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Review

Salmonellae in the air environment: A review

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Abstract: *Salmonella* bacteria, a zoonotic pathogen, are frequently transmitted through food and water, causing foodborne outbreaks and illnesses. Bioaerosols are a growing concern as pathogenic microorganisms could be transmitted to the indoor and ambient air environments. The airborne transmission of pathogenic microorganisms is considered a risk of contamination or a route of infection. *Salmonella* have been found in rare numbers in the air, but their detection indicate their ability to survive in the air environment. Physical, biological and environmental stressors affect the survival of airborne microorganisms. The infectivity of airborne *Salmonella* is determined by its pathogenicity, infective dose and individual health conditions. The accurate assessment of *Salmonella* in aerosols is a problem due to the synergistic influence of many uncontrollable environmental conditions and a lack of standardized analysis and sampling protocols. Knowledge of the airborne transmission of *Salmonella* and factors influencing their viability is critical to understanding their potential health risk and the related control measures. This review provides evidence for the transmission of *Salmonella* in different air environments, focusing on the presence of *Salmonella* in the air as a risk of biocontamination. The sampling, detection and enumeration methodologies of *Salmonella* in the air are discussed with recommended mitigation and control strategies.

Özet: Zoonotik bir patojen olan *Salmonella* cinsi bakteriler sıklıkla gıda ve su yoluyla bulaşarak gıda kaynaklı salgınlara ve hastalıklara neden olmaktadırlar. Patojenik bakterilerin hava ortamına bulaşabilmesine aracılık ettikleri için biyo-aerosoller giderek artan bir sorun olarak ele alınmaktadırlar. Patojenik mikroorganizmaların hava yoluyla bulaşması, kontaminasyon veya enfeksiyon riski olarak kabul edilir. *Salmonella*'nın havada az sayılarda bulunması, hava ortamında hayatta kalma yeteneklerini göstermektedir. Fiziksel, biyolojik ve çevresel stres etkenleri havadaki mikroorganizmaların hayatta kalmasını etkileyen faktörlerdir. Hava ortamında bulunan *Salmonella* üyelerinin bulaşıcılığı patojeniteleri, enfektif doz ve bireylerin sağlık koşullarınca belirlenir. Aerosollerle taşınan *Salmonella* üyelerinin doğru bir şekilde değerlendirilmesi, kontrol edilemeyen birçok çevresel koşulun sinerjik etkisine ve standartlaştırılmış analiz ve numune alma protokollerinin eksikliğine bağlı bir sorun olarak görülmektedir. *Salmonella* üyelerinin hava yoluyla bulaşması ve canlılıklarını etkileyen faktörlerin bilinmesi, potansiyel sağlık risklerinin ve ilgili kontrol önlemlerinin anlaşılması açısından kritik öneme sahiptir. Bu derleme, biyolojik kontaminasyon riski olarak havadaki *Salmonella* varlığına odaklanarak *Salmonella* üyelerinin farklı hava ortamlarında bulaştığına dair kanıtlar sunmaktadır. Hava ortamında bulunan *Salmonella* üyelerinin örnekleme, tespit ve sayımı metodolojileri, önerilen azaltma ve kontrol stratejileriyle birlikte tartışılmıştır.

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Introduction

Aerosols are ubiquitous in the earth's atmosphere and they are central to many environmental issues and public health (Colbeck & Lazaridis 2010, Zhang 2020). Atmospheric aerosols are suspensions of liquid, solid or mixed particles with highly variable chemical composition and size distribution (Putaud *et al.* 2010). Bioaerosols are particles of biological origin (e.g. bacteria, viruses, fungi, algae, biological fragments and pollen) suspended in the air and are an important part of aerosols (Wéry 2014, Smets *et al.* 2016). Bioaerosols, which considerably vary in composition and size (0.2-100 µm) (Stetzenbach 2009), are produced in the environment from a variety of natural and anthropogenic sources (Kim *et al.* 2018, Xie *et al.* 2021), affecting living organisms

through infectivity, allergenicity and toxicity (Cox & Wathes 1995) (Fig. 1). Moreover, bioaerosols could be a source of pollution for plants, animals and surface water (Michalkiewicz, 2019). Biological particles are transported up in the air as free (single cells, spores or aggregates) or attached to non-biological particles (Jones & Harrison 2004), thus leading to considerable differences in their stability, survivability, composition and dispersal mechanisms (Cambra-López *et al.* 2010). Sewage treatment plants, biosolid landfills, spray irrigation (untreated / or insufficiently treated), wastewater (Brooks *et al.* 2004), composting, livestock facilities and herb processing have been considered as potential sources of bioaerosols and pathogenic microorganisms (Hickey & Reist 1975, Skórska *et al.* 2005, Zhang *et al.* 2019, Dai *et al.* 2020). Transmission of pathogenic microorganisms is of a great concern due to their ability to affect worker's and the nearby residents' health.

The transmission of pathogenic microorganisms in the atmosphere has to be paid attention, as their transmission is attributed to the initial health symptoms resembling enteric diseases among workers and population living near sewage treatment plants, biosolid landfills, composting and livestock facilities (Chinivasagam *et al.* 2009). The detection of pathogenic microorganisms in the air environment indicates their ability to persist harsh atmospheric conditions. Nowadays, the potential of aerosolization of pathogenic microorganisms has become a debated issue. The available information on emission, source apportionment and transmission of pathogenic microorganisms into the air environment is scarce (Xie *et al*. 2021).This review aims to highlight the transmission of *Salmonella* in the air environment, factors affecting their survivability, sampling and analysis methods and control strategies.

