Outcomes of Microbiological Challenges in Poultry Transport: A Mini Review of the Reasons for Effective Bacterial Control

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Abstract: This review aims to highlight the main microbiological challenges faced in poultry transport and show the role of effective bacterial control during this process to ensure poultry health and meat safety and to reduce economic losses. Poultry infections are among the most frequent infections in production systems, manifesting themselves in hatcheries, farms, slaughterhouses, and during transport between these integration centers. Although the clinical symptoms of these infections can range from mild to severe, many of them can lead to irreversible conditions, resulting in death and compromising productive results. Bacteria are the main causative agents of these infections, although fungi, viruses, and protozoa may also be involved. During the transport of poultry from farms to slaughterhouses, poultry are very vulnerable to infectious conditions. Therefore, implementing effective antibacterial management, focused on professionals, transport crates, and transport vehicles, is essential to guarantee the survival and quality of poultry until the moment of slaughter.

Keywords: antibacterials; bacterial contamination; broilers; poultry farming; poultry microbiology; slaughterhouse; transport trucks

1. Introduction

Poultry refers to avian species (e.g., Gallus gallus domesticus—chicken; Anas platyrhynchos domesticus—duck) housed in a diversity of production systems (e.g., organic, free-range and conventional structures) that are dedicated to the production of poultry products, specifically chicken meat and table eggs for human consumption and eggs hatching for reproduction [1,2]. Bacterial contamination in poultry can be initiated by ingestion of contaminated feed or water, direct contact with surfaces, other poultry, or contaminated excreta, and inhalation of contaminated particles [3,4]. The spread and severity of bacterial contamination can vary due to several factors, such as flock density, the moment of introduction of harmful bacteria into the environment, bacterial pathogenicity, the health status of the poultry immune system, and antibiotic practices adopted to prevent or control contamination [5–8]. The clinical spectrum of broilers with bacterial infection ranges from non-specific signs to symptoms of acute respiratory illness, such as coughing, ruffled sneezing associated with weakness, depression, and severe dehydration [9]. These infections can cause suffering to poultry and are costly for the production sector.

Although the process of slaughtering poultry is generally considered safe, it is crucial to be aware of the potential complications that can arise during this procedure. Safety concerns arise due to possible failures in sanitary hygiene protocols adopted before, during,
or after slaughter, which can have a significant impact on the microbiological safety of the final poultry meat product. Contamination of poultry meat should not be tolerated, as it represents a serious warning for both the production sector and consumers. In a study conducted by Dantas et al. [10], the level of adherence to hygienic–sanitary standards was examined in a representative sample of 20 meat product slaughterhouses. This study ranged from the assessment of sanitary facilities to the verification of quality control of the final product and its subsequent transportation. Worryingly, the researchers observed that all the slaughterhouses analyzed exhibited deficiencies in relation to the established criteria, with around 90% of the evaluated parameters not complying with the recommended guidelines in one of them. An emblematic example of these deficiencies was the lack of adherence by handlers to basic hygiene practices, such as regular hand washing and the use of personal safety equipment, which could seriously compromise the microbiological integrity of the meat. Silva [11] detected the presence of mesophilic bacteria at levels above established limits at various stages of the broiler slaughter flow. These bacteria were identified in the washing tanks, on the carcasses after the washing process, on the evisceration table, on the knives, on the drip tables, and on the carcasses after the evisceration process, as well as in the cold room. Detailed analysis of the flowchart revealed several nonconformities, while the microbiological evaluation demonstrated a worryingly high level of contamination in several stages of the process.

De Quadros et al. [12] observed that, over the course of a year in a slaughterhouse, of the 4,372,619 broiler carcasses destined for total condemnation, contamination was identified as the main cause, representing 24.84% of the total. Similarly, Pissolitto and Piassa [13] observed that gastrointestinal and biliary contamination were responsible for the highest condemnation rate of broiler carcasses, totaling 1,051,666 carcasses during a period of 90 days in a slaughterhouse. This finding corresponds to a significant percentage of 49.43%. Pathogenic bacteria such as \textit{Campylobacter jejuni} [14], \textit{Salmonella} spp. [15] and \textit{Escherichia coli} [16], have been consistently identified in samples from broilers slaughtered in slaughterhouses. The origin of these contaminations often goes back to deficiencies in hygienic–sanitary management, not limited only to the period during slaughter, as mentioned previously, but also covering the poultry journey from leaving the farm to the slaughterhouse.

