites/shed and three sets of 20 were combined	-	[6]
	2004–2007/US	Poultry	Samples of compost heaps with chicken litter or chicken carcasses	N.A.	-	[30]
Mycobacterium	N.A./Canada	Poultry	Litter samples	44	5%	[16]
	N.A./Nigeria	Layers	Litter samples	N.A.	+	[20]
Salmonella	N.A./Canada	Poultry	Litter samples	44	7%	[16]
	N.A./US	Poultry from 5 premises	Litter samples	198	73%–89%	[31]
	1977/Canada	3 broiler flocks	Litter samples (top 1.27 to 2.54 cm layer)	N.A.	0%–2%	[32]
	1978–1979/Canada	60 broiler houses	Litter samples	15 from each house	30%	[33]
	1980–1981/Canada	Broiler	Litter and feces samples	36 and 2 for litter and feces samples, respectively	19%–89% and 0%–100% for feces and litter, respectively	[21]
	1989–1990/Canada	Broiler	Litter samples	12	76%	[22]
	1994–1995/US	Poultry	Litter samples (64 composted, 18 not composted, and no determination for 4 samples)	86	-	[29]
	1996–1997/US	Poultry	Litter samples intended for dairy cattle feed from 13 dairy ranches	104	-	[28]

Table 1. *Cont.*

Pathogen	Year/Location	Sample source	Sample type	Sample size	Prevalence	References
Salmonella	2002/Nigeria	5 poultry farms	Fecal samples	120	38%	[34]
	2006–2007/Hungary	Broiler	Fecal samples	60	35%–43%	[35]
	N.A./US	Hen	Fecal samples	78	17%–56%	[23]
	N.A./Nigeria	Layer	Litter samples	N.A.	+	[20]
	N.A./US	7 broiler farms	Fecal samples	420	6%–39%	[36]
	N.A./Australia	28 sheds of 28 broiler farms	Litter samples	60 sites/shed and three sets of 20 were combined	71%	[6]
	2004–2007/US	Poultry	Samples of compost heaps with chicken litter or chicken carcasses	N.A.	26% surface and 6.1% internal samples (1st composting phase); absent in all samples (2nd composting phase)	[30]
Staphylococcus	N.A./Canada	Poultry	Litter samples	44	100%	[16]
	1994–1995/US	Poultry	Litter samples (64 composted, 18 not composted, and no determination for 4 samples)	86	-	[29]
	N.A./Nigeria	Layers	Litter samples	N.A.	+	[20]
Streptococcus	N.A./Canada	Poultry	Litter samples	44	100%	[16]

[a] N.A., not applicable; [b] -, no pathogen or selected microorganism was isolated; [c] +, pathogen or selected microorganism was isolated.

E. coli is present in chicken litter with the prevalence rate as high as 100%; however, *E. coli* O157:H7 was not detected in chicken litter samples [28,29] or poultry compost samples [29,30]. The population of *E. coli* in reused chicken litter can reach up to 9.7×10^4 CFU/g while the population for single use litter has been found to be 4.2×10^5 CFU/g [6]. *Campylobacter*, followed by *Salmonella*, is the leading cause of bacterial gastroenteritis due to food consumption [37] and is also likely to be present in poultry wastes. The prevalence of *Campylobacter* in chicken litter or fecal samples can range from 0 to 100% and its average population level was reported to be *ca*. 10^5 CFU/g in fecal samples collected from broiler chicken flocks [19]. *L. monocytogenes* is usually absent (negative) in chicken litter and poultry compost, and this pathogen therefore appears not to be a significant issue in chicken litter or chicken litter-based organic fertilizers [6,30].

There are also growing concerns about the presence of antibiotic-resistant pathogens in animal manures from both on-farm exposure and off-farm contamination. Widespread dispersal of chicken litter or chicken litter-based organic fertilizers harboring antibiotic-resistant foodborne pathogens can be a serious environmental hazard. Furthermore, horizontal transfer of mobile antibiotic resistance genes from one bacterium to another can possibly occur under some conditions [38]. Nandi *et al*. [39] reported that Gram-positive bacteria were found to be the major reservoir of Class 1 antibiotic resistance integrons in poultry litter. As antibiotics are routinely used for disease prevention and growth promotion, a low level of antibiotics may select antibiotic-resistance bacteria in the gastrointestinal tract of the animal and also under *in vitro* conditions when antibiotic-laden manure is applied to the agricultural land [40]. Table 2 lists the presence of antibiotic-resistant bacteria in chicken litter or chicken litter based-organic fertilizers, highlighting the need for better waste management practices by poultry producers.

The prevalence of some antibiotic-resistant bacteria in chicken litter or chicken litter based-organic fertilizers can reach more than 60% for selected microorganisms, while it should be noted that some bacteria, such as *E. coli*, *Enterococcus*, and *Providencia*, are found to be multi-resistant to various antibiotics. It is also known that the increased use of antibiotics in the poultry industry can introduce a selective pressure which leads to the development of resistance or even multi-resistance characteristics in some of the bacterial populations. Moreover, as was observed by Khan *et al*. [41], erythromycin-resistant *Staphylococci*, *Enterococci*, and *Streptococci* were only isolated from litter samples collected from poultry houses that had used the antibiotics. Isolation of antibiotic-resistant foodborne pathogens from chicken litter or chicken litter based-organic fertilizers raises concerns about possible transmission of these bacteria to fresh produce after land application since these pathogens can potentially transfer to the arable land from contaminated chicken litter or chicken litter-based organic fertilizers, and can also further contaminate surface and ground water through runoff. This suggests the poultry industry should follow prudent management options and safety precautions by establishing more effective disinfection guidelines to reduce the population of antibiotic-resistant pathogens and monitoring the potential infection of subsequent flocks with resistant bacteria. In addition, a judicious and moderate use of antibiotics may also help prevent the emergence of antibiotic resistance in pathogenic bacteria. In the meantime, it is of great significance to identify and characterize various isolated antibiotic-resistant pathogens from chicken litter or chicken litter based-organic fertilizers. Therefore, further research is warranted to evaluate the pathogenicity of these antibiotic-resistant isolates, as well as their persistence in manure-amended soil.