Salmonella bacteria

Salmonellae belong to *Enterobacteriaceae*, a family of Gram-negative bacteria represented with facultative anaerobic bacilli with 2-5 µm long and 0.5-1.5 µm wide and are motile by peritrichous flagella (Andino & Hanning 2015). *Salmonella* grow at temperatures in the range of 5-45°C, with ideal temperatures between 35-37ºC, but some species can grow at temperatures as high as 54ºC and as low as 2°C (Gray & Fedorka-Cray 2002) and at optimum pH range of 6.5 and 7.5 (Shaji *et al.* 2023). *Salmonella* can be distinguished from other bacterial species by their biochemical and antigenic features. Salmonellae are a complex group containing ≥2600 serovars based on somatic (O), flagellar (H) and surface capsule (Vi) antigens (Mumy 2014).

Salmonellae are ubiquitous human and animal pathogens and can be divided into 2 groups, typhoidal *Salmonella* (TS) and Non-typhodial *Salmonella* (NTS) (Wang *et al.* 2023a). *Salmonella enterica* ser. Enteritidis (*S*. Enteritidis) and *Salmonella enterica* ser. Typhimurium (*S*. Typhimurium), belonging to NTS group, are responsible for the majority of human salmonellosis (Ashurst, *et al.* 2022). NTS group is responsible for ~ 93 million cases of gastroenteritis and 155,000 fatalities annually and is frequently zoonotic (Gordon 2011, Cosby *et al.* 2015). The natural habitat of *Salmonella* is the gastrointestinal tract of humans and animals. Historically, transmission of *Salmonella* and enteric zoonotic infections (e.g. Q-fever, brucellosis, and avian and swine influenza) via aerosols has been neglected (Kallapura *et al.* 2014).

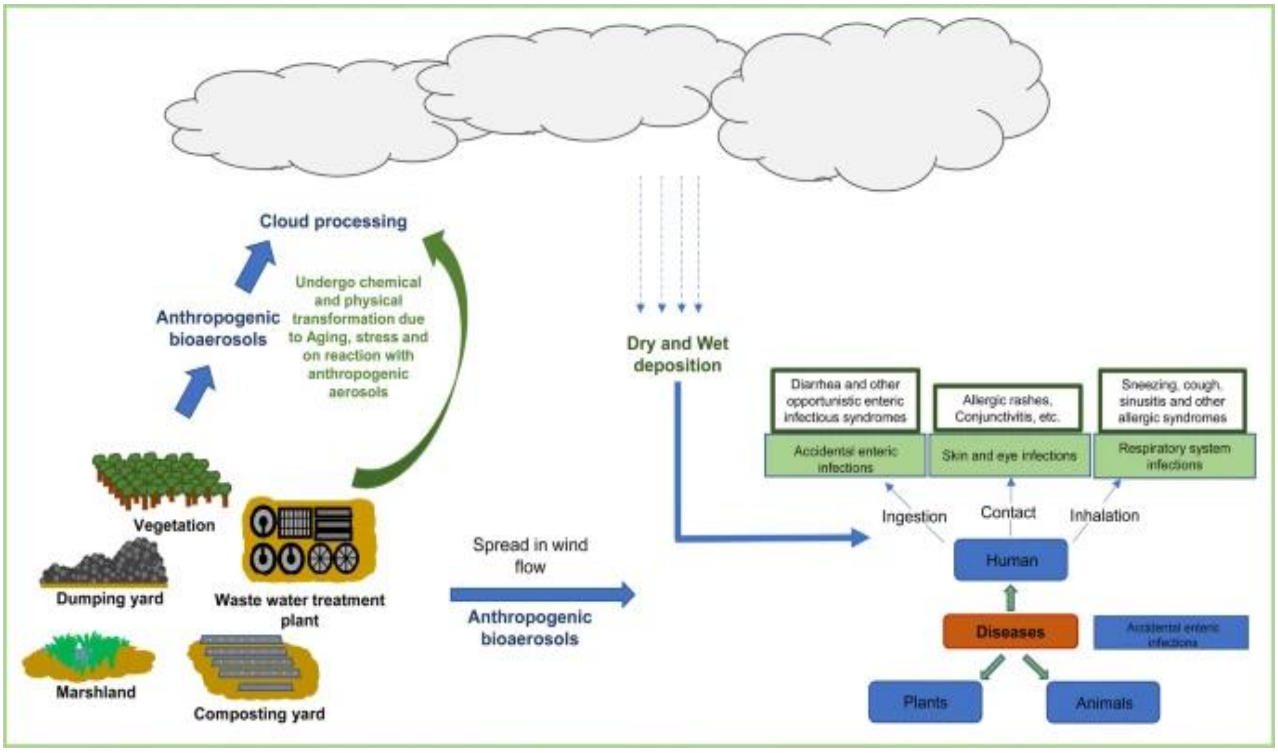

Fig. 1. Diagram of bioaerosols emission sources and fate (Krishnamoorthy *et al.* 2020).

Transmission of *Salmonella* aerosols is less pathogenic and rarely occurs (Shuval *et al.* 1986). The possibility of transmission and survival of *Salmonella* (Oliveira *et al.* 2006, López *et al.* 2012) in aerosols should be considered.

Salmonella aerosol is a concern route for vegetables contamination and foodborne outbreaks. *Salmonella* directly enter water and agricultural environments via waste and sewage irrigation (Heaton & Jones 2008) or indirectly via *Salmonella* aerosols. *Salmonella*, pathogenic *Escherichia coli* and *Listeria monocytogenes* have been linked to bacterial outbreaks of foodborne diseases associated with ready-to-eat fruit and vegetables (Thomas *et al.* 2024).

A low number of *Salmonella* cells may be sufficient to cause disease in a large number of people (Werber *et al.* 2005). For instance, ~ 13 CFU/g is enough to cause salmonellosis outbreaks. Infectious dose of outbreaks of salmonellosis is found between 10 and 1,000 cells (Blaser & Lee 1982, Vought & Tatini 1998). The infectious dose of *Salmonella* via respiratory pathway is lower than the oral route (Darlow *et al.* 1961). Inhalation of *S.* Typhimurium by mice was reported to cause disease in animals in a dose dependent manner, where the lowest dose was reported as ~150 CFU that could produce a disease (Wathes *et al.* 1988).