The main function of trucks (Figure 1) on the farm–slaughter route is to ensure the transport of poultry with maximum quality, ensuring their health until slaughter and high standards of confidence in poultry transport practices. Sanitizing poultry transport crates must begin before catching the poultry. In this process, the crates need to undergo pre-cleaning before sanitization. These control measures aim to minimize the exposure of chickens to contaminated crates, aiming to reduce the risk of contamination as much as possible [17]. Decontamination must also be carried out throughout the truck and the professionals involved must carry out personal hygiene [18,19]. These decontamination guidelines may be sufficient to ensure more effective control in preventing or maintaining infections during routine microbial outbreaks.

Given that transported broilers are normally healthy, it is crucial to prevent the presence of contaminating bacteria, especially those known for their resistance to major antimicrobial agents, such as \textit{Salmonella} spp. [20]. This review aims to highlight the main microbiological challenges faced in poultry transport and show the role of effective bacterial control during this stage to ensure poultry health and poultry meat safety and to reduce economic losses.
2. Transport: From Farm to Slaughterhouse

Poultry undergo at least two transport routes throughout their lives, varying from short distances (<15 km) to extensive journeys (>90 km) lasting several hours [21]. These movements are carried out in trucks, first from the hatchery to the farms and then from the farms to the slaughterhouses. The last move is especially concerning due to the microbiological challenges. In Brazil, for example, broilers are transported to slaughter in open trucks, making poultry more vulnerable to microbial contamination [21,22]. Broiler-rearing normally occurs in a random geographic distribution, and may be located in regions quite distant from the slaughterhouse. In this way, poultry are transported in varying conditions and combinations of distances and times, which, in most cases, can lead to losses, directly reflecting on the quality of the final product (meat) [23]. Transporting broilers is often seen as stressful, but it is a crucial element of the poultry industry. The success of this stage is closely linked to the on-farm sanitary conditions [23]. Therefore, it is recommended to carry out a microbiological diagnosis. Although many farms follow adequate sanitary standards, failures can occur and compromise the entire production batch. Given this concern, researchers have dedicated themselves to investigating microbiological issues associated with poultry, transport crates, and vehicles used for transportation. The relevance of microbiological diagnoses transcends the mere guidance of technologies for the health control of poultry farms. Such diagnoses will undoubtedly be fundamental tools to improve microbial monitoring of pre-slaughter transport across the country.

3. Bacterial Infection during Poultry Transport

Trucks are often used to transport healthy broilers for slaughter in a confined space. In Brazil, travel time from a farm to a slaughterhouse can vary, for example, from approximately forty minutes over short distances, depending on traffic conditions, and from just over two hours on longer journeys [21]. The longer the broilers remain confined in the truck, the greater the risk of bacterial infection [23]. This risk can be influenced mainly by the quantity of broilers transported, human handling, whether the truck is open or closed, the air quality inside and outside the vehicle, the environmental conditions during transport, and the level of decontamination of the truck. Therefore, the process of transporting broilers can be a vector of pathogenic microorganisms, which can facilitate the transmission of infectious diseases between poultry. Poultry infections during transport can begin while the poultry are being caught. This may occur because hands can serve as a vehicle for microorganisms that, in turn, contaminate poultry through direct contact during handling. According to Dianin [24], professionals’ hands can contain, on average, $2.25 \pm 0.46 \text{ CFU/cm}^2$ of mesophilic aerobes, $0.10 \pm 0.47 \text{ CFU/cm}^2$ of Enterobacteriaceae,
1.70 ± 0.00 CFU/cm² of total coliforms and 1.70 ± 0.00 CFU/cm² of *E. coli*. Additionally, a significant source of pathogens, especially notable in the pre-transport period, is the failure of professionals to bathe before entering poultry facilities.

