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Monitoring airborne pathogen transmission for enhanced safety at food processing facilities

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REVIEW PAPER

ABSTRACT

Food manufacturing and processing are part of the nation's critical infrastructure. Due to the recent global spread of the SARS-CoV-2 virus, the potential contamination of the food chain and the resulting public health implications are of high consequence to society. The current primary food manufacturing and processing facilities already have various mechanisms such as hazard analysis and critical control point (HACCP) system in place. However, the widespread microbial infections in these facilities raise concerns that they will not only threaten the welfare of food processing workers, but also have a potentially greater consequence on the public if the food is contaminated with an infectious agent.

Despite the increasingly recognised role of the environment in the spread of microbes, the effect of air properties remains poorly understood. Heating, ventilation, and air conditioning (HVAC) systems in meat processing facilities not only provide a means of transport for viruses and bacteria but may also deposit them on surfaces where they can survive for days. To maintain a stable and safe food chain supply during the pandemic, the challenges to ensure safe food supply and protect the workers' health must be quickly addressed through sustainable, safe and economic approaches. With these two imminent challenges in mind, the overall goal of this review article is to provide a comprehensive overview of the role of the environment in the impaction and resuspension of bioaerosols, focusing on airborne bacteria and viruses. The review includes the latest results of modeling the spread of microbial aerosols in the airflow and the development of preventive measures to mitigate virus contamination in the unique environment of meat processing operations. By understanding how the environmental factors and seasonality affect the infectivity and spread of airborne pathogens, mitigation measures can be designed to minimise future infections within and beyond these facilities.

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1. INTRODUCTION

The Centers for Disease Control and Prevention (CDC), estimates that each year in the United States about 48 million (1 in every six) people get sick, 128,000 are hospitalised, and 3,000 die of food-borne diseases (CDC, 2018). An estimated 600 million – almost 1 in 10 people in the world – fall ill after eating contaminated food and 420,000 die every year, resulting in the loss of 33 million healthy life years (WHO, 2022).

Some of the main factors that are responsible for food-borne disease emergence are associated with modern living conditions and globalisation, such as ecological changes, intensive agriculture, anomalies in the climate, human demographical changes and behavior, travel and commerce, technology and industry, microbial adaptation and change; and the breakdown of public health measures (King, 2006; Vignolo et al., 2008). Furthermore, concerning pathogens, their most striking feature is their diversity and selection for drug resistance, suggesting that infections will continue to emerge and will probably increase, which emphasises the urgent need for effective surveillance and control (Douwes et al., 2003; Morse, 2004; Menetrez et al., 2009). Considering that most of the meat consumed in developed countries is produced in developing countries, the existent gap in monitoring and surveillance systems for quality and safety of foods can have a significant impact on food-borne illnesses (Cahill and Jouve, 2004). Consumers are increasingly demanding food that is free from pathogens, has undergone minimal processing, contains fewer preservatives and additives yet has maintained its sensorial quality. As a response to these conflicting demands, current trends in the food industry include the investigation of alternative inhibitors for use in foods.

A recent survey on the incidence of infection by pathogens transmitted through food in the US showed a decline in infections caused by *Campylobacter*, *Listeria*, *Shigella* and *Yersinia*; however, those caused by Shiga toxin-producing *Escherichia coli* 057 and *Salmonella* did not decrease significantly, while *Vibrio* infections increased (State of CDC, 2007). Undoubtedly, the major threat to food safety is the emergence of "new" pathogens. The presence of *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Vibrio parahemolyticus* as foodborne microorganisms has been related to the increase in outbreaks when compared to traditional food pathogens (Bassler et al., 1995; Church, 2004; Elmi, 2004).