Sources of Salmonella aerosols

Wastewater and sludge applications

The primary concern of wastewater treatment plants (WWTPs) is to remove contaminants and inactivate pathogenic organisms to protect environment and human health. The enteric bacteria, viruses, protozoa and helminths are the common groups of microorganisms present in municipal wastewater (Akin *et al.* 1978). The enteric bacteria are commonly found in wastewater, with *Escherichia coli* and *Enterococcus feacalis* frequently at concentrations of ~10^9/l and ~10^8/l, respectively. However, concentrations of *E. coli* and total coliform are significantly 1 to 3 folds higher in the influent than the effluent (Ajonina *et al.* 2015). Salmonellae are the most prevalent pathogenic bacterial species in raw wastewater with a concentration ~ 5000 bacteria/l (Foster and Engelbrecht 1973). *Salmonella* concentrations averaged 130 bacteria/100 ml in the raw sewage water and 3 bacteria/100 ml in the treated sewage water (Langeland 1982). The presence and concentration of pathogenic microorganisms in sewage are determined by their prevalence among other populations and their ability to persist treatment processes. Biological wastewater treatment plants leave ~1-10% of *Salmonella*, *Mycobacterium* and viruses in the treated wastewater (Sorber & Sagik 1979). The aerosols containing pathogenic and non-pathogenic microorganisms are generated during wastewater treatment processes, as wastewater undergoes turbulent mixing or mechanical agitation (Sorber & Guter 1975, Sánchez-Monedero *et al.* 2008, Liu *et al.* 2020).

The transportation of bioaerosols is a function of time and distance (Pepper & Gerba 2015). Concentrations of total aerobic bacteria, total and fecal coliforms, fecal enterococci and coliphage were reported to significantly increase in the air within the perimeter of a WWTP in USA after operation (Fannin *et al.* 1985). Aeration tanks and trickling filters at WWTPs are the main sources emitting microorganisms into the ambient air (Han *et al.* 2020). High airborne microbial concentrations were found near aeration tanks and mechanical agitation, ranging within few to more than $8x10^4$ CFU/m^3 (Korzeniewska *et al.* 2008). Concentrations of mesophilic bacteria, bacteria-associated certain waterborne virulence factors, mesophilic fungi and thermophilic fungi were 1.7×10^4 CFU/m^3, 2×10^3 CFU/ m^3, 1.7×10^3 CFU/m^3 and 4.5×10^1 CFU/m^3, respectively in aerosols emitted by aeration tanks of an activated sludge plant (Bauer *et al.* 2002).

Airborne Gram negative bacteria, fecal indicators (*E. coli* & *Clostridia*), *Salmonella* and *P. aeruginosa* were detected at WWIP but in lower counts than Gram positive bacteria. *Escherichia coli* and *Salmonella* were found up to a distance of 300m and 10 m downwind of the aeration tanks, respectively, and a higher number of positive findings were observed during higher wind velocity and low sunshine (Müller 1980). Coliforms were found up in the air to a distance of 0.8 mile downwind of trickling-filter sewage treatment plant (Adams & Spendlove 1970). The dissemination of *Salmonella* by the air was low in relation to *Salmonella* content of the sewage itself (Müller 1980).

A microorganism can be released into the air from aerated sewage only when its concentration exceeds 10^3 cells/cm^3 in the sewage (Teltsch *et al.* 1980), a higher number of a given microorganism in sewage has a higher aerosol emission rate (Sawyer *et al.* 1993). Composition of airborne microflora is closely related with the type and number of microorganisms present in sewage waste (Ossowska-Cypryk 1991). The majority of the released aerosols do not travel very far distances. However, smaller particles tend to travel a considerable distance away from the source point (Mckinney 2004). The composition and size of microbial aerosols are influenced by type of treated wastewater, treatment technology, ambient conditions and shear stress force (Heinonen-Tanski *et al.* 2009). The highest emission of *P. fluorescens*, *E. coli*, *Enterococcus* sp. and *Salmonella* was detected in the air at the first stage of the purification in a municipal wastewater plant, Toruń, Poland (Paluszak *et al.* 2003). *Salmonella* and *Shigella* were not isolated from the air samples despite their presence in sewage water (Sekla *et al.* 1980).

Airborne microbial contamination greatly differed in the vicinity of aeration tank, maturing composting plant and 100 m downwind of municipal treatment plant in Poland, where the concentrations of *E. coli*, *Enterobacter* and *Salmonella* were ~10^1CFU/m^3 in average (Breza-Boruta & Paluszak 2007). The highest microbial air contamination was found in the pretreatment of wastewater (screening, aerated grit removal and pumping) in a WWTP in Finland, where somatic coliphage and

enterococci were found in higher numbers and no *Salmonella* bacteria were detected (Heinonen-Tanski *et al.* 2009). The ratio between *Salmonella* to coliphage densities in sewage aerosols was 1:100,000 (Grunnet & Tramsen 1974) and *Salmonella* bacteria were not recovered in any of the air samples collected at a WWTP in Egypt (Abdel Hameed 1992). Airborne pathogenic enteric bacteria (*S.* Enteritidis and *S.* Boydii), reovirus and enterovirus were isolated in 2%, 46% and 9%, respectively of the total samples collected at different sites in sewage sludge treatment plants in Italy (Carducci *et al.* 2000).

A given quantity of pathogens present in sewage aerosols could represent a source of a threat to workers who are daily exposed to aerosols associated with a variety of infectious microorganisms (Grisoli *et al.* 2009). Wastewater treatment processes bring the workers in contact with multiple pathogens and infectious agents such as viruses (*Hepatitis*-A, *Polio, Coxsackie, Echo, Rota* and *Adeno*), bacteria (*Salmonella* spp., *Shigella* spp., *Campylobacter jejuni, Yersinia enerocolitica, Legionella pneumophila, Helicobacter pylori, Listeria monocytogenes* and *Mycobacterium xenopi*) and protozoa (*Giardia lamblia, Entamoeba histolytica* and *Helminthes)* (Mulloy 2001).