Infections in broilers can also occur during transport, especially associated with the packaging of poultry in specific crates for this purpose [24,25]. This may be associated with exposure to high temperatures during transport, inadequate washing of trucks and transport crates, as well as the use of contaminated water to clean vehicles [26]. Transport crates are essential for transporting poultry; however, after each use, these crates often become contaminated with microorganisms due to contact with non-sterile environments. For example, poultry excreta, which are non-sterile materials, can accumulate in significant quantities in crates. In addition to *E. coli*, excreta may be contaminated with a variety of other strains of *Salmonella*, including, but not limited to, *S. Virchow*, *S. Hadar*, and *S. Ohio*. Less common, but still present, are *S. Mbandaka*, *S. Typhimurium*, *S. Brikama*, *S. Senftenberg*, *S. Goldcoast*, *S. Altona*, *S. Havana*, *S. Agona*, *S. Heidelberg*, *S. Infantis* and *S. Brandenburg* [27]. An increase in *Campylobacter* spp. in broiler transport crates was also recorded [28–30]. Other microorganisms that can be isolated from broiler transport crates are *E. coli*, coagulase-negative *Staphylococcus*, *Streptococcus* spp., *Proteus mirabilis*, *Morganella morganii*, *Hafnia alvei*, *Bacillus* spp., *Citrobacter* farmeri, *Shigella sonnei*, *Citrobacter koseri*, *Micrococcus* spp., *Shigella fleneri*, *Providencia stuartii*, *Shigella* Groups A, B, C, *Yersinia pseudotuberculosis*, *E. fergusonii*, *Citrobacter diversus*, *Citrobacter sedlaki*, *Shigella dysenteriae*, and *Enterobacter agglomerans* [25]. Dianin [24] carried out an evaluation of hygiene indicators in broiler transport crates, revealing the following count averages: 8.52 ± 1.31 CFU/cm² of mesophilic aerobes, 5.96 ± 1.03 CFU/cm² of Enterobacteriaceae, 5.73 ± 0.71 CFU/cm² of total coliforms and 5.51 ± 0.57 CFU/cm² of *E. coli*. This reinforces the central role of crates in infecting poultry during transport.

A crucial aspect of poultry health lies in their interaction with bacteria, a dynamic that can lead to infections and subsequent detrimental effects on their survival, regardless of their stage of development or the environment in which they are found. Research by Anushri et al. [31] highlights the impact of *Salmonella* spp. infection in broilers and layers, manifesting in a range of microscopic and macroscopic lesions primarily affecting the liver, intestine, spleen, and ovary. Microscopic observations reveal congestion, cellular infiltration, hemorrhagic foci, and necrosis in the liver, along with lymphocytic follicle depletion and fibrinoid necrosis in the spleen. Intestinal lesions include hemorrhage, epithelial degeneration/desquamation, and hyperplasia of goblet cells, while the ovary exhibits hemorrhage and cellular infiltration. Macroscopic manifestations encompass enlarged, congested, necrotic livers with hemorrhagic foci, pericarditis, and pericarditis, as well as enlarged, congested spleens and hemorrhagic gastroenteritis in the intestine.

Similarly, Surjagade et al. [32] shed light on the repercussions of *E. coli* infection in broilers, underscoring the discordance between infection and survival. Microscopically, broilers infected with *E. coli* exhibit fibrinous pericarditis, focal necrosis, cellular infiltration, and congestion in the liver, alongside fibrin covering and mononuclear cellular infiltration in the heart, and congestion, hemorrhage, edema, fibrinous exudate, and cellular infiltration in the lungs. Spleen lesions comprise multifocal necrosis, congestion, fibrinous exudate, and lymphocyte depletion. Macroscopic observations reveal fibrinous pericarditis, congestion, and hepatoenomegaly in the liver, fibrinous pericarditis and congestion in the heart, congestion in the lungs, splenomegaly, and cloudy air sacs with fibrinoid mass deposition.