Bacteria have posed a serious problem to the commercial and private food industries for centuries. However, only recently have aerosolised bacteria been seen as a large threat to human health and shelf life of food. The beef industry faces a particularly hard problem in maintaining a clean environment in the slaughterhouses that process the cattle. Of particular importance is *Salmonella* and Shiga toxin producing *E. coli* (STEC), due to their prevalence and severe pathogenic qualities (Cray et al., 1995, 1998; Hancock et al., 1997; Chapman et al., 2001; Conedera et al., 2001). STEC and *Salmonella* annually cause an estimated 470 deaths in the United States alone. An estimated 77.1 billion dollars are lost each year as a direct cause of contaminated food. Heating, ventilation, and air conditioning (HVAC) systems not only provide necessary moisture for bacterial life but are also utilised as a means of transport for these

microscopic organisms. The direction of airflow can be very important to the sanitation of a facility. Most of the harmful bacteria are initially introduced by cattle (Paiba et al., 2002; LeJeune et al., 2004). Depending on where the cow resided before entry into the facility as well as the time of the year will affect the number of bacteria it introduces. The mechanical layout of a slaughterhouse facility and the ventilation design will directly affect whether its end products are more likely to become contaminated or not.

It is impossible to keep airborne bacteria, mold and viruses in food processing areas at zero level. Some of the major sources of contamination in food processing facilities are wastewater, rinse water and spilled products that become aerosolised. Heating, ventilation and air conditioning (HVAC) systems contribute to airborne microorganisms under normal operation because they provide fertile areas for growth due to moisture. Worker activity (talking, sneezing and coughing), equipment operation, sink and floor drains, and high-pressure spraying are major sources of bioaerosols (Heldman, 1974; Salem and Gardner, 1994).

This review article summarises the current approaches to understand the role of the environment in the spread of microbial infections at processing facilities, focusing on meat packing and to use the insights gained to guide the design of preventive measures.

2. RESULTS AND DISCUSSION

With the introduction of the Bioterrorism Act of 2002, food processors' ability to detect and prevent food contamination faces increasing scrutiny, and food processing plants must meet stringent environmental standards. Previous studies characterising bioaerosol concentrations in food processing plants are limited. Various methods have been used for collecting and concentrating bioaerosols, however, there is a notable absence of standardised or validated measurement methods (Cox and Wathes, 1995; Crook and Sherwood-Higham, 1997; Millner, 2009).

The two principle means of monitoring the microbiological population of air at food processing plants are passive monitoring and active sampling. Passive monitoring uses settling plates, which are inexpensive and easy to use, however, they will not detect smaller aerosol particles and cannot sample specific volumes of air, so the results are not quantitative. They easily become overgrown in heavily contaminated conditions and can be used only at low-risk factories. The most prevalent active sampling techniques employed at a variety of food processing plants have included direct impaction of bioaerosols on filters, multistage impaction, and liquid impingement (Jensen et al., 1992; Griffiths et al., 1997). Each of these methods have their own advantages and disadvantages, with the greatest concern with all sampling techniques being the survivability and viability of the microorganisms, which can impair further identification and enumeration when relying on traditional culture-based techniques (Lange et al., 1997). To improve microorganism detection and enumeration, some studies have combined the use of traditional culture-based techniques with molecular methods such as real-time quantitative polymerase chain reaction (real-time qPCR), which allows for the identification and quantification of non-culturable microorganisms (Rahn et al., 1992; Tsen and Chen 1992; Zeng et al., 2004; Nayak et al., 2005; Dungan and Leytem, 2009).