Municipal sewage sludge is utilized worldwide on agricultural lands to solve the problem of sewage disposal, water scarcity and environmental contamination. However, the increase of wastewater in land application has magnified problems such as production of aerosols containing pathogens and contamination of crop and ground and surface waters (Bitton 1980). In the United States, ~33% of the produced municipal sludge is applied onto agricultural lands (Mclamarra & Pruitt 1995), increasing accumulation of pathogens and toxic substances that may be released into the air environment.

Climatic and environmental factors differently affect the survival of airborne pathogenic bacteria. Temperature, relative humidity, oxygen content, UV radiation and reactive chemical radicles are the main factors affecting viability of airborne microorganisms (Ruiz-Gil *et al.* 2020). Salmonellae in sewage sludge spread on grass and may survive up to 72 weeks, and neither aerobic stabilization nor anaerobic digestion significantly reduces the contamination with Salmonellae (Hess & Breer 1975). Raw sludge from municipal sewage may release more airborne pathogens than aerobic/or anaerobic digestion, lime stabilization and thermal drying sludge (Straub *et al.* 1993). The application of raw sludge on agricultural lands has been prohibited in many countries due to its hazardous effects that may be presented by direct contact/or inhalation of infectious aerosols (Cole *et al.* 1999).

Low concentrations of *Salmonella*, coliforms and enteroviruses were detected in air samples collected downwind wastewater spray -irrigated fields. *Salmonella* was detected in 78% and 18% of wastewater and air samples ~40m downwind, respectively, and enteroviruses in 71% and 44% in wastewater and aerosols, respectively, as an indication of the prevalence of enteroviruses than *Salmonella* in aerosols (Teltsch *et al.* 1980). This is attributed to viral contamination may be more resistance to inactivation processes than enteric bacteria and may be concentrated in aerosols than suspending fluid (Baylor *et al.* 1977). Table 1 shows the concentrations of *Salmonella* bacteria in wastewater and aerosols at WWTPs.

Biosolids and composts

Composting is used to stabilize biosolids, as organic substrates are subjected to microbial degradation. Composting produces substrates suitable for cultivation or aids in the disposal of wastes (Fig. 2). Application of composted sludge improves soil quality, but the microbiological safety should be considered (Brooks *et al.* 2005). The risk of infection posed to biosolid handlers reached 34% and 2% annually from exposure to Coxsackievirus A2I and *Salmonella*, respectively (Tanner 2004).

Growth and death rate of pathogens in biosolids, including *Salmonella*, depend on several factors such as moisture content, temperature, available nutrient, associated flora and indigenous microorganisms (Sidhu *et al.* 2001). Most of enteric pathogenic bacteria are non-spore formers and relatively sensitive to environmental factors (Vilanova & Blanch 2005). *Salmonella*, *E. coli* and fecal coliforms can regrow in moist conditions after treatment (Lang *et al.* 2007).

Table 1. Concentrations of *Salmonella* in wastewater and aerosols at WWTPs.

Environment	Concentration	Reference
Wastewater	2-60 MPN/100 ml	Katzenelson & Teltsch (1976)
Wastewater	*Salmonella*: coliforms 2:60 MPN/100 ml	Teltsch et al. (1980)
Aerosols	*Salmonella*: coliforms 3.2×10^{-2} : 5.410^{-2} MPN/m^3 43:1076 CFU/m^3	
Dry sewage sludge Raw wastewater Treated wastewater	140-14000 CFU/100gm 130 bacteria/100 ml 3 bacteria/100 ml	Langeland (1982)
Bulk sludge	0.3-17000 CFU/gm	Hussong *et al.* (1985)
Raw sewage	5000 CFU/ml	Prazmo (1980)
Aerosols/aeration tank	$\leq 10^1$ $CFUm^{-3}$	Breza-Boruta & Paluszak (2007)
Aerosols	*Salmonella*: Coliphage 1:100,000	Grunnet & Tramsen (1974)
Aerosols	≤1 CFU/m^3	Heinonen-Tanski *et al.* (2009), Abdel Hameed (1992), Pillai *et al.* (1996).

The bacterial concentrations were reported to range between 10^4-10^6 CFU/g in a well-managed compost, decreased over time to 150 CFU/g and increased over 6 weeks in poorly managed composts (Ogden *et al.* 2001). Wastewater biosolids generally contain *Salmonella* at a range of 10^2-10^3 CFU/g dry weight (Epstein 1997) and ~10^5 CFU/g in dewatered anaerobically digested sludge (Russ & Yanko 1981). *Salmonella* bacteria are known to survive composting process in low concentration (Gibbs *et al.* 1997) and can form filaments under moderately low-water conditions and upon rehydration can achieve high bacterial loads within a short period of time (Stackhouse *et al.* 2012). The active indigenous flora of compost establishes a homeostatic barrier against *Salmonella* which is considered an invader. However, in the absence of indigenous compost flora, the inoculated *Salmonella* may grow to potentially hazardous levels (Sidhu *et al.* 2001).

Microorganisms are released into the air when compost piles are formed or dismantled. The potential of aerosolization of pathogenic microorganisms from biosolids has become an important debated issue worldwide. The nature of the airborne microflora depends on the existing contamination of the starting materials and microbial development between disposal and composting (Lacey *et al.* 1996). The elevated temperature in composting kills-off coliforms and pathogens, however inadequate compost turning leads to temperature stratification and survival of pathogens (*Salmonella*) in cooler layers which may be emitted into the air during mechanical agitation/ or by wind action (Millner *et al.* 1980).