The introduction of bactericides and bacteriostatics during the transport of poultry brings another threat to the control of bacterial infections: the resistance of bacteria to these agents. This phenomenon is characterized by a decrease in the sensitivity of bacteria to antibacterial drugs. The control of bacterial infections can be compromised, especially when resistant bacteria become predominant in infected poultry. This risk is amplified by the conditions in which the poultry are transported, as during the journey to the slaughterhouse, they are exposed to multidrug-resistant bacteria. *E. coli*, *Staphylococcus* spp., *Streptococcus* spp.,
Proteus mirabilis, Citrobacter spp., Morganella spp., Hafnia alvei, Bacillus spp., Micrococcus spp., Providencia spp., Yersinia spp., E. fergusonii, and Enterobacter spp. were bacterial strains isolated from broiler transport crates that presented a multiple antimicrobial resistance phenotype [25]. For example, E. coli showed resistance to 19 antibiotics (amoxicillin, ampicillin, nalidixic acid, cepalexin, cephalothin, cefazolin, ceftazidime, ciprofloxacin, chloramphenicol, doxycycline, enrofloxacin, erythromycin, streptomycin, gentamicin, neomycin, sulfonamide, teicoplanin, tetracycline, and vancomycin) [25]. Likewise, Salmonella spp. presented a resistance profile against cephalosporins [33]. This indicates that during the transport of broilers, bacterial contamination is strongly associated with resistance to different antibiotics, which can compromise food safety [25,33]. To complicate the situation, multidrug-resistant strains of different bacteria can pose a risk of infection and spread to uninfected broiler chickens transported in the same crates [33]. This could become widespread, resulting in significant discards and serious economic losses.

4. Strategies to Prevent Microbial Contamination during Poultry Transport

Enjoying the benefits of available antibacterials to protect poultry health, and indirectly human health, in addition to optimizing poultry productivity and minimizing economic impacts, is a viable strategy for controlling bacterial infections during the transport of poultry to the slaughterhouse. Studies have conclusively demonstrated the effectiveness of certain antibacterial agents in mitigating contamination of duck and broiler transport crates, as well as trucks dedicated to this purpose [19,30]. Additionally, these substances are effective in cleaning workers’ hands [34]. Below, we present recent examples that illustrate the ability of these antibacterial solutions to directly suppress bacteria.

Bacterial contamination in confined spaces, such as transport crates, represents an aggravating factor for the health of poultry, due to the high spread, pathogenicity, and resistance of certain bacteria, which can evolve into worrying systemic scenarios. Poultry fatalities resulting from the confinement of poultry during transport to slaughter have highlighted the urgency of sanitizing transport crates. Professionals and researchers are collaborating to develop antibacterial management protocols, aiming to guide and prepare the poultry sector in the face of bacteriological dangers (Table 1). These protocols must be carefully coordinated and applied with specific measures to prevent and control poultry infection due to cross-contamination.

<table>
<thead>
<tr>
<th>Washing Protocol</th>
<th>Findings</th>
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<tr>
<td>1. Pre-wash carried out with a set of spray nozzles.</td>
<td>The washing protocol has the potential to reduce the microbial load in chicken transport crates, potentially mitigating the risk of introducing Campylobacter spp. into broilers.</td>
<td>[17]</td>
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<td>2. Immersion for 90 s in a tank with cold water and 0.5% sodium hypochlorite.</td>
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<td>3. The main wash with high-pressure nozzles that spray cold water on all surfaces of the crate.</td>
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<td>4. Removing excess water with channel blowers.</td>
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<td>5. Disinfection with 0.5% sodium hypochlorite.</td>
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<td>6. Drying with a dehumidifier.</td>
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<tr>
<td>1. Physical removal with water spray of fecal material, dirt, and debris.</td>
<td>The cleaning and sanitization protocol was ineffective in reducing Campylobacter spp.</td>
<td>[30]</td>
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<td>2. Water spray.</td>
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<td>3. Application of chlorinated alkaline detergent.</td>
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<td>4. Rinsing with water spray.</td>
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<tr>
<td>5. Application of 1% benzalkonium chloride-based disinfectant.</td>
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<td>The water used in the tunnel was pumped at a pressure of 10 kg/cm² with an average temperature of 45 to 50 °C, pH 6.75 to 7.02, and chlorination at 0.3 to 2 ppm.</td>
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Transport plays a crucial role in the daily routine of many poultry professionals, especially those responsible for taking poultry to slaughter. Trucks intended for transporting poultry are not free from contaminants, as they are surrounded by an environment full of contaminants agents, which are present from the air to the contact surface where they travel. The approach to bacterial control in trucks must focus on essential measures to interrupt the chain of transmission, especially of pathogenic or opportunistic bacteria. To meet this demand, technologies and strategies are available to ensure the microbiological quality of poultry transport vehicles [19,40].