Traditional bioaerosol sampling methods, such as spore traps, impingers, nucleopore and gelatin filters (Errington and Powell, 1969) are greatly limited by the low recovery of spores due to biological stress during the sampling process (Lin and Li, 1998) or by the small volume of air sampled and hence the quantification that can be achieved. For example, the six-stage

Andersen impactor (Graseby Andersen, Atlanta, GA) that has long been recommended as a sampler for enumerating and sizing viable airborne microorganisms (Jensen et al., 1992), is used at an airflow rate of 27 L min^{-1} to collect bioparticles for 15 min periods (Olanya et al., 1997). The low sample flow rates likely lead to poor aspiration of larger bioparticles and failure to capture representative samples of bioaerosols when utilised for such short periods of time. Additionally, these traps will collect ubiquitous non-target species that can interfere with evaluating the sample for viability. The Burkard cyclone sampler, that collects air at 16.5 L min^{-1} through an orifice and deposits the particulates in a tube at the base of the cyclone stream, was found ideal to sample airborne *Aspergillus flavus* particles in dry climates in comparison to filter samplers and rotorods, used at the same airflow rate (Bock and Cotty, 2006). In a field study involving eight portable impactors (Bioculture, SMA, Microflow, SAS-180, Millipore Mair T, RCS High Flow, MAS-100 and Gelatin Filter), it was found that a majority of them under-performed compared to a BioStage impactor (SKC Inc., Eighty Four, PA), which is an equivalent to the Andersen N-6 viable impactor (Yao and Mainelis, 2007). The recently developed batch type cyclones (Cage et al., 1999; Sigaev et al., 2006) operate up to 400 L min–1 with controlled fluid levels, however, the retention of collected samples is reduced with time and is dependent upon the type and size of the particle (King et al., 2009). Commercial BWWC units are also available from Research International Inc (Monroe, WA) and Zaromb Research Corp (Burr Ridge, IL). The SASS system of Research International Inc. is designed to sample aerosol at 265 L min^{-1} and concentrate the particulate matter into a liquid batch of 5 mL, which is also volume-controlled to compensate for evaporation. The Zaromb Research Corp PHTLAAS system samples at a flow rate of 300 L min^{-1} and collects particulate matter in an initial batch of 25 mL of liquid; however, there is no evaporation compensation, and the liquid volume diminishes with time, so the maximum sampling time in a laboratory environment is typically only about 30 min. The virtual impactor XMX/2L (Dycor Technologies, Edmonton, Alberta, Canada), samples large volumes of air (up to 850 L min^{-1}) and can be coupled with a liquid impingement module to provide concentrated liquid samples for analysis. However, typical sampling times are short (5 min) due to the evaporation of the collection liquid and the collection efficiency is low (~30% for 5 μm bioparticles) (Fahlgren et al., 2010).

2.1. Microbial testing of air and surfaces

The meat industry operates with strict rules to maintain safe and hygienic environments. The microbiology of meat carcasses is highly dependent on the conditions under which animals were reared, slaughtered and processed; the condition of animals at slaughter, contamination spread and sanitation during the process and time-temperature of storage are important factors that will determine the microbiological quality of meat (Wells et al., 1991; Rahn et al., 1997; Shere et al., 1998, 2002; Savell et al., 2005; Kramer et al., 2017) and may also have affect the workers' health (Larsson et al., 1988; Robbins et al., 2000).

Air samples taken before and during three separate pork and beef slaughter processes at the bleeding area, hide removal or dehairing area, back splitting area and holding cooler using an Andersen N6 single-stage impactor (Sutton, 2004) showed that the total airborne bacterial counts were less than three logs before slaughtering and greater than three logs during slaughtering.

Lues et al. (2007) found 10^4 CFU m^{-3} microbial counts for potentially pathogenic bacteria and fungi in a chicken slaughtering facility, using a SAS Super 90 air sampler that collects airborne microorganisms onto 55-mm Rodac plates at 28 L min^{-1} airflow. Fungi are known

contaminants of indoor spaces (Clark et al., 1983; Lacy and Crook, 1988; Gorny et al., 2002). Higher counts of airborne microorganisms found in the receiving-killing and defeathering areas indicate the importance of controlling microbial levels before processing to prevent the spread of organisms. Due to its clump-like structure, *S. aureus* was found to be able to readily adhere to surfaces in poultry slaughtering facilities (Lutgring et al., 1997).