Table 2. Antibiotic-resistant bacteria in chicken litter or chicken litter based-organic fertilizers.

Pathogen	Year/Location	Sample source	Sample type	Sample size	Comments [b]	Reference
Coliforms	N.A. [a]/US	4 turkey farms (8 houses), 10 adult broiler breeder chicken farms (43 houses), and 30 broiler chicken farms (110 houses)	Litter samples	N.A.	In turkey litter, the percentage of NAL-resistant coliforms ranged from 0.6% to 61.9%. Two farms had houses containing coliforms resistant to ENR and SAR. There was also multiple resistance to AMP, TIO, CAM, KAN on all 4 turkey farms. There were no NAL-resistant isolates from any of the 10 adult broiler breeder chicken farms. All of the 30 broiler chicken farms with NAL-resistant isolates were also resistant to SAR.	[42]
E. coli	2004–2007/US	Poultry	Chicken litter, carcasses, pine shavings, pine fines, and fresh wood chips	30 compost samples of chicken litter and carcasses, 42 compost samples of chicken litter and pine shavings, 18 compost samples of chicken litter with pine fines, and 24 compost samples of chicken litter, carcasses, and fresh wood chips	Isolates from California chicken litter/horse track had higher levels (63%) of resistance to AMP as compared with poultry compost in South Carolina (0%). *E. coli* isolates from poultry composts on South Carolina farms were found to be more resistant to TET (50%) as compared with isolates in compost from California, which had no resistance to this antibiotic.	[43]
	N.A./Canada	Broiler	Litter samples	9	All isolates were multiresistant to at least 7 antibiotics. Resistance to AMO, TIO, TET, and SA was the most prevalent.	[44]
Enterococcus	2006/US	3 broiler farms	Litter samples	N.A.	Resistance levels to CLI and ERY were 68%, 18%, respectively. No isolates were found to be resistant to VAN.	[45]
	N.A./US	60 chicken houses	Litter samples	N.A.	ERY-resistant bacteria were only isolated from litter samples collected from farms that had used the drug.	[41]
Providencia	N.A./US	Turkey	Fecal samples	11	Isolates were found to be resistant to TET, MAC, and SA groups.	[46]
Staphylococci	2006/US	3 broiler farms	Litter samples	N.A.	Resistance levels to CLI and ERY were 0% and 57%, respectively.	[45]
	N.A./US	Poultry	Litter samples	60	ERY-resistant bacteria were only isolated from litter samples collected from farms that had used the drug.	[41]
Streptococcus	N.A./US	Poultry	Litter samples	60	ERY-resistant bacteria were only isolated from litter samples collected from farms that had used the drug.	[41]

[a] N.A., not applicable; [b] NAL: nalidixic acid, ENR: enrofloxacin, SAR: sarafloxacin, AMP: ampicillin, TET: tetracycline, CAM: chloramphenicol, KAN: kanamycin, AMO: amoxicillin, TIO: ceftiofur, SA: sulfonamide, CLI: clindamycin, ERY: erythromycin, VAN: vancomycin, MAC: macrolide.

The gastrointestinal tracts of animals are the natural habitats for most of the enteric pathogens. After being defecated in feces, these pathogens are immediately exposed to a hostile environment with numerous microorganisms to compete for limited nutrients. Botts *et al*. [47] and Tucker [48] found that *S*. Pullorum and *S*. Gallinarum persisted much longer in fresh chicken litter than in built-up litter. Other studies have also shown that some pathogens in fresh chicken manure can initially grow to higher numbers under favorable environmental conditions. Himathongkham and Riemann [49] reported that *E*. *coli* O157:H7 and *L*. *monocytogenes* were able to multiply by as much as 100-fold for a period of 2 days in fresh chicken manure at 20 °C, whereas *S*. Typhimurium populations remained stable. Therefore, special attention should be paid to the initial disinfection processing so as to effectively eliminate pathogens from chicken litter.

When animal wastes are introduced into the agricultural field, the antagonistic effect of indigenous soil microorganisms and the hostile condition of soil microcosm are possible factors influencing the length of time that pathogens can persist [50]. According to the Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption (Proposed Rule) proposed in the U.S. Food and Drug Administration (USFDA) Food Safety Modernization Act (FSMA) [51], growth of human pathogens in biological soil amendments of animal origin could result in the amendment acting as an inoculum that spreads pathogens to covered produce growing area, leading to a likelihood of produce contamination. Previous studies have reported the growth and persistence of human pathogens in chicken manure and manure-amended soil. Islam *et al*. [52] reported that *S*. Typhimurium persisted for 203 to 231 days in soils amended with poultry compost, dairy compost, and alkaline-pH-stabilized dairy compost. As pathogens most commonly associated with fresh produce outbreaks, including *E*. *coli*, *Salmonella*, and *Listeria*, are unlikely to survive at detectable population levels in soil after 270 days [51], it is proposed by FSMA that the waiting period (application interval) between the application of untreated biological soil amendments of animal origin (such as untreated chicken litter) and the harvest of covered produce should be 9 months provided the material is used in a manner that does not contact covered produce during application and minimizes the risk of contamination after application.

