Microbial Composition in Bioaerosols of a High-Throughput Chicken-Slaughtering Facility

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ABSTRACT The microbial composition of the air in various areas of a high-throughput chicken-slaughtering facility was investigated. Over a 4-mo period, 6 processing areas were sampled, and the influence of environmental factors was monitored. The highest counts of microorganisms were recorded in the initial stages of processing, comprising the receiving-killing and defeathering areas, whereas counts decreased toward the evisceration, air-chilling, packaging, and dispatch areas. Maximum microbial counts were as follows: coliforms, 4.9×10^3 cfu/m^3; *Escherichia coli* 3.4×10^3 cfu/m^3; *Bacillus cereus*, 5.0×10^4 cfu/m^3; *Staphylococcus aureus*, 1.6×10^4 cfu/m^3; *Pseudomonas aeruginosa*, 7.0×10^4 cfu/m^3; presumptive *Salmonella* spp., 1.5×10^4 cfu/m^3; *Listeria monocytogenes*, 1.6×10^4 cfu/m^3; and fungi, 1.4×10^4 cfu/m^3. 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 downstream. This should limit the risk of carrying over contaminants from areas known to generate high counts to areas where the final food product is exposed to air and surface contamination.

Key words: bioaerosol, chicken slaughtering, South Africa

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INTRODUCTION

Large facilities that produce a variety of ready-to-cook poultry products are increasingly dominating the poultry slaughtering industry (Thaler, 1999). The poultry industry has changed from providing mainly whole chickens for domestic consumption to cut and deboned poultry packed in bulk containers for domestic and export markets (Raj et al., 2001; Turcsan et al., 2003). Although much research has been done on microbial contaminants associated with the various stages of processing poultry and meat products (Buys et al., 2000; Borch and Arinder, 2002), only few investigations have reported on the indoor air quality of these plants (Rylander et al., 1994; Whyte et al., 2001). Poultry processing plants are prone to indoor air contamination, and microorganisms settle on and contaminate working surfaces, equipment, and the hands of workers (Whyte, 2002). In addition, air plays a significant role in the transmission of pathogens and may be implicated in contamination of poultry meat at various stages of slaughtering and processing (Geornaras et al., 1996; Ellerbroek, 1997; Whyte et al., 2001). Fecal contamination of chicken carcasses with *Escherichia coli* sporadically causes problems at abattoirs (Summer, 2004), whereas food pathogens such as *Listeria monocytogenes*, *Bacillus cereus*, and especially *Staphylococcus aureus* are often associated with poultry products (Donnelly, 1994). *Staphylococcus aureus* is often used as an indicator of food-handler hygiene, because it is found extensively in slaughtering environments and originates particularly from employees' hands, noses, or hair (Bennet and Lancette, 1995; Shale, 2004). Environmental pathogens such as *Pseudomonas aeruginosa* also occur liberally in food processing environments, because they are able to survive at low temperatures (Geornaras et al., 1995; Eisel et al., 1997; Forsythe, 2000).

Bioaerosols implicated in respiratory-associated hazards have received much attention, but the potential of food-associated microorganisms and foodborne pathogens in bioaerosols to cause food spoilage or food-associated infections needs to be elucidated. Airborne microorganisms have been indicated to cause allergic reactions, irritation of the eyes and upper respiratory tract, respiratory infections, and other symptoms such as fatigue, especially in individuals working in the offloading, receiving, and defeathering areas (Savilahti et al., 2000; Chang et al., 2001; Huang et al., 2002). This study was undertaken to determine the levels and distribution of airborne microorganisms in various processing areas in a high-throughput chicken abattoir during processing, to concomitantly monitor various environmental factors in the different areas over a specific period, and to evaluate their influence on the microbial population associated with the bioaerosols.