The biosolid land application generates bioaerosols through soil agitation and weathering of biosolid. Biosolids left on the soil surface are subjected to drying; rendering it friable, becoming airborne with the associated pathogens (Pillai 2007). At a municipal solid waste recycling and composting plant stations in Quebec, Canada, the concentrations of airborne total culturable bacteria and Gram-negative bacteria were above 10^4 CFU/m^3 and 10^3 CFU/m^3, respectively, at six of the nine work stations (Marchand *et al.* 1995). *Salmonella* and *Enterobacter* bacteria were found in the air samples only in the vicinity of the compost piles in Poland (Breza-Boruta & Paluszak 2007). The generation and disposal of bio-wastes potentially increase aerosolization of a wide variety of microbial pathogens.

Fig. 2. Photograph of a drying sewage sludge used as fertilizer.

Livestock houses

Livestock houses have significant hazards to biocontamination of food (Hutchison *et al.* 2004), water (Devane *et al.* 2018) and soil (Nolan *et al.* 2020). Pathogenic microorganisms are shed in animals' excretions, secretions or exhaled in breath, litter (e.g. straw, sawdust or wood chippings) and feed (Chien *et al.* 2011). Poultry litter and manure can pose a serious threat to environmental and human health and need to be managed properly (Gržinić *et al.* 2023). *Salmonella* bacteria are ubiquitous in farm environment, and bioaerosols may be released into the air environment as free/ or associated dust particles (Zhao *et al.* 2014). In agricultural livestock farming, bioaerosols account for well over 90% of airborne dust (Aengst 1984), reaching ~10^7 CFU/m^3 (Dungan 2010). The concentration of airborne total bacteria was 6.43 log CFU/m^3 in broiler houses, 5.1 log CFU/m^3 in pig buildings and 4.3 log CFU/m^3 in cattle buildings, and the overall concentrations of *Enterobacteriaceae* ranged between 3 and 4 log CFU/m^3 (Seedorf *et al.* 1998). In animal houses, the majority of airborne microbial composition is non-pathogenic and Gram-negative bacteria constituted 0.02 and 5.2% of the total amount of aerobic bacteria (Zucker *et al.* 2000).

There is evidence that enteric pathogens are important in airborne transmission of diseases among animals (Pepper & Gebra 2015). *Salmonella* Typhimurium aerosols are transmitted among calve houses (Hinton *et al.* 1983). *Salmonella* Typhimurium could survive for long periods in the air, and calves and mice exposed to *Salmonella* developed gastrointestinal symptoms, proving that pathogens could be spread by aerosolization (Wathes *et al.* 1988). *Bordetella bonchiseptica*, *Brucella suis*, *Haemophilus* spp., *Corynebacterium equi*, *Listeria moncytogenes*, *Mycobacterium* spp., *Mycoplasma* spp., *Pasteurella* spp., *Escherichia coli*, *Salmonella* spp., *Staphylococcus aureus*, *Streptococcus suis* and *Leptospira* Pomona are some pathogenic bacteria in pigs and poultry houses that can be airborne/or aerosol transmitted (Wathes 1995).

The aerosolized *Salmonella* Enteritidis could infect laying hens (Baskerville *et al.* 1992). *Salmonella* colonized and persisted in different tissues in broilers following exposure to aerosolized *Salmonella* (Pal *et al.* 2021). Chickens and animals are direct or indirect sources of *Salmonella* through feces and dust (Venter *et al.* 2004, Gale & Velazquez 2020). *Salmonella* infection has been experimentally proven to occur by oral, intracloacal, intratracheal, intraocular, navel and aerosol administration (Cox *et al.* 1990). The hatchery is the most significant contributor of *Salmonella* with a prevalence of 48.5%. Litter, feces, and indoor environment of poultry house are the other 3 major contributing factors with prevalences of 25.4, 16.3, and 7.9%, respectively (Wang *et al.* 2023b).

Cross-contamination of *Salmonella* from contaminated to uncontaminated eggs could be spread by fan-driven air (Berrang *et al.* 1995). *Salmonella* Typhimurium DT104 strain could be efficiently

transmitted to eggs up to 15 times more when laying hens were inoculated via aerosol route than crop route (Leach *et al.* 1999). Airborne transmission of *Salmonella enterica* serovar Typhimurium was demonstrated in chicks hatching in a cabinet containing infected hatchmates (Cason *et al.* 1994). *Salmonella* Enterifidis from infected chicks in an isolation cabinet rapidly transmitted to chicks physically separated from the infected seeder chicks but sharing the same air (Gast *et al.* 1998). However, the transmission mechanism of airborne *S*. Enteritidis has not been fully defined in chick's cabinets. *Salmonella* move through the air by itself or via water droplets, dust, dander or feathers need further studies (Cox *et al.* 1990, Davies & Wray 1996, Holt *et al.* 1999). These types of media may have important role in the transmission process of Salmonellae. Higher *Salmonella* and *E. coli* concentrations were detected in litter samples with water activity ≥ 0.90 and moisture ≥ 35% in a broiler litter (de Rezende *et al.* 2001). Airborne bacterial levels were linked to their densities in litter/or dust, *Salmonella* and *E. coli* averaged 10^4 MPN/g and ~10^8 CFU/g of litter, respectively (Davies & Wray 1994). *Salmonella* concentrations ranged between 10^3-10^5 MPN/g in litter and 2.2×10^{-1}- 44×10^{-1} MPN/m^3 inside the air environment of a poultry house (Chinivasagam *et al.* 2009). The prevalence of Salmonellae isolated from both poultry farm and processing plant environments were 5.4% and 4.7%, respectively with no *Salmonella* bacteria detected in the air samples (Alzenki *et al.* 2007). *Salmonella* Typhimurium was detected in the air at dehairing and evisceration locations in an Irish pig slaughtering plant (Pearce *et al.* 2006).