3. Food Safety, and Human and Animal Health Issues Associated with Chicken Litter or Chicken Litter-Based Organic Fertilizers

Poultry is one of the commodities most commonly associated with foodborne disease outbreaks in the preceding years. During 2009–2010, the commodities in the 299 outbreaks associated with the most illnesses were eggs (27% of illnesses), beef (11%), and poultry (10%) [53]. Although foodborne disease outbreaks caused by bacterial pathogens reported so far have rarely been linked directly to chicken litter or chicken litter-based organic fertilizers, their risks to contaminate food or environment is considerably high. And there have been some food safety and human health issues associated with chicken litter in recent years.

Feeding poultry litter to dairy and beef cattle is a means of disposing of a waste product while concurrently supplying a low-cost protein feed to cattle [28]. Processed chicken litter has been used as a feed ingredient for almost 40 years in the US [15]. Cattle have the ability to digest low-cost feedstuffs, such as chicken litter, that are not suitable for other livestock species. However, from the hygienic

perspective, raw chicken litter may contain some bacterial pathogens, as noted above. Salmonellosis has been reported in cattle that were fed improperly composted broiler litter [54,55]. Chicken litter was also implicated as a possible source of chronic histoplasmosis case in 2003, which was caused by inhaling fungal spores released by *Histoplasma capsulatum* when the litter was spread on a pasture [56]. Moreover, some of the fungal species that are indigenous to the manure or litter can result in the production of mycotoxins. The specifications suggested by the Association of American Feed Control Officials (AAFCO) require that processed animal waste products as feed ingredient must be free of human pathogenic microorganisms, which could be harmful to animals or could result in residues in human food products or by-products of animals at levels in excess of those allowed by State or Federal statute or regulation [15]. Hence, proper processing to reduce the amount of these microorganisms or render the waste absent of pathogens is required. In addition, feed additives such as antibiotics are also added into poultry diet, which can be excreted as waste by-products used for cattle feed. Although the use of non-therapeutic levels of antibiotics in animal feed is approved and regulated by the USFDA [57], there is still limited information about the specific types and amounts of antibiotics that should be used for this purpose.

Pathogens can be transmitted to humans directly through contact with poultry litter or indirectly through contaminated poultry products. Water may also become contaminated by runoff either from poultry facilities or from excessive land application of poultry waste [58]. Runoff can possibly carry pathogens from the original site of animal manure-applied agricultural fields to water bodies serving as irrigation, drinking, or recreational water sources [59]. Clear understanding of the transport of pathogens potentially present in poultry wastes and its runoff is essential for the establishment of effective control strategies to reduce the adverse impact on environment, food safety, and public health. Sistani *et al*. [60] compared two methods of poultry litter application, surface broadcast and subsurface banding, to investigate the influence of application methods on *E*. *coli* concentration in runoff from tall fescue pasture. *E*. *coli* concentration was found to be significantly higher in runoff from broadcast application than subsurface banding treatment. They concluded that subsurface banding of poultry litter into perennial grassland can greatly reduce pathogen losses in runoff as compared to surface-broadcast application. Therefore, the traditional surface-broadcast application of chicken litter onto agricultural land may result in high levels of pathogens on the soil surface that could be potentially transferred to runoff water.

Adequate control of pathogens may require multiple control interventions to achieve significant reduction of pathogens in a poultry waste management system. And there is a need for some good management practices to reduce potential human exposures to these pathogens by effectively controlling them during chicken litter or chicken litter-based organic fertilizer processing.

4. Control of Pathogens in Chicken Litter or Chicken Litter-Based Organic Fertilizers

As previously stated, a variety of pathogenic bacteria may be present in animal wastes including those destined for composting, presenting a risk of human infection when they are utilized for land application. Composting is commonly employed as a pathogen control technique to recycle animal wastes back into the soil to improve its fertility [61]. Heat treatment after composting or without composting is also recommended to reduce or eliminate potential bacterial pathogens in animal wastes.

Meanwhile, some physical, chemical, and biological methods have been developed as alternative disinfection techniques for animal waste processing.

4.1. Composting

The interest in composting has greatly increased in recent years because of the need for environmentally acceptable animal waste treatment technologies and also the demand for organic fertilizers in organic agricultural production system. Composting of animal wastes is a spontaneous bio-oxidative process to produce more uniform, concentrated, and safe final products compared to fresh manure, allowing for easy spreading in the soil and also significant elimination of pathogens [62]. Furthermore, initial capital as well as operating and maintenance costs for composting are lower compared with other treatment techniques [63]. Composting can thus be considered as an effective technique which adds value to poultry waste for agricultural applications.

4.1.1. Composting Process

Before land application, chicken litter is usually built-up inside the chicken house during the growing season of broilers. Build-up is a common method of storing solid animal manure or used as bedding materials until it can be composted or applied to cropland as fertilizer. Barker *et al*. [64] observed that the middle and bottom sections of the built-up broiler litter bed provided a less favorable environment for anaerobes and coliforms than the top section, as the temperature required to reduce or eliminate bacterial loads are not achieved as they are at deeper layers.

Compared to build-up, composting is a controlled process of mixing organic wastes with other ingredients in an appropriate ratio to optimize microbial growth [65]. Composting is typically the biological decomposition process of biodegradable organic wastes in a predominantly aerobic environment by a consortium of microorganisms. Generally, it is a fast biodegradation process, which takes 4–6 weeks of microbial action to break down organic materials to stable and usable organic substances called compost. Composting allows easy handling and elimination of pathogens (including human and plant pathogens), along with the volume reduction of the wastes and the destruction of weed seeds. However, disadvantages of composting are also documented, such as loss of nitrogen and other nutrients during composting, cost of installation and labor, odor, and requirement for available land for storage and operation [62,66]. The cost of transporting chicken litter is a major obstacle facing the more efficient use of this poultry by-product. After composting, the bulk density of chicken litter is increased, which can reduce the cost of transportation [3]. Since composting can result in considerable nitrogen loss, compost producers may need to add amendments, such as straw, peat, woodchip, paperwaste, aluminum sulfate, and zeolite, to the litter to reduce ammonia volatilization during this process [67]. Moreover, although the objective of composting is generally to achieve a stable waste product, it may also affect both the total content and the composition of metals, such as cadmium, copper and zinc, in poultry manure by-products [68].