2.2. Bioaerosol sampling with the WWC

Wetted wall cyclone (WWC) air sampling methods provide the opportunity to overcome the challenges associated with other collectors by continuously sampling a large volume of air for longer periods of time (weeks to months) and concentrating the collected bioaerosols into a liquid solution. To accommodate the needs of bioaerosol sampling systems, effective WWC units that have airflow rates of 1,250, 300, and 100 L min^{-1} have been developed (Hu and McFarland, 2007; McFarland et al., 2010). The autonomous WWC aerosol samplers atomise a liquid spray in the aerosol inlet, creating droplets with a volume median diameter of about 40 μm, which are drawn with the sampled airflow and ambient particulate matter (PM) into the cyclone inlet. The liquid droplets and sampled particles (including bioaerosols) become entrained in a liquid film on the inner wall of the cyclone. Upon effluxing from the impaction zone, the liquid is transported along the cyclone wall to the region of the skimmer, where the entrapped liquid is aspirated from the cyclone by an external pump. The WWC collects and concentrates ambient particles into a small volume of collection liquid, which increases the concentration of the particles in the sample by a factor of 10^6 as compared to ambient air. The WWC units have been demonstrated to operate efficiently at extreme temperatures ($-22-50\,^{\circ}\text{C}$), and in both dry and wet air environments.

Two beef harvesting establishments in Texas, a teaching facility and a family-owned rural abattoir were sampled three times in one year, collecting samples during the spring, summer and fall seasons. At the establishments, two WWC units were set up to continuously sample air for one day at the dehiding area and at the chiller entrance area at a flow rate of 100 L min^{-1}. Two dynamic samplers were moved along the straight processing line from one end of each facility to the other, collecting air for 30 min periods at the bleeding, delimbing and dehiding steps, and in the chiller area. The samples were tested by microbial plating and whole-cell qPCR method. From all the positive samples, DNA was extracted and analysed by Illumina sequencing to delineate the microbiome in the collected samples. Results show significant fluctuations in the concentrations of the different bacterial species (Beck et al., 2019).

The autonomous WWC system operates efficiently in environments with elevated PM concentrations and efficiently collects relevant, viable pathogens. Detected variations in the number of bacteria collected in real-time during a given test also allow for monitoring and predictive modeling of environmental changes.

Salmonella and Shiga toxin producing *E. coli* (STEC) have been recognised as pathogens of concern in meats because of the prevalence of these microorganisms in the gastrointestinal tract and hide of livestock. Bacterial ingestion from contaminated food products causes a great economic burden from the costs of hospitalisation and mortality of those who become infected, as well as the social impact on families and communities. During the harvesting process, these pathogens may become aerosolised from the carcasses by various mechanisms, including worker activity and airflow from the HVAC systems. Of greatest concern in the meat industry is the possibility of generating bioaerosols containing bacterial pathogens. To identify likely sources of airborne contamination with foodborne pathogens and improve plant sanitation programs, air

samples inside the facilities have been collected using different bioaerosol collectors. However, many collectors are not capable of dynamic sampling of large air volumes that are required for tracking the movement of the pathogens in the facilities. The wetted wall cyclone collectors have been successfully applied for the spatial and temporal sampling of bioaerosols, enabling the quantitation, speciation, and tracking of the particles.

2.3. Computational fluid modeling (CFD)

Processing facilities, particularly the fabrication rooms of meat packing plants, provide a supportive environment for infectious particles due to pressure hosing that creates high moisture and fat content in indoor air. Disassembly and fabrication room workers standing next to each other are exposed to the risk of infectious droplets transmission by asymptomatic carriers who may spread microbes unintentionally to other workers through sneezing, coughing or talking. Several studies found a correlation in meat processing plants between infection risk and indoor air properties, including indoor temperature, relative humidity, and ventilation, in addition to outdoor airflow rate, and high number of workers in close physical proximity (Herstein et al., 2021; Pokora et al., 2021; Dyal et al., 2022).