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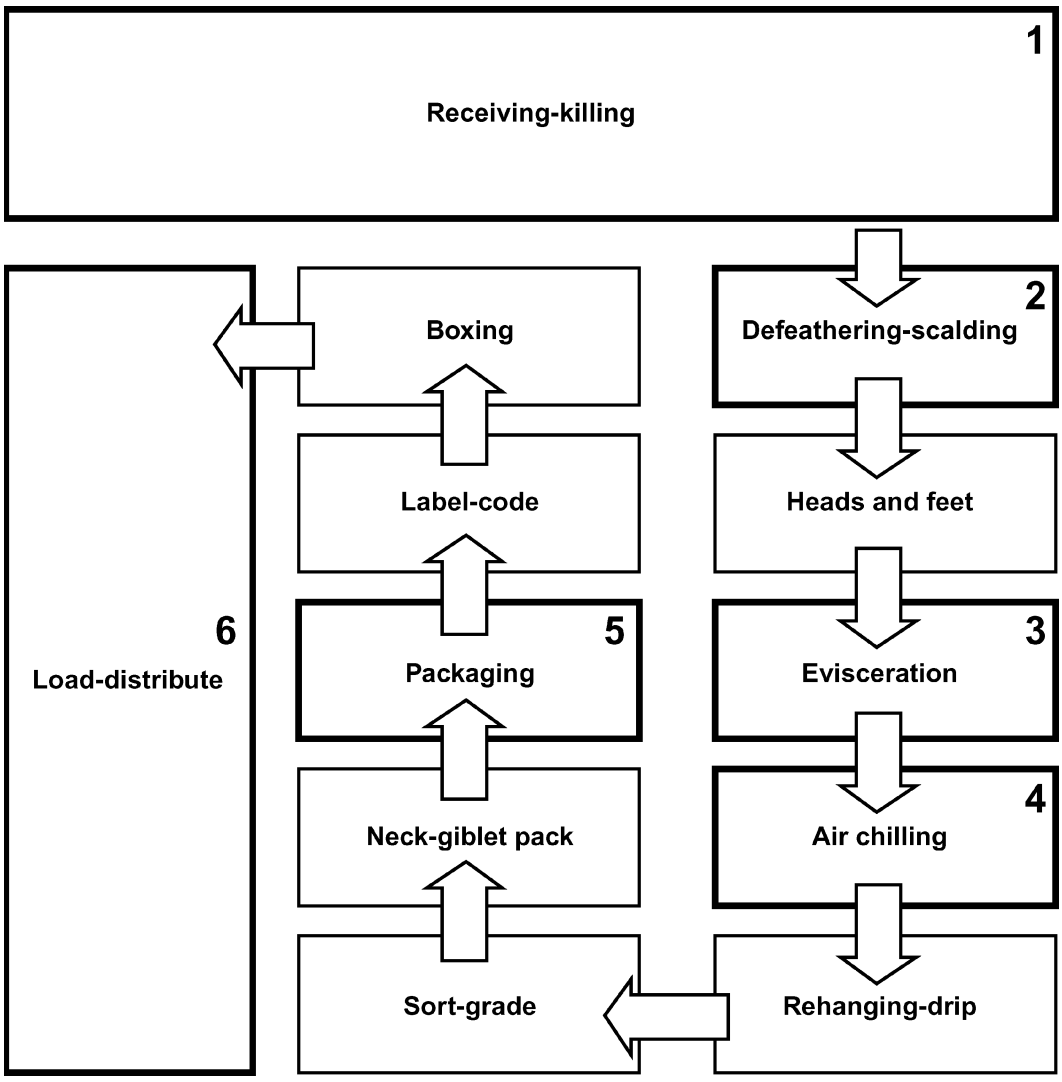

Figure 1. Schematic representation of the slaughtering processes at a chicken processing plant, with numbers 1 to 6 representing sampling areas.

MATERIALS AND METHODS

Sampling Site

Air samples were collected from a high-throughput chicken abattoir (±32,000 birds slaughtered per day) situated in the industrial area of Kroonstad in the Free State province, South Africa. This facility employs approximately 400 workers and operates 16 h per day. Operations carried out at this plant include the receiving and killing of birds, scalding, defeathering, evisceration, head and feet removal, spin-chilling and air-chilling of carcasses, whole bird packaging, and portion packaging of the final product (Figure 1).