The number of total airborne aerobic bacteria and Gram negative bacteria varied between 780 and 20100 CFU/m^3 and 39 and 1030 CFU/m^3, respectively, in Chinese rabbit houses (Duan *et al.* 2006). The median of airborne mesophilic bacteria at the processing area of the moving rail was 1.7×10^6 CFU/m^3 with no *Salmonella* species detected from the air samples at a poultry house in Styria, Australia (Haas *et al.* 2005). On the other hand *Salmonella* bacteria only represented ~ 0.56% of the total airborne bacterial colonies in a small poultry house in Egypt (Abdel Hameed *et al.* 2010). *Salmonella* were identified in 10% of total airborne bacterial colonies, with *S.* Choleraesuis, *S.* Typhi and *S.* Typhimurium constituting 5.5% of the total bacterial counts in three pig and three beef plants in USA (Cosenza-Sutton 2004). The concentration of airborne *Salmonella* in a poultry production unit constituted up 3.3% of total bacterial cell counts measured by 4, 6-diamidino 2-phenylindole, ranging from 2.2×10^1 to 3×10^6 *Salmonella* targets/m^3 using *Salmonella*-specific *invA* genes of DNA (Fallschissel *et al.* 2009).

Salmonella bacteria have been isolated from the settled dust within unoccupied poultry shed as a result of the residual effect (Chinivasagam *et al.* 2009). *Salmonella* can survive for ~ 53 weeks in dust (Davies & Wray 1994) and 26 months in thin layers of litter of dried feces and feed (Davies & Breslin 2003). Pathogenic microorganisms were found in low counts, suggesting that air environment is not a significant source of enteric biocontamination. However, the transmission of airborne *Salmonella* within the livestock environment may impact the bird's and worker's health. Table 2 shows levels of *Salmonella* in raw and aerosols at livestock facilities.

Factors influencing the survival of Salmonella aerosols

The air environment is not an optimal medium for the survival of microorganisms. Aerosolized pathogenic bacteria are subject to considerable stressors leading to cell injury and/or death in both Gram positive and negative bacteria (Heidelberg *et al.* 1997). The persistence of airborne microorganisms depends on their tenacity. The tenacity (the ability to survive the airborne) of different microbial species depends on meteorological factors (temperature and humidity, UV radiation, solar radiation), air pollution, free radicals and ozone-olefin reaction products (Open Air Factor, OAF) (Stärk 1999, Clauss *et al.* 2016). These factors lethally affect microbial viability and infectivity through chemical, physical and biological modifications to phospholipid, protein and nucleic acid moieties (Karra & Katsivella, 2007).

Gram negative bacteria, including Salmonellae, are rapidly die-off in the airborne state (Cox 1995). Some bacteria (anaerobic species) are highly sensitive and cannot grow in the presence of oxygen (Tang 2009). Desiccation is experienced by Gram-negative bacteria, ~ 90% immediately loss their viability after aerosolization, due to denaturation of outer phospholipid bilayer membranes (Cox 1989).

Table 2. Levels of *Salmonella* in raw and aerosols at livestock facilities

Environment	Level	Reference
Swine house units	No *Salmonella*	Elliott *et al.* (1976)
Chick dust	10^4 CFU/g	Davies & Wray (1994)
Chick dust	10^3 - 10^5 MPN/g	Chinivasagam *et al.* (2009)
Poultry house- air	0.22 - 4.4 MPN/m^3	
Poultry house-air (DAPI)	$2.8 \times 10^5 \pm 1.9 \times 10^5$ cell/ m^3	Fallschissel *et al.* (2009)
Poultry house-air (culture method)	$3.3 \times 10^2 \pm 1.2 \times 10^2$ CFU/m^3	
Duck stalls (molecular method)	2.5×10^1 - 3×10^6 genes/m^3	
Small poultry house	~0.56% of total bacterial isolates	Abdel Hameed *et al.* (2010)
Poultry house- picking area	2 - 598 CFU/m^3	Heber *et al.* (2006)

Microorganisms generated from liquid suspension undergo desiccation (loss of water) and those generated as dust particles partially rehydrated (Cox 1995, Cox & Wathes 1995).

Long distance transport of microorganisms in the air depends on atmospheric dispersion, dilution, deposition, particle size and meteorological conditions (Gregory 1973). The immission concentrations of bioaerosols decreased exponentially with increasing distance from the source of emission. In the air environment, bioaerosols are exposed to wind and weather and their extent dependens on the tenacity, size and composition of bioaerosol particles (Clauß 2020). The behavior of *Salmonella* in the air environment remains unpredictable (Carrique-Mas & Davis 2008). Temperature affects the molecular structure of the microorganism and consequently its inherent thermodynamic instability (Maillard reaction), involving the elimination of water molecules (Stärk 1999). At warmer temperatures, phospholipid membranes of Gram negative bacteria undergo many complex transition, separation and aggregation phases, leading to changes in biological functions. However, at cooler temperature, exothermic crystallization of lipid moieties together with protein subunit formation leads to loss of viability (Cox 1989). The effect of relative humidity on airborne microorganisms is difficult to determine, however surface damage (inactivation at high RH) and rehydration (inactivation at low RH) are the most influential factors (de Jong *et al.* 1973).

Airborne *Salmonella* are affected by sunlight and other environmental factors, because *Salmonella* bacteria are enteric microbes, adapting to live in a protected environment (Müller 1980). Aerosol particles play a crucial role in the transmission of airborne bacteria, as particles may protect microorganisms from harsh environmental conditions. A significant positive relationship was found between concentrations of aerosol sizes of 0.5-1.0µm and *Salmonella* species in a dairy house (Aminul Islam *et al.* 2020).