The process of composting is typically divided into four main phases based on temperature and active microbial community: mesophilic, thermophilic, cooling, and maturation phases [69]. Microbial activity is critical for a satisfactory composting process, in which mesophilic, thermotolerant, and thermophilic bacteria, actinomycetes, and fungi are all extensively involved [70]. Aerobic microbial decomposition

generates sufficient heat to increase the temperature of compost mixtures to the thermophilic zone (45 to 75 °C). Temperatures reached in a well-managed compost operation should be within a range of 55 to 65 °C [71]. Such temperatures are well above the thermal death points of mesophilic pathogens, such as *E. coli* O157:H7 and *Salmonella* spp. [72]. Besides high temperature, other mechanisms are also known to get involved in the inactivation of foodborne pathogens during composting, including microbial antagonism, production of organic acids, pH change, desiccation and starvation stresses, exposure to ammonia emission, and competition for nutrients [1].

Currently, within the United States, composting of animal wastes is not regulated by any federal agencies. The National Organic Program, administered by the U.S. Department of Agriculture (USDA), includes a composting standard in 7 CFR 205.2 that is aimed to maximize soil fertility and is required to achieve "USDA Certified Organic" status [73]. Similar to USEPA specifications described in 40 CFR Part 503 for regulating land application of Class A composted sewage sludge [74], the FSMA [51] proposed standards for two specific scientifically valid controlled composting processes: (1) Static composting that maintains aerobic conditions at a minimum of 55 °C for three days and is followed by adequate curing, which includes proper insulation; and (2) turned windrow composting to maintain aerobic conditions at a minimum of 55 °C for 15 days with a minimum of five turnings, and is followed by adequate curing, which includes proper insulation. However, a slightly different temperature and time criterion is recommended by the guidelines for composting dead poultry proposed by USDA [65] and adapted from those developed by McCaskey [75], which requires that when the compost has achieved a temperature greater than 50 °C for at least five days, the composting process is adequate to eliminate *L. monocytogenes*, *E. coli* O157:H7, and *S.* Typhimurium.

Composting has been proved to be an effective method to produce organic fertilizers in order to treat the ever-increasing volume of poultry wastes [76,77], which converted the soluble nutrients to more stable organic forms, thus increasing their bioavailability while reducing their susceptibility to loss when applied to agricultural land. Several studies have demonstrated that composting can be an effective way of lowering the level of foodborne pathogens in chicken litter. In some cases, moreover, common foodborne pathogens can be completely eliminated during the composting process. Martin *et al*. [29] reported that no *E. coli* O157:H7 and *Salmonella* spp. was detected in 64 composted poultry litter samples. Brodie *et al*. [78] also observed the complete elimination of *Salmonella*, *C. jejuni*, and *L. monocytogenes* from poultry compost when temperature exceeded 55 °C. In the work of Macklin *et al*. [79], chicken litter samples inoculated with *Salmonella* and *C. perfringens* were collected after seven days to determine the population of inoculated bacteria that survived. These pathogens were completely eliminated from the composted samples, while it was still recoverable from the samples that were uncomposted. Results from the study of Silva *et al*. [80] also indicated that the final compost of poultry manure was free of fecal coliforms and *Salmonella* spp., although a thermophilic phase (temperature > 40 °C) was not verified in the compost pile. Additionally, the findings of Guan *et al*. [81] demonstrated that composting of chicken manure, when managed to produce sufficiently high temperature, could reduce or degrade heat-sensitive genetically modified *Pseudomonas chlororaphis* and their transgenes. Therefore, available information on composting of poultry wastes indicates that composting is a suitable and environmentally sound method of reducing or eliminating foodborne pathogens. Furthermore, it should be emphasized that proper composting

management is needed to ensure that the process achieves the target level of the time–temperature combination for killing pathogens.

4.1.2. Pathogen Persistence and Regrowth after Composting

The die-off of pathogens during composting may not be extensive or uniform throughout the composting heaps or piles and depends on some environmental factors, such as microbe type, manure physico-chemical characteristics, aeration, and temperature pattern [62]. Therefore, in some cases, a traditional composting technique may not always be sufficient to ensure complete inactivation of pathogens within the entire compost mass. Consequently, persistence of pathogens in poultry compost has been reported. The surface of fresh compost has been identified as the critical location for pathogens to extend the survival or serves as the source of cross-contamination with the rest of the compost mass during heap turning or with the ambient environment. Shepherd *et al*. [30] detected that 26% and 6.1% of the surface and internal samples from poultry compost heaps were positive for *Salmonella* during the first phase of composting, respectively. Their results indicated that the conditions at the compost surface are suitable for pathogen survival, and that the complete composting process, including both heating and curing phases, should be confirmed before the compost is considered a finished, pathogen-free product. In another study, compost temperature of 55 °C was unable to inactivate *Salmonella*, *E. coli* O157:H7 and *L*. monocytogenes for more than eight days in poultry compost [82]. Macklin *et al*. [79] also recovered *C. perfringens* from five out of six interior chicken litter samples after seven days during in-house composting. Erickson *et al*. [83] conducted a field study on the fate of three avirulent pathogen surrogates (gfp-labeled *E. coli* O157:H7 and *L. innocua* and avirulent *S*. Typhimurium) in static composting piles of chicken litter and peanut hulls. *Salmonella* was detected by enrichment in sub-surface samples of static composting piles up to 14 days. Indicator microorganisms were only detected by enrichment in surface samples during the summer after four days of composting, while *E. coli* O157:H7 and *L. innocua* were still detectable by direct plating after 28 days in compost piles during the fall and winter trials. All three bacteria could be detected by enrichment in surface samples for 56 days of composting during the winter.