Sampling Protocol

Samples were collected in duplicate at monthly intervals over a 4-mo period (August to November) from 6 processing areas, which included receiving-killing, defeathering, evisceration, air-chilling, packaging, and dispatch (Figure 1). Bioaerosol samples were collected by impaction on agar plates using a SAS Super 90 air sampler (PBI Int., Milan, Italy) that collects airborne microorganisms onto 55-mm Rodac plates [Merck (Pty.) Ltd., Modderfontein, South Africa]. The air sampler was precalibrated and adjusted to a flow rate of 28 L/min. All removable parts of the air sampler were sterilized by autoclaving before sampling and sterilized between samples with 70% ethanol.

Isolation of Microorganisms

Samples were analyzed for the presence of *S. aureus*, coliforms, *E. coli*, *P. aeruginosa*, *L. monocytogenes*, *B. cereus*, *Salmonella* spp., and fungi.

Enumeration of *S. aureus* was done on Baird Parker Agar (Biolab Diagnostics, Merck, Wadeville, South Africa) after 48 h of incubation at 36°C. Typical *S. aureus* colonies were recognized as gray-black and shiny, with clear zones surrounding the colonies.

Violet red bile MUG agar (Biolab Diagnostics) was used to isolate *E. coli* and other coliforms (International Standards Organization, 1993). Inoculated plates were incubated at 37°C for 24 h; *E. coli* colonies were detected as dark red and coliforms as small pink colonies.

Cetrimide Agar (Biolab Diagnostics) with added glycerol was used for culturing and enumerating *P. aeruginosa.* After 48 h of incubation at 25°C, typical yellowish *P. aeruginosa* colonies were identified.

Enumeration of *L. monocytogenes* was performed on *Listeria* selective agar [Merck (Pty.) Ltd.], and plates were incubated at 37°C for 48 h. *Listeria monocytogenes* colonies were recognized as greenish-gray with a black core and halo.

Bacillus cereus selective agar (Scharlau Chemie SA, Barcelona, Spain) was used for culturing and enumeration of *B. cereus.* Agar plates were incubated at 30°C for 18 to 24 h and examined for typical *B. cereus* colonies, recognized as large (±5mm in diameter) turquoise-peacock blue colonies and small zones of egg yolk precipitate.

Brilliant green agar [Merck (Pty.) Ltd.] plates were inoculated for culturing and enumerating *Salmonella* spp. and incubated at 42°C for 48 h. *Salmonella* colonies are typically reddish-pink opaque and surrounded by brilliant red zones.

Potato dextrose agar (Biolab Diagnostics) plates, acidified with tartaric acid to pH 3.5, were inoculated for culturing and enumeration of fungi and incubated at 25°C for 5 d. Yellowish-white colonies were identified as yeasts, whereas mold growth was recognized by its mycelial appearance.

Control Strains

Bacterial strains included as controls were *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *L. monocytogenes* ATCC 19117, *B. cereus* ATCC 14579, and *Salmonella enteritidis* ATCC 13076.

Environmental Parameters

Temperature, RH, wind velocity, and airborne particulates were evaluated from August to November (winter to spring), and the readings were done in triplicate at a height of 1.5 m above the floor (Venter et al., 2004). Direct reading instruments used for monitoring were as follows: 1) area heat stress monitor (QUESTemp°15; Quest Technologies Inc., Oconomowac, WI) to measure temperature as well as RH, 2) airflow anemometer (Airflow Instrumentation LCA 6000 VT; High Wycombe, Buckinghamsire, UK) for measuring wind velocity, and 3) handheld aerosol monitor (1005/1060; PPM Enterprises Inc., Knoxville, KY) for determining airborne particulates (Venter et al., 2004).

Statistical Processing

Data were represented as the means of samples collected during the 4 sampling months at 3 different points in each area. Box plots were constructed to show the 25th and 75th percentiles as well as the means. For normally distributed data, general ANOVA and correlations were used to measure significant differences among means of the environmental factors and bioaerosols. The data consisted of 4 observations (August to November) with duplicate samples. Stepwise regression was used in determining the significance of the independent variables (environmental factors) in various regression models. The *t*-statistics indicated the independent variable to be significant if the related P-value < 0.05. A statistical program, *STATISTICA* Base (version 7.1, StatSoft Inc., Tulsa, OK), was used to calculate r- and P-values.