Climate change and global warming have contributed to the spread of pathogens. Several studies have recognized the importance of increased ambient temperature and precipitation in the spread and persistence of *Salmonella* in soil and food. The impact of extreme weather events on *Salmonella* infection rates among the most prevalent serovars has not been evaluated worldwide (Jiang *et al.* 2015; Morgado *et al.* 2021). Dust storms have positive (e.g. fertilization of aquatic and terrestrial ecosystems) and negative (e.g. transport of toxins and pathogenic microorganisms) effects. S*almonella* proliferate rapidly at higher temperature, increasing their spread through different environmental media (Akil *et al.* 2014). Emergence or resurgence of numerous infectious diseases is influenced by environmental factors such as climate or land use change (Mills *et al.* 2010). However, the impact of extreme weather events on *Salmonella* growth and persistence in the air environment should be fully evaluated.

Airborne Salmonella: Sampling and analysis techniques

The detection of airborne pathogenic bacteria is of great concern. The efficiency of collection depends on the sampling strategy, analysis technique and media used. Different air sampler types exist and not all are suitable for collecting a specific microorganism. The ideal air sampler is efficiently able to recover all microorganisms from the air and allow all the required analysis to be performed. Currently, there is a lack of standardized techniques to quantify airborne microorganisms. The advantages and drawbacks of different sampling methods (filtration, impingement, impaction, and sedimentation) have been previously reviewed (Buttner *et al.* 1997, Griffin *et al.* 2011, Adell *et al.* 2014).

The collection and analysis methods may represent a stress factor on microbial viability. Sampling technique, type of medium, cut-off diameter of sampling device and its detection limits play important stress factors on the survivability of microbial aerosols. Non-detection of *Salmonella* bacteria from the air environment could be attributed to their low concentrations at point sources (Kocwa-Haluch 1996). Moreover, the presence of many competing bacteria limits isolation of *Salmonella* in air samples (Carrique-Mas & Davies 2008). Several official organizations for standardization have developed reference methods for the isolation of *Salmonella.* Conventional detection methods for *Salmonella* bacteria are based on culturing techniques, using pre-enrichment broths, and selective enrichment media, followed by biochemical and serological reactions. Liquid impinge sampler using pre-enrichment broths (buffered peptone, selenite, tetrathionate brilliant green, Muller-Kauffmann tetrathionate and Rappaport-Vassiliadis soya) have been preferred to collect airborne *Salmonella* (ISO 2002). The efficiency of the enrichment broths depends on type of sample, addition of antibiotics, portion of the inoculum used and incubation temperature (35-37^{o}C). Isolation of Salmonellae is enhanced by incubation of pre-enrichment broth into selective enrichment media (Carrique-Mas & Davis 2008) to detect low levels of pathogens; enabling reproduction of the injured cells and subsequently overestimate pathogens density (Sidhu & Toze, 2009).

Salmonellae can be isolated using numerous low-selective media (MacConkey agar, deoxycholate agar), intermediate-selective media (*Salmonella-Shigella* [SS] agar, Hektoen [HE] agar) and highly selective media (selenite agar with brilliant green), (Cooke *et al.* 1999). Most of the conventional plating media (e.g. brilliant green agar) are non-specific, developing a large number of false positive Salmonellae (*Citrobacter* and *Proteus*). XLD and HE agar are the most popular media for isolating *Salmonella* and their differentiation abilities rely on the characteristics of *Salmonella* (Rambach 1990).

Salmonella colonies are isolated and screened using different biochemical reactions. The main biochemical reactions are Triple sugar iron (TSI) agar (alkaline slant,

with acid, gas and H_2S in the butt), lysine iron agar (Alkaline slant with alkaline, rare gas and H_2S in the butt), oxidase reaction (–ve), predominantly lactose-negative and urease reaction (–ve) and confirm with polyvalent anti-sera (Table 3).

Airborne microbial concentrations cannot be accurately determined using only culture-dependent method; because microorganisms could be viable but non cultivable (Alvarez *et al.* 1995). The selective enrichment media may not restrict the growth of undesirable microorganisms (Albrecht & Kämpher 2006). The majority of naturally occurring pathogenic microorganisms cannot be cultivated using the traditional cultivation techniques (Amann *et al.* 1995). A range of chromogenic media has been developed for the detection of *Salmonella*, based on combination of chromogenic substrate and conventional biochemical reactions. These media produce distinctive colonies; making *Salmonella* identification easier and faster. Rambach agar and *Salmonella* detection media (O'Neill *et al.* 2003) and BBLTH CHROM agar are the common chromogenic media used (Eigner *et al.* 2001). Chromogenic media offer a much higher degree of specificity than conventional media which are based on absence of lactose fermentation within *Salmonella* and/or their ability to generate hydrogen sulphide.

The culture independent technique, based-on DNA amplification by polymerase chain reaction (PCR) is used to complement /or replace culture based technique (Gugliandolo *et al.* 2011). The qualitative ISO 6579:2002 technique is the most sensitive and specific method among presence /absence PCR/ or ELISA for detecting *Salmonella* in the environmental samples (Eriksson & Aspan 2007). Molecular base methods offer advantages of a more rapid, sensitive and specific detection of pathogenic microorganisms (Kolb *et al.* 2005).

qPCR is a potential method for specific/ or genus specific quantification of aerosol samples (Dutil *et al.* 2007, Oppliger *et al.* 2008, Fallschissel *et al.* 2009). The qPCR analysis of airborne microorganisms gives higher counts than conventional cultivation methods as molecular method determines cultivable and non-cultivable cells. The accuracy and detection limit of qPCR are influenced by DNA extraction and analytical phases (Hospodsky *et al.* 2010). The drawback of the PCR is related to its inability to provide information on pathogen viability which is necessary to investigate microbial infectivity (Zeng *et al.* 2016). The most frequently target species-specific and virulence associated genes used in the PCR of *Salmonella* are shown in Table 4.