One suggestion in the study by Huneau-Salaün et al. [19] to prevent or intervene with excessive contamination in trucks includes the complete removal of organic matter, from the tires to the roof of the vehicle, followed by the application of a detergent solution, washing with high-pressure water, and sanitizing with products based on glutaraldehyde and quaternary ammonium. Likewise, in addition to pre-washing and rinsing, sanitizing trucks with 1% quaternary ammonia, applied for 10 min, can also be an effective measure to reduce the bacterial load in broiler transport trucks [40]. In addition to aldehydes and

### Table 1. Cont.

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<tr>
<td>Rinse with tap water followed by steam flow at ambient pressure at 100 °C.</td>
<td>The combined application of rinsing and steam has been shown to be very effective, reducing the number of <em>Campylobacter</em> spp. on the crate floor by more than 99.9%. The antibacterial protocol significantly reduced the number of <em>C. jejuni</em>, <em>Enterobacteriaceae</em>, and total aerobic bacteria in chicken transport crates. The maximum reduction of 3.12 log10 CFU/cm² of <em>S. Enteritidis</em> was achieved in crates treated with 15 s of tap water, followed by a 40 s treatment with slightly acidic electrolyzed water containing 50 mg/L of chlorine. Reduction of 2.32 log10 of aerobic bacteria and 4.48 log10 of <em>S. Typhimurium</em> was observed on the surfaces of broiler transport crates treated with high-pressure water rinse followed by peroxyacetic acid plus foaming agent.</td>
<td>[35] [36] [37] [38]</td>
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<td>Irradiation with 265 nm UV-C light.</td>
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<td>1. Cleaning with tap water (5 to 15 s). 2. Spraying with slightly acidic electrolyzed water (pH 5.85 to 6.53/20 to 40 s) with chlorine concentrations ranging from 30 to 70 mg/L. (1) Low-pressure water rinse, (2) peroxyacetic acid + foaming agent, or (3) high-pressure water rinse followed by peroxyacetic acid + foaming agent. Contact time of 10 min followed by a rinsing with low-pressure water.</td>
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<td>1. Immersion in a tank with continuous water renewal. • Automatic renewal every 2 min for 10 s. • Total volume of water in the tank: 1020 L. • 20 L water exchange per renewal cycle. • Immersion time for broiler transport crates: approximately 4 s. 2. Replacement of immersion tank with potable water jet application. • Jet application on the broiler crate transport line. • Time for a crate to pass completely under the jet: approximately 4 s. • Jet flow rate: 0.8 L/s. 3. Initial immersion in tank with water renewal and additional step of immersion in quaternary ammonium. • Shutdown of sanitizer sprayers during passage through the sanitization machine. • Immersion step in quaternary ammonium carried out manually. • Solution of quaternary ammonium compound at 1500 ppm. • Contact time with sanitizer: 5 s.</td>
<td>All four samples that tested positive for <em>Salmonella</em> spp. were negative after the application of Protocol 2. No samples presented <em>Salmonella</em> spp. before and after the application of Protocol 3.</td>
<td>[39]</td>
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quaternary ammonium compounds, other antibacterial compounds can be effective in sanitizing poultry transport trucks. Among these, acids stand out, such as acetic acid; alcohols, such as ethanol; alkaline agents, such as sodium hydroxide; biguanides, such as chlorhexidine; halogens, such as chlorine; oxidizing agents, such as hydrogen peroxide; and phenols, such as ortho-phenylphenol [41].