RESULTS AND DISCUSSION

Microbial counts demonstrated a similar pattern in the prevalence of the various microbiota throughout the various processing stages (Figure 2, panels A to H). There was a definite decline in the numbers from the receiving-killing area downstream toward the dispatch area, whereas the air-chilling area had the lowest microbial counts. The high prevalence of airborne microorganisms in the receiving-killing area is attributed to the flapping of wings and the excessive movements of the birds (Lutgring et al., 1997).

Counts of airborne *S. aureus* reaching 10^4 cfu/m^3 were found in various areas (Figure 2, panel A), except in the air-chilling area, where average counts were 3.9×10^1 cfu/m^3. Due to its clump-like structure, *S. aureus* is able to readily adhere to surfaces (Lutgring et al., 1997; Plaatjies, 2004), and in previous studies, the microbiota from the receiving area were described as consisting of mainly *Staphylococcus* spp. (Holt et al., 1994; Ellerbroek, 1997).

Airborne fungi counts of up to 1.3×10^4 and 1.2×10^4 cfu/m^3 were detected in the receiving-killing and defeathering areas (Figure 2, panel B). Fungal spores are very small, relatively resistant to drying, and are readily aerosolized. Although clear correlations between the presence of airborne fungi and their effect on human health have not unambiguously been identified in epidemiological studies, indoor fungi is regarded as a potential health hazard (Bornehag et al., 2001; Burge, 2001).

The highest total coliform counts were found in the defeathering area (average 1.6×10^3 cfu/m^3; Figure 2, panel C), whereas *E. coli* predominated in air samples from the packaging area (average count 1.5×10^3 cfu/m^3; Figure 2, panel D). Geornaras et al. (1996) also reported on the dominance of *E. coli* in a similar processing plant, comprising 95.6 and 93.7% of the isolates from the defeathering and packaging areas, respectively. *Escherichia coli* is exclusively a large-intestine organism and is used in gauging the level of processing or postprocessing contamination by fecal sources (Levinson and Jawetz, 1994), whereas the other members of the coliforms are found in the environment also and are more commonly used as indicators for assessing microbial proliferation in foods (Department of Health, 2000). The relatively high prevalence of *E. coli* in the air of the receiving-killing and the defeathering areas could have resulted from fecal particu-