Besides the survival of pathogen during composting, there is also a concern over the possibility of regrowth due to the outside recontamination under open air composting environments and during storage as well. In the study of Kim *et al*. [10], *E. coli* O157:H7 increased from *ca*. 1 to 4.85 log CFU/g in autoclaved dairy compost after seven days, suggesting that a small portion of pathogenic cells that survive the composting process or are cross-contaminated from the environment could multiply to high populations under favorable conditions. In addition, studies have also demonstrated that pathogen growth in compost is influenced by several environmental variables, such as moisture content, temperature, background microflora, and nutrient availability of the composted solids [10,77]. To ensure the microbiological safety of composted chicken litter, environmental factors supporting potential pathogen regrowth after composting need to be identified and monitored. Additionally, outdoor composting is generally exposed to fluctuating environmental conditions, animal intrusion, and reduced efficiency of composting due to climatic conditions, and is not homogeneous in nature and prone to having "cold-spots" that are not properly treated, even with complete turning [84]. It is thus possible that

composting can result in a treated chicken litter that may continue to harbor a low level of human pathogens of food safety concern.

4.1.3. Composting Stress and Stress-Induced Cross-Protection

Composting is a very complex and dynamic biological process, which may pose a significant challenge and create many hostile stresses for the survival and growth of human pathogens. Bacterial stress is generally defined as a physical, chemical, or nutritional condition insufficiently severe to kill but leading to sublethal injury of microbes [85]. Some common types of stress associated with the composting process include desiccation, heat shock, and acid stresses [11,12,86].

(1) Desiccation stress. During composting, moisture level in the compost mixture, especially at the surface of compost pile, is reduced rapidly due to evaporation and the self-heating during the thermophilic phase [9]. Water loss through the desiccation process is an important factor affecting the survival and persistence of bacterial pathogens in low-water-activity environmental habitats, such as soil, sand, and compost surface [86].

(2) Heat shock stress. Heat shock occurs when microorganisms are exposed to temperatures above their normal growth range [87]. Temperature during composting process increases gradually, from ambient temperature to the mesophilic range and then to the thermophilic phase, which may consequently cause heat shock or stimulate a concomitant genetic and physiological heat shock response in some population of pathogenic bacteria [86]. Especially during the extended mesophilic phase of composting, some bacterial cells may become acclimatized to sublethal high temperatures before lethal temperatures are reached, allowing them to survive and, in some cases, multiply under stressful conditions. In support of this notion, results of Singh *et al*. [11] revealed that heat-shocked *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* at 47.5 °C survived longer in dairy compost than non-heat-shocked cells at composting temperatures of 50, 55 and 60 °C.

(3) Acid stress. Acid stress can occur in low pH conditions when H^+ ions cross the bacterial cell membrane and create an acidic intracellular environment. Acid resistance is especially crucial for foodborne pathogens that must survive the hostile acidic condition in the stomach before entering and colonizing the small intestines or colon [88]. Pathogenic cells present in compost of animal origin may become acid-adapted as they are exposed to acidic condition when passing through the gastric tract.

The presence of stressed microorganisms in manure and compost could pose significant public health concerns when applying the contaminated compost to arable land. Stressed cells in chicken litter or chicken litter-based organic fertilizers can initially go undetected during routine microbiological analysis; however, subsequent resuscitation under suitable environments may allow for significant growth and also possible production of toxins and other virulence factors [89]. Many stressed pathogens either retain or exhibit enhanced virulence and invasion, thus making their detection crucial to ensure food safety [90,91]. *S.* Enteritidis PT4 with enhanced heat and acid resistance has been reported to be more virulent in mice and more invasive in chickens than the non-resistant reference strain [92]. Interestingly, some stresses that are originally part of the host's defense system are very

similar to those occurred in the natural environment [93]. Therefore, it is reasonable to speculate that pathogens may treat stresses encountered during poultry waste composting or build-up as a signal for the expression of virulence factors. When pathogen-contaminated poultry wastes or their composted products are used as organic fertilizer and soil amendment, pathogens with increased virulence could transfer to produce in the field and thus cause serious foodborne disease outbreaks. However, further research is needed to verify this hypothesis.

Bacteria typically respond to stresses by altering their cellular morphology, membrane composition, biological metabolism, and virulence. Such stressed microorganisms produce a series of stress responses that can afford cross-protection against other stresses, indicating that the adaptation to a single sublethal stress may also enhance the tolerance to multiple lethal stresses. In fact, bacteria, especially foodborne pathogens, are frequently exposed to environmental stresses that cross-protect them against various other stresses [85]. Bacterial cells can gradually adapt to the hostile sublethal conditions, causing an adaptive response accompanied by a temporary physiological change that may result in an enhanced stress tolerance [94]. The general stress response identified in most Gram-negative bacteria, such as *E. coli*, *S.* Typhimurium, and *P. aeruginosa*, is regulated by the sigma factor, RpoS (σ^S) [95]. Induction of RpoS makes bacteria more resistant to environmental stresses, such as high and low temperatures, prolonged starvation, osmotic shock, pH stress, and oxidative stress [96]. Bacteria defective in the gene (*rpoS*) for RpoS synthesis have proved to be more sensitive to different adverse conditions [97]. Van Hoek *et al*. [98] reported that a fully functional RpoS system can provide an advantage for survival in the manure-amended soil environment. In their study, *E. coli* O157 isolates capable of long-term survival in manure-amended soil were all absent of mutations in their *rpoS* gene; however, the strains not capable of long-term persistence were mutant in their *rpoS* gene.