Based on the mechanical layout, a novel CFD model of a fabrication room including air properties and stationary objects (columns, belts, condensers and fans) was prepared (Kumar et al., 2023) to help identify locations that are potentially at lower or higher risk for exposure to viruses that can travel far up in the air (Bourouiba, 2020).

In the study of Kumar et al. (2023) a pressure-based solver was employed for the simulations, utilising the Navier–Stokes realisable k-ε turbulence model (Zou et al., 2018; Armand and Tâche, 2022; Jing et al., 2023; Santamaría Bertolín et al., 2023). For developing airflow in the facility, initially steady state simulations were performed, which were later used for transient simulations.

The Rosin–Rammler diameter distribution approach (Bailey et al., 1983) was adopted for injecting droplets with a mean diameter of 90 µm and a spreading parameter of 1.99 µm. The cough and sneeze show similar pressure responses with different intensities (Gupta et al., 2009; Busco et al., 2020).

The airflow models created based on the facilities' layout and HVAC design coupled with the bioaerosol concentrations enable the visualisation of the pathogen transport in the facility and offers insight into designing appropriate mitigation measures. These measures can include air curtains above the entrances and plastic separation panels between workers to reduce the spread of aerosolised pathogens.

2.4. Other airborne contaminants

Bioaerosols are often responsible for the dissemination of pathogens in food products, including meat, field crops, fruits, vegetables and nuts. The presence of food-associated microorganisms and foodborne pathogens in bioaerosols requires further studies concerning their potential role in food spoilage and food-associated infections. In addition, antimicrobial agents that are highly toxic to bacteria and fungi have been used extensively on plants, resulting in nanoparticle residues that may become airborne during food processing, posing potential health hazard (Wang et al., 2007; FDA, 2018).

To reduce the pathogen concentrations in processing plants, factors affecting bioaerosol concentrations should be explored. Due to the lack of standardised sampling methods in

bioaerosol research, previous studies in which bioaerosol concentrations have been measured cannot be responsibly compared. Furthermore, the low sampling volume airflow rates of existing collectors coupled with the high levels of stress placed on the sampled pathogens using the most common bioaerosol collectors likely lead to inaccurate estimations of the presence and viability of bioaerosols in food processing facilities (Jackson, 1991; Esbensen, 2004).

2.5. Wastewater-based epidemiology

Another potential aspect of monitoring the changes in bioaerosol concentration at the facilities is through the analysis of wastewater effluent that is collected in tanks, separately from the human waste collected in pits. The wastewater effluent contains bioparticles that become entrained in water droplets during extensive hosing and cleaning of the facility, potentially resuspending bacteria and viruses from the surfaces, offering a new approach to identify a correlation between virus levels in wastewater and clinically confirmed cases in the surrounding city area (Zhang and King, 2023).

To overcome these limitations, it is important to focus on the development of improved bioaerosol sampling techniques to monitor bioaerosol concentrations in food processing plants as a function of environmental conditions, ventilation and operating systems, and exposure to pathogens to more fully characterise how diseases are propagated through food processing systems. The results of such research will deliver a significant and sustained long-term impact on agriculture and food systems.

3. CONCLUSIONS

The overall goal of determining the levels and distribution of potentially hazardous airborne microorganisms in various areas of high-throughput food processing plants is to provide the scientific information base for future efforts at enhancing the microbial safety of foods by preventing and/or mitigating contamination during processing. Airborne contaminant levels should be analysed with data collected during concomitant monitoring of various environmental factors to evaluate their influence on microbial populations associated with bioaerosols and validate the computational models for their transport.

Conflict of interest: M.D. King is a member of the Editorial Board of the journal, therefore she did not take part in the review process in any capacity and the submission was handled by a different member of the editorial board. The submission was subject to the same process as any other manuscript and editorial board membership had no influence on editorial consideration and the final decision.

ACKNOWLEDGEMENTS

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