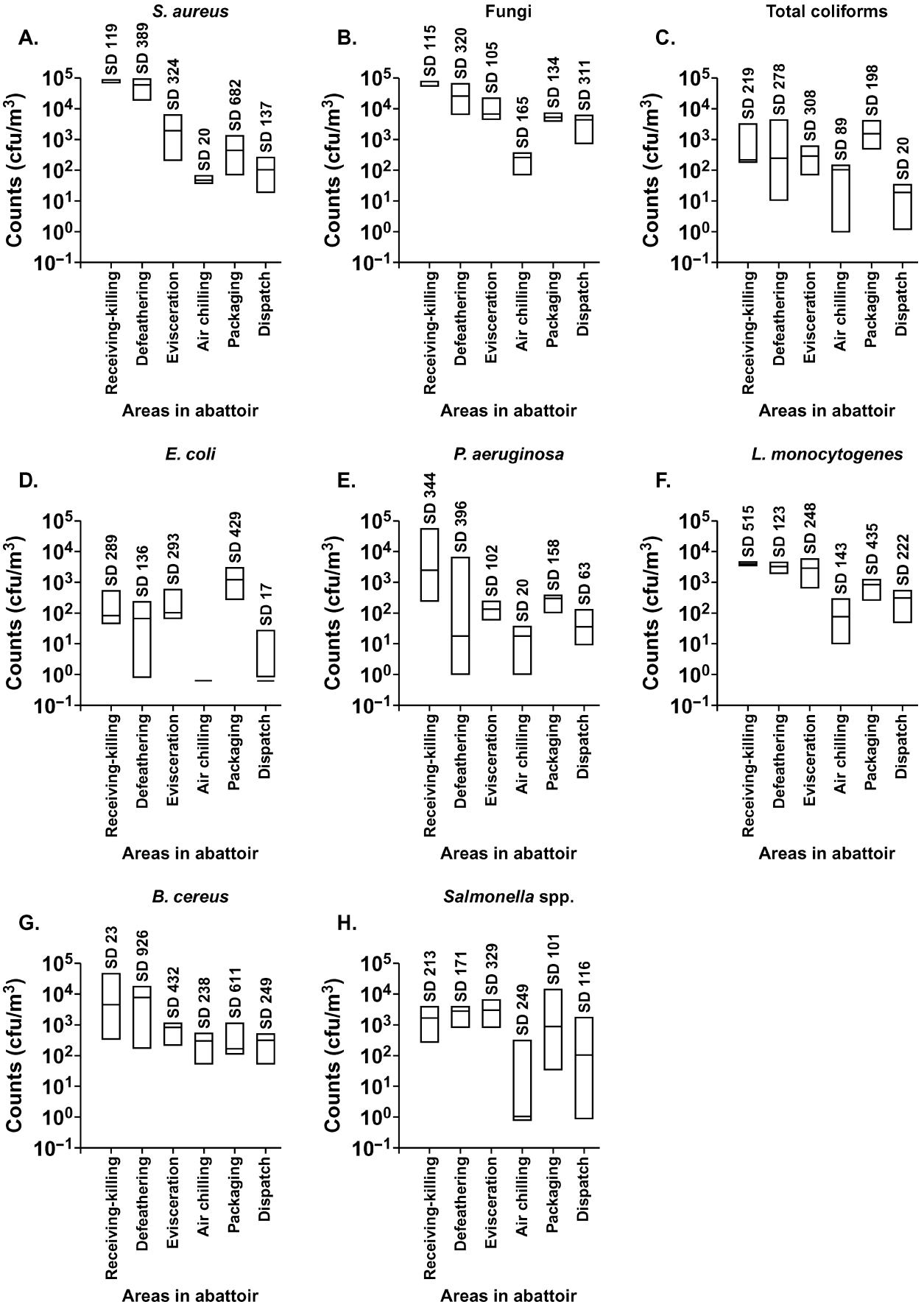

Figure 2. The distribution of microorganisms in the air of 6 different sampling areas of a high-throughput chicken abattoir: *Staphylococcus aureus* (panel A), fungi (panel B), total coliforms (panel C), *Escherichia coli* (panel D), *Pseudomonas aeruginosa* (panel E), *Listeria monocytogenes* (panel F), *Bacillus cereus* (panel G), and *Salmonella* spp. (panel H).

lates spreading by the flapping of wings. Fecal contamination is often caused when the lower intestinal tract is ruptured during processing, resulting in the release of contaminating fecal material and the consequent high counts of *E. coli* in the postprocessing areas. However, if handled in the correct manner, birds should have a minimum of waste material in their digestive tracts (Summer, 2004).

In the processing flow, the highest counts of presumptive *Salmonella* spp. were detected in the first 3 areas (averages 5.5×10^3, 3.3×10^3, and 3.8×10^3 cfu/m^3). In the last 2 areas downstream (packaging and dispatch), average counts of 2.3×10^3 cfu/m^3 and 5.6×10^2 cfu/m^3 were recorded, whereas the counts found in the air-chilling area were 1.2×10^1 cfu/m^3 (Figure 2, panel H). Apart from false positive counts of *Salmonella* in the receiving-killing area reported by Gallo et al. (1988), *Salmonella* has seldom been isolated from any of these areas (Lutgring et al., 1997). High prevalence of *P. aeruginosa* was detected in the receiving-killing area (1.9×10^3 cfu/m^3; Figure 2, panel E) and could have been introduced upon the arrival of the birds at the processing plant. Airborne *P. aeruginosa* was also found in the evisceration and the packaging areas, with counts ranging from 1.5×10^2 to 3.3×10^2 cfu/m^3 (Figure 3, panel E). *Pseudomonas* spp. are often found along with other gram-negative bacilli in poultry meat and has been called one of the most notorious bacteria to cause spoilage of refrigerated foods, originating predominantly from water or soil on equipment (Geornaras et al., 1996; Ellerbroek, 1997; Buys et al., 2000).