4.2. Other Treatments

The common practice of applying poultry wastes to soil as a source of nutrients to crops is of paramount importance in sustainable agriculture. While composting, to some extent, is an effective method for reducing pathogen concentrations in animal manure, pathogens can still survive in the composted products and contribute to soil contamination. Additional appropriate processing and control measures should therefore be adopted to minimize the pathogen contamination of chicken litter. The FSMA also suggests that such biological soil amendments with a residual population of pathogens after composting should require a multiple hurdle approach to minimize the likelihood of introducing pathogens to the field upon which they are applied [51]. There are a variety of physical, chemical, and biological approaches that have the potential to effectively disinfect chicken litter. Several treatment processes have been designed to operate at conditions capable of disinfecting bacterial pathogens in chicken litter, and the extent to which they actually reduce these microorganisms have also been characterized in laboratory and pilot scale field studies.

4.2.1. Physical Treatment Techniques

Physically heat-drying manure or compost to low moisture content after composting or without composting can reduce the volume and weight, which thus lowers transportation costs, though it requires significant energy inputs. Heat-dried products can be much easier to handle and spread uniformly

over an agricultural field, especially after they have been further processed into pellets. Table 3 presents the temperature–time requirements and acceptance criteria for the physical heat-drying of soil amendments.

Although a number of organizations or federal agencies offer independent protocol to ensure safe and effective heat treatment for animal manure or biosolids, there were no defined heat sources (dry *vs*. moist heat), varying time–temperature requirement, and microbial standards among groups. Research on the inactivation effects of thermal processing on pathogens in chicken litter has yielded generally satisfactory results. The main factors influencing pathogen reductions in animal manure by these processes are temperature, duration of treatment, and moisture level [2]. Wilkinson *et al*. [1] could not detect any *S*. Typhimurium in fresh poultry litter (30%, 50% and 65% moisture contents) after 1 h wet heat treatment at 55 or 65 °C in water bath. Kim *et al*. [2] found that a 7-log reduction of *Salmonella* spp. in fresh chicken litter (30%, 40% and 50% moisture contents) could be achieved by dry heat treatment at 80 °C in a conventional oven for 44.1 to 63.0 min. When they investigated the effect of chicken litter freshness on heat resistance profiles of *Salmonella*, *Salmonella* cells in aged chicken litter survived significantly longer than those in fresh chicken litter under any conditions. Ghaly and Alhattab [99] reported that the drying process at 40, 50 and 60 °C reduced the populations of bacteria, yeast and mold, and *E*. *coli* in chicken manure by 65.6%–99.8%, 74.1%–99.6% and 99.9%, respectively. Salmonellae were detected in the raw manure and the dried manure samples collected from the 3 cm deep manure layer after drying at 40 °C but not at 50 and 60 °C. Their results indicated that the higher the drying temperature and/or the thinner the manure layer, the more destruction of microorganisms in the dried manure. It should be noted that the aforementioned results on temperature–time combination requirements for eliminating *Salmonella* varied among studies. However, comparisons between these different studies should be conducted with precaution due to the differences in the composition and moisture level of chicken litter material, *Salmonella* strain, and also heating source. For example, Messer *et al*. [100] found that four different kinds of bacterial pathogens in chicken litter were destroyed by dry heat at different temperatures and within different times. *Arizona* spp., *S*. Pullorum, *S*. Typhimurium, and *E*. *coli* were destroyed by heat at 47.2 °C for 30 min, 62.8 °C for 30 min, 62.8 °C for 60 min, 68.3 °C for 30 min, respectively. Also, the physiological stage may contribute to the difference in heat resistance of pathogens in chicken litter. The work by Chen *et al*. [101] demonstrated that *Salmonella* cells adapted under a desiccation condition survived much longer in aged chicken litter as compared to non-adapted cells when exposed to the same dry heat treatment at 70, 75, 80, 85 and 150 °C. An obvious variability in heat resistance profile among *Salmonella* serotypes was also observed during thermal exposure, since *S*. Senftenberg and *S*. Typhimurium exhibited higher levels of heat resistance than *S*. Enteritidis and *S*. Heidelberg.

Table 3. Temperature–time requirements and acceptance criteria for the physical heat-drying of soil amendments.

Source	Soil amendment	Temperature-time requirement	Acceptance criterion	
			Moisture level	Microbial level
USEPA [74]	Biosolids	Either the temperature of the biosolids >80 °C or the wet bulb temperature of the gas in contact with the biosolids as the biosolids leave the dryer >80 °C	<10%	For Class A biosolids, fecal coliforms: <1000 MPN/g dry weight or *Salmonella*: <3 MPN/4 g dry weight
National Organic Program [102]	Animal manure	>65 °C for >60 min	<12%	Fecal coliforms, *Salmonella*, and *E. coli* O157:H7: negative
European Union [103]	Animal manure	>70 °C for >60 min	N.A.	*E. coli* or *Enterococaceae*: <1000 MPN/g *Salmonella*: absence in 25 g of sample
California Leafy Green Products Handler Marketing Agreement [104]	Animal manure	Either the process has been validated by a recognized authority or is subject to 150 °C for 60 min	<30%	Fecal coliforms, *Salmonella*, and *E. coli* O157:H7: negative or less than detection limit

[a] N.A., not applicable.