In commercial poultry farming practices, the negligence of some professionals in washing their hands has been noticed, especially before and after contact with poultry. This practice is unacceptable due to the high risk of contamination, which can result in production losses and compromise the future commercialization of the product for consumption. Durmuşoğlu et al. [34] observed that total viable colony counts on the hands of poultry workers can range from 7.1 to 8.2 log$_{10}$ CFU/hand, and Enterobacteriaceae counts can range from 4.5 to 5.9 log$_{10}$ CFU/hand when hygienic practices are not followed. The authors highlighted that simply washing hands with water for a period of 5 to 7 s, until visible contamination is removed, is not adequate for an effective reduction in the microorganisms present. To achieve a significant decrease in the number of microorganisms, the authors recommend incorporating antimicrobial agents into the water.

Racicot et al. (2013) proposed some hand-washing protocols adapted to the poultry environment. These included sanitization with alcohol gel containing 62% ethanol, washing with water and antibacterial soap containing triclosan followed by drying with disposable paper and sanitizing with the same alcohol-based gel, using degreasing cream with the addition of water-free pumice stone followed by drying with disposable paper and sanitizing with the same alcohol-based gel, and employing antimicrobial wipes followed by sanitizing with the same alcohol-based gel. The general considerations for these protocols depended on the level of bacterial hand contamination. When contamination was low, all protocols were effective. However, when contamination was high, the combination of soap and water followed by hand sanitizer was most effective in controlling aerobic bacteria counts. Conversely, the use of waterless degreasing cream followed by sanitizer gel yielded better results in reducing coliforms. Detergent and antimicrobial wipes are not recommended.

In this study, we emphasize the critical moments for cleaning and sanitizing hands, which we believe are ideal to ensure the minimum influence of hands on the microbiological quality of the poultry and the safety of the professional during the poultry transport stage. These critical moments include before entering the poultry house, before carrying out management procedures, after the risk of exposure to poultry excreta, after direct handling of the poultry, after contact with poultry house surfaces, after contact with the transport crates, and after contact with the truck areas.

Although interventions aimed at reducing poultry infection and mortality rates during transport, including the handling, washing, and application of effective antibacterials by poultry professionals, are laborious and expensive, it is crucial to persist in ongoing efforts to minimize the number of poultry killed during transport. It is essential to recognize that the success of antibacterial intervention is not only limited to washing and sanitizing hands, crates, and transport trucks appropriately and efficiently, but also optimizing biometeorological parameters, such as ambient temperature, to ensure maximum comfort of poultry [23].

In many countries, for example, the transport of broilers is carried out in open trucks, without control of meteorological variables, which can result in an increase in the internal temperature of the truck’s load during the journey [21,22]. On long journeys, lasting more than two hours, the temperature of the load can reach around 31 °C, which represents a high level of stress for the broiler [21]. In addition to compromising well-being and production performance, heat stress can impair the functions of poultry immune cells, making them more susceptible to infections and putting their lives at risk. For example, Hirakawa et al. [42] demonstrated that heat stress compromises the immune system in broilers, resulting in the deterioration of the development and functional maturation of T and B cells in primary and secondary lymphoid tissues, significantly impairing the poultry
immune resistance. Modifying transport conditions to reduce heat within transport loads can improve antibacterial protocols during this crucial step.

5. Conclusions
This review reported the presence of bacteria on workers’ hands, in crates, and in vehicles transporting poultry. These transport elements have been shown to be significant vectors of pathogens, which can place financial stress on the poultry system. Based on these conclusions, there is a need for rigorous surveillance and the implementation of effective bacterial control procedures during the preparation, handling, and transportation phases of poultry to slaughterhouses. Such measures are essential to ensure the efficiency of the process, ensuring that consumers do not consume chicken meat below the minimum microbiological quality standards, as well as to reduce financial losses in poultry farming.

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