Average levels of airborne *L. monocytogenes* in the receiving-killing, defeathering, and evisceration areas were 9.3×10^3, 3.9×10^3, and 2.5×10^3 cfu/m^3, respectively (Figure 2, panel F). Although *Listeria* spp. are psychrotrophic and proliferation is not inhibited by normal refrigeration (Rammel, 2003), *L. monocytogenes* counts were lowest in the air-chilling area. This organism is nonspore-forming, notably resistant to pH fluctuations, high salt concentrations, and desiccation, and the majority of the more than 22 species identified are found in food-processing plants (Ryser and Marth, 1991; Miller, 1992). *Bacillus cereus* counts in the air samples of the receiving-killing and the defeathering areas were relatively high (1.8×10^4 and 8.04×10^3 cfu/m^3, respectively), which again could be attributed to wing flapping (Figure 2, panel G). *Bacillus cereus* is a common airborne and dustborne contaminant that readily multiplies in meat products. The production of spores enable these organisms to withstand unfavorable conditions such as low temperatures or heat and may improve the chances of *B. cereus* to be present in high numbers (Stevenson and Segner, 1992; Whyte et al., 2001). In a study done by Nel et al. (2003), *B. cereus* levels were reported to rapidly increase when a product was exposed to poor handling and processing procedures. The most common airborne *Bacillus* spp. were, for many years, dismissed as harmless contaminants with weak to nonexistent pathogenicity. However, infections are increasingly reported, and because the spores are abundant in the environment and usual methods of disinfection and antisepsis are powerless in controlling them, these organisms are becoming a serious health risk (Talaro and Talaro, 1999).

Microorganisms detected in the indoor air of processing plants are frequently found to be derived from humans but may also emanate from intramural sources or enter from the outside environment. High concentrations of microorganisms may reflect on insufficient ventilation in relation to the number of persons and activities involved (Verhoeff et al., 1992; Otten and Burge, 1999). This is often the result of ventilation systems that are not properly maintained and that generate excessive condensation and moisture (Law et al., 2001; Lee et al., 2002; Guo et al., 2004). The importance of the airflow pattern in food processing plants can be overlooked, and ventilation systems are sometimes designed without taking into consideration the settling of particles (Holmberg and Chen, 2003). In a room with a low air exchange rate, microbial concentrations have been reported to be twice as high as in rooms with higher exchange rates (Barlett et al., 1999). Buildings such as the poultry slaughtering facility should, therefore, be designed to achieve proper air distribution and to control the dispersal of particles and microbes (Goodman, 1999).

The overall RH in area 1 (receiving-killing) reached an average of 71% (Figure 3, panel A), which could have been influenced by rainy conditions prevailing during the second sampling interval. The evisceration (77%) and packaging (64%) areas had the highest mean RH. Multiple processes carried out in the relatively small evisceration area, which included the final washing of carcasses and the weighing and packaging of livers, feet, and heads, could contribute to elevated RH. It is preferred that RH not exceed 100%, which would elevate condensation processes and cause high levels of discomfort (Wang et al., 2001; Cappella, 2004; Williams, 2004). The South African Bureau of Standards (2001) also stated that the RH should be controlled to protect food quality. Although the majority of microorganisms survive better in an environment with high RH, *B. cereus* appears to prefer a dry environment, because RH and *B. cereus* counts in the receiving-killing area indicated a strong negative relationship ($r = -0.9838$; P-value = 0.016). Relative humidity also had a negative effect on the dispersal of airborne *Bacillus* spores. The presence of *P. aeruginosa,* on the other hand, showed an increase associated with an increase in RH ($r = 0.9687$; $P = 0.031$).