According to the FSMA [51], chicken manure may be physically heat-treated to create a dried, pelleted material that is functionally sterile due to the high heat used during production; however, it has been observed that if the heat treatment is not uniform, the end product may still harbor human pathogens and pose a likelihood of the material being recolonized by these pathogens, leading to the possible contamination of any covered produce to which it is applied. Moreover, physically heat-treated poultry manure pellets would be expected to have limited microorganism content including competitive native microflora of foodborne pathogens, which may provide an opportunity for the potential growth of foodborne pathogens in the event of pellet contamination. The significant energy costs for heat-drying manure or compost at high temperature are also in great contrast to the self-heating generated by microbial respiration during the composting process. The physical heat-drying of manure or compost may largely increase the volatilization of ammonia–nitrogen and reduce the total nitrogen level in the finished products. Additionally, manure or compost that is physically heat-dried at a high temperature instead of going through a curing phase at an ambient temperature is not considered to be as microbiologically active as the composted products.

Besides traditional thermal processing, some other non-thermal physical methods have also been studied to control foodborne pathogens in chicken litter. Barbour *et al*. [105] evaluated the impact of soil solarization on reduction of indicator microorganisms of fecal contamination in chicken manure-treated soils. The percentage reductions in counts in chicken manure-treated soil collected at 20 cm depth after solarization were: 26.3% (*S. aureus*), 45.5% (total bacteria), 71.3% (fungi), 81.8% (*C. perfringes*), 92.6% (fecal coliforms) and 100.0% (non-lactose fermenting bacteria). Significantly, UV disinfection may be an effective treatment for reducing pathogen concentrations in animal wastes,

while retaining the nutrient content for crop production. Oni *et al*. [106] investigated the effect of UV radiation on the survival of *Salmonella* when present on dried turkey manure particles. They observed that *Salmonella* exposed to UV in a thin layer of cells in saline that were directly placed in a petri dish showed a 5-log decline within 80 min, as compared to the 1.5-log decrease in turkey manure dispersed as a thin layer on a petri dish, suggesting the presence of manure particles significantly ($p < 0.05$) protected *Salmonella* from UV exposure.

4.2.2. Chemical Treatment Techniques

Alum treatment has been widely used to reduce pathogens before land application of chicken litter [107], and it can also be applied as an effective way to reduce ammonia volatilization and water-soluble phosphorus runoff from poultry litter in chicken houses [108]. Pathogens in poultry wastes, such as *Salmonella* and *Campylobacter*, have been shown to be reduced significantly or eliminated by alum treatment [109–111]. Rothrock *et al*. [107] used denatured gradient gel electrophoresis (DGGE) and quantitative real-time polymerase chain reaction (PCR) to characterize pathogenic microbial communities in alum-treated poultry litter. Alum addition (10% wt/wt) resulted in significant reductions in *C. jejuni*, *E. coli*, and *Clostridium*/*Eubacterium* populations by the end of the first month. The concentrations of *Salmonella* spp. were below detection limit ($<5 \times 10^3$ cell/g litter) throughout the entire experiment. Similarly, when Gandhapudi *et al*. [108] studied the immediate effects of alum on potential nitrification in poultry manure, slurries with alum-treated poultry manure reduced the population of fecal bacteria, presumably because of the pH stress that suppressed bacterial growth. However, labor requirements to apply alum to chicken litter and mix them sufficiently may be a limiting factor.

Lime in the form of quicklime (CaO) or hydrated lime [$Ca(OH)_2$] has also been reported to effectively disinfect and stabilize chicken litter by raising the pH of the waste to 12 or increasing the temperature by exothermic reaction, which are beyond the tolerance ranges of most enteric pathogens [112,113]. The disinfection efficacy of quicklime is also attributed to the dehydration in the poultry litter. With reduced water availability, bacteria will need more energy to absorb water from the litter for metabolic processes, making survival more difficult or even impossible [114]. However, there may be some additional costs to consider such as human labor to mix and haul the lime. Maguire *et al*. [115] applied 10% quicklime to 20% solid broiler litter and reduced the total plate counts from 793,000 to 6500 CFU/mL, which, as they indicated, could reflect the fate of many organisms, such as *Salmonella*. Bennett *et al*. [116] found that the addition of lime (5%, 10% and 20%) to poultry litter significantly reduced the recovery incidence of *S*. Enteritidis within 24 h. Nevertheless, Bennett *et al*. [117] verified that the effects of 0%, 1%, 2% and 5% of lime addition on turkey litter did not lead to a reduction in *Campylobacter* or *Salmonella*, which are contradictory to the above findings, but lime treatment did reduce the population of aerobic bacteria. This difference may be explained by differences in the composition of poultry litter sample and also the lime concentration applied.

Ammonia can also cause a significant reduction of non-spore-forming pathogens in a stacked manure pile [118,119]. There is, however, a lack of comprehensive research concerning this area. The limited information available on the effect of ammonia gas on common pathogens in chicken litter shows that generally it results in a significant reduction of pathogens. Himathongkham and Riemann [49]

evaluated the effect of drying and/or exposure to ammonia on *S*. Typhimurium, *E*. *coli* O157:H7 and *L*. *monocytogenes* in chicken manure. Drying to a moisture content of 10% followed by gassing with ammonia for 72 h as in an amount of 1% of the manure wet weight resulted in an 8-log reduction of *S*. Typhimurium and *E*. *coli* O157:H7, and 4-log reduction for *L*. *monocytogenes*. However, some environmental issues associated with potential pollution impacts of applying ammonia to chicken litter include ammonia emissions as aerial contaminants [120].

Fontenot *et al*. [121] investigated the effects of various processing techniques on sterilizing broiler litter. Heating at 150 °C for 1 or 2 h, dry heating at 100 °C for 48 h, autoclaving, beta-propiolactone treatment, or ethylene oxide fumigation was ineffective in completely sterilizing the litter. The only effective method was dry heat at 150 °C for a minimum of 3 h. Extensive studies were conducted by Caswell *et al*. [122] investigating the effectiveness of paraformaldehyde addition and ethylene oxide fumigation on pasteurization of broiler litter. They found that ethylene oxide treatment and dry heat at 150 °C following the addition of paraformaldehyde were all effective in reducing coliforms and total bacteria in broiler litter from >30,000 CFU/g to <2000 CFU/g. Seltzer *et al*. [123] found that the addition of 1, 3, or 7 g of paraformaldehyde per 100 g of fresh chicken feces reduced total bacteria counts from 2.2×10^9 CFU/g for untreated feces to 1.6×10^8, 1000 and 0 CFU/g, respectively.