Salmonella as air bioindicator

The criteria considered in selecting a microbial indicator include 1) the ability of a microorganism to survive in the environment of concern, 2) the correlation between the presence of the indicator and pathogens, 3) ease and speed of detection, and 4) non-pathogenicity of the indicator. The presence of fecal coliform is a good indicator of the possible presence of associated pathogenic bacteria, particularly *Salmonella*. However, pathogens are difficult to assay and seldom occur at readily detectable concentrations but high levels of coliforms and total bacterial counts may indicate the existence of enteric pathogens (Sorber & Sagik 1979). In contrast to Gram positive bacteria, Gram negative bacteria have a thinner cell wall; therefore they are more sensitive to dehydration and not viable in the air state for a long time. Gram negative bacteria represent ~1 - 10% of the airborne total bacteria (Matkovi̧ć *et al.* 2007), however Gram-negative bacteria may include pathogens such as Salmonellae. As a result of their thicker cell wall and the accompanying greater "robustness" towards the airborne state, most of the bacteria from the air detected via cultivation methods are Gram-positive bacteria (Zhao 2011).

Table 3. Appearance of *Salmonella* bacteria on different selective media.

Selective medium	Appearance
Bismuth sulfite agar	Fully developed colonies, convex, 1-3 mm in diameter, black with lustrous surface, form a shallow, soft, black pit with light edge
Brilliant green agar	Transparent pink colonies surrounded by a brilliant color
MacConkey and SS agar	Colonies usually colorless, transparent with light tan, light pinkish or yellow appearance tan centers, 1-5 mm
XLD medium	Pink to red with black center colonies
Hektoen enteric agar	Green or blue green colonies

Table 4. The target species specific and virulence associated genes used in the PCR of *Salmonella*

Target gene	Primer	Sequence (5^{-}-3^{-})	Amplicon size (bP)	Reference
Invasion plasmid Antigen-B (*ipa*B)	*ipa*B-F *ipa*B-R	GGACTTTTTAAAAGCGGCGG GCCTCTCCCAGAGCCGTCTGG	314 429	Kaniga *et al.* (1995)
-	ST11 ST15	AGCCAACCATTGCTAAATTGGCGCA GGTAGAAATTCCAGCGGGTACTG	-	Adell *et al.* (2014)
-	Sef.B127L	5'-AGATTGGGCACTACACGTGT-3'	535	Wang *et al.* (2009)
-	SefB661R	(5'-TGTACTCCACCAGGTAATTG-3'	535	Santos *et al.* (2021)

Enterobacteriaceae are sensitive towards the airborne state, as they already die before/or during sampling and thus are barely detectable. The survival of coliforms in the air environment is still controversial. Coliforms have lower survivability in the air environment than *Salmonella* (Teltsch *et al.* 1980) and do not fulfill the main requirement of microbial indicator "its ability to survive in the environment is equal to/or more than the tested pathogenic microorganism". The stability of coliform in the air environment appears to be lower than certain viruses (Scarpino 1975). *Salmonella*, *Citrobacter*, *Clostridium*, *Proteus*, *Edwardsiella* and *Klebsiella* species have been associated with the presence of fecal contamination (Kromoredjo and Fujioka 1991) and Clostridia are better indicator of airborne pathogens (Hill *et al.* 1993).

Control measures

The practical control measures are crucial in livestock and waste applications to prevent release and spread of pathogenic microorganisms into the air environment (Hendriksen *et al.* 2004). Biosecurity management includes a set of practical measures to prevent and limit the spread of infections to humans and animals (Amass 2005). Biosecurity includes replacement of animal and husbandry (Andres & Davies 2015), dust reduction, air filtration and proper air disinfectants (Stärk 1999), electrostatic filtration, fogging and oil based spray, negative air ionization, vacuum cleaning, ventilation and wet scrubbers (Holt *et al.* 1999, Ritz *et al.* 2006). Rodent and insect control and disinfection between flocks are recommended to reduce *Salmonella* in farms (Gosling *et al.* 2014). Pressurized steam followed by forced hot air reduces levels of *Salmonella* and *Campylobacter* in transport cage flooring and reduce cross-contamination of broilers (Reina *et al.* 2024). Assessing biosecurity includes measuring the potential routes for disease transmission. Air is yet another vector by which pathogens can contaminate the final products. The adjacent nearby residential areas require higher standards of amenity. The width of a buffer zone > 400m between waste and livestock applications and residential areas should be taken in consideration during city planning.

The International Life Sciences Institute (ILSI) outlined a number of measures that should be considered with regard to air entering production floors (Beuchat *et al.* 2011), including a positive pressure air system to prevent the contaminated air infiltrating controlled production areas and eliminating residual moisture (Podolak *et al.* 2010). Filtering air entering production zones may also be effective as well as continuous monitoring of *Salmonella* in the air is important to maintain the appropriate state of the environment.

Conclusion

Salmonella bacteria are potentially generated into the air from livestock farms and waste application facilities. These facilities are hotspots associated with high infection risks of aerosols- containing *Salmonella*. Salmonellae are found in aerosols in detectable counts. Transmission of *Salmonella* via the air pathway is less pathogenic and rarely occurs, however airborne *Salmonella* may represent a threat to public health, but no greater than that of the same count of pathogens ingested. The low count of *Salmonella* in the air is attributed to their enteric adapted to living in the protected environment, short time survives and occurrence is sporadic related to the incidence of disease infection. The efficiency of sampler, analytical technique and nutrient medium in use are important factors in detecting airborne *Salmonella*. The qPCR is fast, rapid and accurate for quantification of *Salmonella* in air samples. More sensitive laboratory methodological techniques should be created. The absence of correlation between the presence of Salmonellae and fecal coliforms make them fail to fulfill one of the main requirements of microbial indicator for air biocontamination. There is an urgent to identify more reliable alternative indicators which could be used for potential public health risk assessment. The development of new diagnostic tools (less labour and more rapid and sensitive) and vaccines targeting specific pathogenesis factors could be used in comparative investigations and control *Salmonella* transmission and infection. Finally, the presence of *Salmonella* in the air may have a hypothetical potential to cause infection.

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