Average temperatures in the receiving-killing and defeathering areas were below 18°C, and in the packaging and dispatch areas, they were below 21 and 22°C (Figure 3, panel B). The evisceration area had an average temperature of 23°C and the air-chiller 4°C throughout the 4-mo sampling intervals. According to poultry regulation, meat should be subjected to uninterrupted chilling to reduce the core temperature of chilled meat to 4°C within 12 h, and meat that is being frozen may not be removed from the freezer before a core temperature of –12°C has been reached (Republic of South Africa, 2000). In addition to

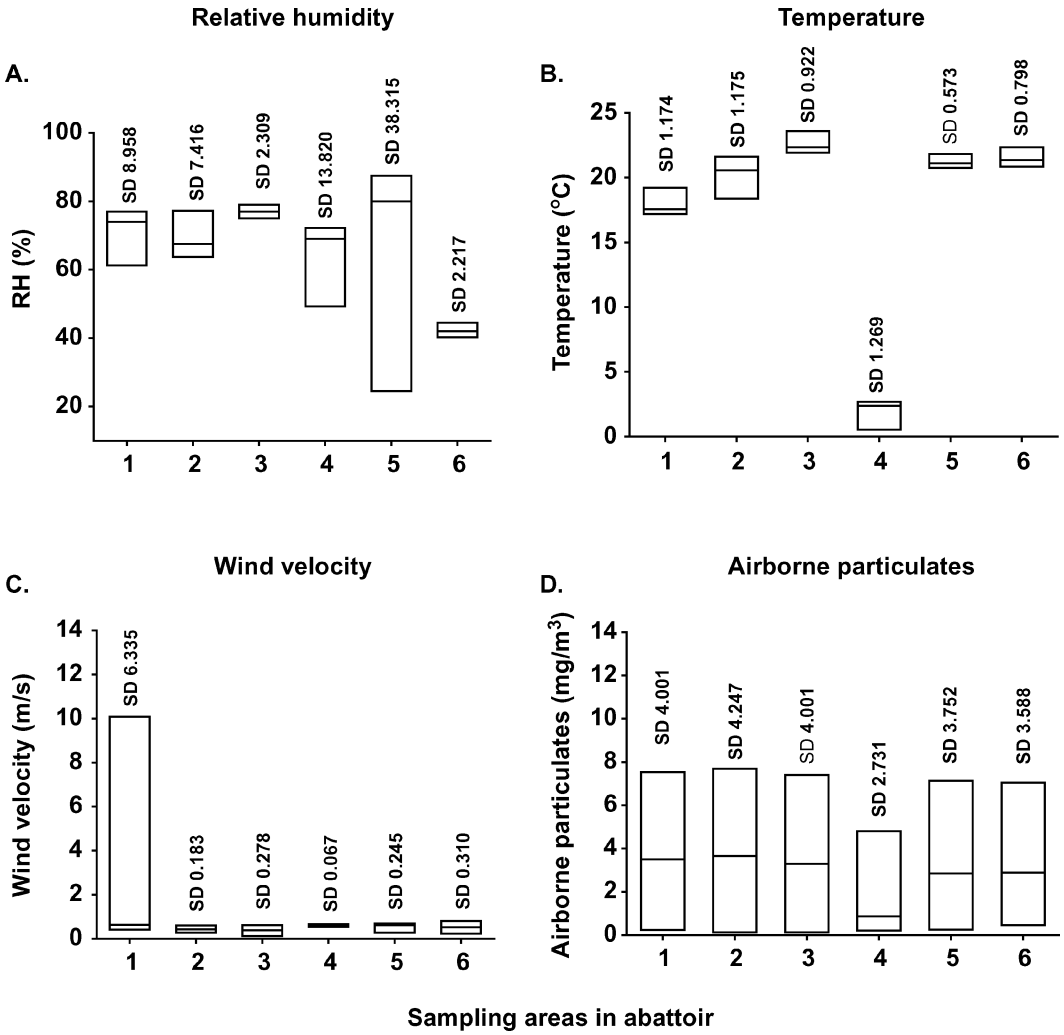

Figure 3. Environmental factors monitored in the 6 processing areas during the 4-mo sampling period. These areas are as follows: 1 = receiving-killing; 2 = defeathering; 3 = evisceration; 4 = air chilling; 5 = packaging; 6 = dispatch.

the microorganisms being inhibited at low temperatures, their adhesion to surfaces is also influenced by temperature (Czechowski, 1990; Pompermayer and Gaylarde, 2000). By employing the statistical program *STATISTICA* Base (StatSoft Inc.), a positive correlation (r = 0.9738; P = 0.026) was observed between the temperature and *S. aureus* counts in the receiving-killing area and between fungi counts and temperature in the evisceration area (r = 0.9761; P = 0.024).