Chlorine is an effective disinfection method commonly used for drinking water; however, the high organic matter found in chicken litter may largely diminish the effectiveness of chlorine. Murray *et al*. [124] concluded that chlorination, while initially reducing the total population of bacteria in sewage, may greatly increase the proportions of antibiotic-resistant pathogenic bacteria. It is still uncertain whether chlorination specifically induces modifications and changes in antibiotic resistance in some bacterial populations. Additionally, Munir *et al*. [125] reported that chlorination did not contribute to a significant reduction of antibiotic resistant genes and antibiotic resistant bacteria. The efficacy of chlorination can be substantially impacted by the presence of suspended organic matter, which may significantly lower the effective chlorine concentration which bacterial pathogens can be exposed to, and therefore require a higher amount of chlorine [14]. Without extensive pre-treatment, it is possible that manure-derived waste will have substantial interfering substances that will make chlorination less effective. Furthermore, the chemical reactions between chlorine and organic matter, nitrogen-containing compounds, or ammonia in chicken litter or chicken litter-based organic fertilizers when they are exposed to each other may also generate carcinogenic by-products, posing great risk to human health when applying them to agricultural land [126].

While effective in reducing pathogen levels, most of the current chemical treatment techniques are not economically feasible for large-scale chicken litter processing. Meanwhile, the interferences of high loads of endogenous non-pathogenic microorganisms in chicken litter may also influence the efficacy of chemical treatments on the killing of target pathogens.

4.2.3. Biological Control Techniques

To ensure that pathogens are eliminated from chicken litter or chicken litter-based organic fertilizers before application to agricultural land, some innovative biological control approaches have also been explored. The results obtained in the study of Erickson *et al*. [127] revealed the significant inactivation effect of soldier fly larvae (*Hermetia illucens* L.) against foodborne pathogens in chicken

manure. After two days, *Salmonella* population in chicken manure containing larvae had decreased by 4-log CFU/g. A 6-log reduction in the population of *E. coli* O157:H7 in chicken manure was also observed after adding larvae for three days. Yongabi *et al*. [128] designed a simple plastic anaerobic digester to disinfect contaminated poultry feces while providing biogas and pathogen-free fertilizer. Following anaerobic digestion of poultry feces for 37 days, both coliform and *E. coli* counts decreased drastically. It was also reported by Krylova *et al*. [129] that high levels of ammonium (>30 g/L) during anaerobic digestion of poultry litter resulted in a decrease in the numbers of all physiological microbial groups. A study on bacteriophage has also demonstrated its effectiveness for biological control of pathogens in compost. Heringa *et al*. [130] applied a five-strain bacteriophage mixture to dairy manure compost inoculated with *S*. Typhimurium. They found that bacteriophage treatment resulted in a greater than 2-log-unit reduction within 4 h. And since bacteriophages are host-specific, unlike physical or chemical treatments, it will not significantly alter the background microbial community in animal wastes. Therefore, application of bacteriophage to chicken litter or chicken litter-based organic fertilizers may be another promising biological control technique during the preharvest stage.

5. Conclusions

Raw chicken litter has been widely applied to arable land as organic fertilizer or soil amendment to improve the soil fertility and structure. To prevent possible microbiological safety issues for the environment and food crops grown in the field, practical and effective treatments should be developed specifically for raw chicken litter prior to land application. Composting, commonly used on farms, can inactivate large populations of human pathogens; however, studies have revealed that some pathogens can survive the composting process due to improper composting or cross-contamination. As a result, a small population of pathogenic cells may survive or regrow in the finished compost products under favorable conditions. Physical, chemical, and biological treatments can be alternative ways for pathogen inactivation but may not always lead to the complete elimination of foodborne pathogens in chicken litter or chicken litter-based organic fertilizers. Furthermore, some cells may become stress-adapted during build-up or composting, which cross-protect them against these subsequent treatments. Based on the hurdle concept, each kind of treatment can be used in combination with other disinfection strategies to potentiate microbial lethality. In order to effectively inactivate pathogens in chicken litter, it would be plausible to design a multi-step treatment with composting as the first step to kill large populations of pathogens, and then apply additional treatments to further eliminate the remaining cells. These systems with multiple treatments involved can be efficient in eliminating pathogens in chicken litter when proper control measures are in place and adopted.

Although chicken litter is considered a potential source of foodborne pathogens, this does not suggest that every portion of the litter contains all the various kinds of pathogens that have been reported or that they will be present at maximally reported prevalence. Nonetheless, treatment techniques should still be developed to inactivate the most resistant and persistent types of pathogens possibly to be encountered. Most of the studies on physical, chemical, and biological treatment techniques have attempted to quantify reductions of different bacterial pathogens or indigenous microorganisms in chicken litter or its composted products. Some estimates of pathogen reductions are uncertain and based only on limited lab studies with few pathogens, including indicator microbes

(primarily fecal coliforms). However, it is still not clear whether the fate of such fecal indicator bacteria properly represents the responses of various human pathogens. Moreover, not all fecal coliforms or tested pathogens arise from animal feces, and they have some non-fecal environmental sources, which makes it more difficult to investigate the fate of pathogens in animal wastes during different treatments. Therefore, future studies should focus on evaluating pathogen survival for different treatments using a wide range of conditions commonly encountered during build-up or composting.

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Conflicts of Interest

The authors declare no conflict of interest.

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