An important function of airflow or wind velocity is to ensure proper mixing of the temperature and humidity throughout the processing environment (Godish, 1995; Goodman, 1999). The wind velocity in the receiving-killing area reached an average of 3.7 m/s (Figure 3, panel C), which could have resulted from an enhancing effect by the air from the outside environment. Elevated wind speed could then result in the dispersal of airborne particles (Holmberg and Chen, 2003). Because low air exchange affects humidity that could lead to high dust concentrations, the air supply to processing areas with a high prevalence of contaminants should be filtered to at least 2 μm (Goodman, 1999; South African Bureau of Standards, 2001). The majority of airborne microorganisms observed in this study correlated with the airflow in the receiving-killing area, and airflow also contributed to the dispersal of *B. cereus* spores. In the defeathering area, however, a negative correlation was found with *B. cereus* counts. This was also evident with *P. aeruginosa* found in the packaging areas.

The highest concentrations of airborne particulates were measured in the receiving-killing and defeathering areas (3.76 and 3.83 mg/m^3; Figure 3, panel D). The high levels of particulates in the receiving-killing area could again be associated with wing flapping, whereas the high concentrations in the defeathering area could be caused by the absence of a physical segregation between the receiving-killing and defeathering area. More variation was demonstrated over time with the airborne particulates than with the other 3 parameters. A strong correlation was found between airborne particulates and the presence of *Salmonella* spp. in the receiving-killing area (r = 0.9439; P = 0.056). Because *Salmonella* has rarely been isolated from other processing areas than receiving-killing, it is suggested that this organism could have been

introduced into the facility via dust spread by the birds (Mitchell, 2000). Airborne particulates were also the only environmental factor to have a significant influence on the dispersal of coliforms (r = 0.9869; *P*-value = 0.013) or *Salmonella* spp. (r = 0.9867; *P* = 0.014) in the air-chilling area.

The RH and airborne particulates demonstrated significant influence on the presence of microorganisms. With *B. cereus* as a selected independent variable, the RH in the receiving-killing area showed significance at *P* = 0.0167 and the airborne particulates at *P* = 0.0032. The values for *P. aeruginosa* (*P* = 0.0016) and *B. cereus* (*P* = 0.0633) in the receiving-killing area were significantly different when airflow was used as a parameter estimate. The presence of *S. aureus,* fungi, and coliforms in the defeathering area could not be correlated with the environmental factors monitored. In the packaging area, *S. aureus,* fungi, coliforms, *L. monocytogenes, B. cereus,* and *Salmonella* spp. showed no linear relationship with any of the environmental factors, whereas airflow in the packaging area with general ANOVA showed a linear relationship between *P. aeruginosa* and airflow in the same area (*P* = 0.0077). *Staphylococcus aureus* and coliforms in the dispatch area showed no significant difference, because no variables met the 0.5 significance level in this area of the abattoir. Correlations between environmental factors and bioaerosol composition have previously been demonstrated by Venter et al. (2004) in chicken egg production plants, and the implications of parameters such as RH, temperature, wind velocity, and airborne particulates and the relationship with the airborne microbial loads require further investigations.

In conclusion, the elevated counts of airborne microorganisms observed in the receiving-killing and the defeathering areas emphasize the importance of controlling the microbial levels before processing, in addition to the control measures implemented in the processing facility. Opposed to well-documented hazards of bioaerosols containing respiratory-associated microorganisms, the presence of food-associated microorganisms and foodborne pathogens in bioaerosols requires further in-depth studies, concerning their potential role in food spoilage and food-associated infections. In a poultry-slaughtering facility, pathogens that are known to become airborne should be reduced to minimal levels to limit the carryover from areas known to generate high counts to downstream areas where the product is exposed to air and surface contamination. Poultry products are prone to microbial contamination, and the focus should not only be on surface cleaning and disinfecting of utensils, but also on reducing or eliminating microbial air contamination, and adaptations should take the climate and other environmental characteristics of the region into consideration while addressing the hygiene requirements. Suggestions made on the findings in this study may contribute to improved practices during raw poultry processing.

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