

Biosecurity Practices for Reducing Antimicrobial Use in Commercial Broiler Farms in Korea

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Farm biosecurity is valuable for reducing the indiscriminate use of antimicrobials. However, its relationship with antimicrobial usage can be difficult to assess because of multiple factors. This study evaluated the impact of biosecurity practices on productivity, frequency of antimicrobial use, and development of antimicrobial resistance. Among factors related to biosecurity, mortality rate <2% within one week of age, survival rate >98%, and production index >350 in farms in which: bedding was not reused or only reused once, regular advice and biosecurity training was provided by poultry veterinarians, distinctions between clean and dirty areas were strictly enforced at all times, workers used farm biosecurity manuals, or disinfection guidelines were fully implemented, including cleaning before introducing new flocks and daily disinfection throughout growth, were significantly higher than those in farms without these measures ($p < 0.05$). The absence of biosecurity practices increased antimicrobial use to one (25.7%), two (39.2%), and three (25.7%) times ($p < 0.05$). In farms that implemented biosecurity practices, the antimicrobial administration was significantly increased to two times (44.2%) ($p < 0.05$), with only 17.4% of farms using antimicrobials three times. The prevalence of environmental *Escherichia coli* resistant to multiple cephalosporins and chloramphenicol, which are not used on broiler farms, was significantly reduced by biosecurity practices ($p < 0.05$). Our findings indicate that improved biosecurity practices decrease antimicrobial use, decrease the incidence of antimicrobial-resistant bacteria, and help to eliminate resistant bacteria in farm environments.

Key words: antimicrobials, antimicrobial-resistant bacteria, biosecurity practices, broiler

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Introduction

Biosecurity is important in poultry production systems to decrease the incidence of infectious diseases; a key concept is the avoidance of pathogen transmission both between and within farms[1]. In Korea, approximately 98% of broilers are raised in vertically integrated broiler operations[2], each with different basic biosecurity and sanitation strategies. Jung et al.[3] reported that commercial broiler farms in Korea showed significant differences in production environments, antimicrobial administration frequencies, and biosecurity practices. Five major integrated production operations were different in disinfectant use, vehicle

management, outdoor access management, and personnel management throughout the growth period[3]. Although implementing biosecurity is not expensive, many commercial chicken farmers still choose the inferior practice of prophylactic antimicrobial applications. Postma et al.[4] and Mallioris et al.[5] have shown that farm biosecurity is a valuable tool that limits indiscriminate antimicrobial use, promoting health, production, and welfare.

Antimicrobials are an important aid to maintaining poultry health, but they are primarily prophylactically administered to entire broiler flocks. Inappropriate antimicrobial use in uninfected broilers has resulted in both antimicrobial residues in meat and the development of antimicrobial resistance (AMR)[6]. Tsegaye et al.[7] reported a disease prevention strategy based on biosecurity improvements that is an economical approach. Robertson[8] demonstrated that implementing farm biosecurity reduced infectious diseases in farm animals, potentially decreasing both the frequency of antimicrobial administration and AMR development.

In Korea, researchers have reported that antimicrobial-resistant bacteria have spread widely in the poultry industry; the causes of this spread have not been identified beyond anti-

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crobial use[9,10]. Recently, Jung et al.[11] reported that environmental dust and feces provide important reservoirs of antimicrobial-resistant bacteria, highlighting the need to strengthen management regulations for cleaning, disinfection, and litter disposal. In the present study, we investigated the relationships between production environment, management practices, disinfection, and productivity in commercial broiler farms; we also evaluated the impact of biosecurity practices on the frequency of antimicrobial use and the development of AMR.

Materials and methods

Data sources

We studied 160 commercial farms from 2021 to 2023; 86 farms partially or fully implemented biosecurity practices, including farm management and disinfection guidelines, while 74 did not, during the broiler grow-out period. Farm management guidelines included regular visits and advice from poultry veterinarians, strict enforcement of dividing clean and dirty areas, and regular training of farm workers using farm biosecurity manuals throughout the growing period. Disinfection guidelines included thorough cleaning to remove feathers, droppings, and bedding; disinfecting water pipelines before introducing new flocks; and imposing daily general requirements such as footbath disinfection, spray disinfection between poultry houses, and drinking water disinfection period.

Productivity of the 86 farms was analyzed in terms of mortality rate of <2% within one week of age, survival rate of >98%, and production index of >350, depending on significantly different factors, in accordance with farm management and disinfection guidelines. Production indices were calculated using the formula: $[\text{survival rate (\%)} \times \text{average weight (kg)} / \text{growing period (d)} \times \text{feed conversion ratio}] \times 100$. Stocking densities per 3.3 m² and downtimes between flocks were determined based on values that would evenly distribute the farms across groups without prejudice for the purpose of comparative analysis with other factors.

Frequency of antimicrobial administration and incidence of antimicrobial-resistant *Escherichia coli* (*E. coli*) in environmental dust were compared between 86 farms that partially or fully implemented biosecurity practices and 74 farms implemented no biosecurity practices. Biosecurity practices and antimicrobial usage during the broiler grow-out period were subjected to situational analysis, using a questionnaire previously described by Jung et al.[3] covering production environment, antimicrobial and disinfectant use, biosecurity practices, and management practices.

Sampling

As per standards set by the National Poultry Improvement Plan (NPIP)[12], dust samples were collected from the farm environment when broilers were transported to slaughterhouses. Fifteen locations per house were swabbed using sterile surgical gauze moistened with buffered peptone water (BPW; Difco, Sparks, MD, USA), yielding dust samples of approximately 10 g each. All samples were placed in sterile bags and transported to

the laboratory at 4 °C.

Bacterial isolation

E. coli was isolated and identified following standard microbiological protocols published by the Ministry of Food and Drug Safety[13]. Briefly, 10 g of dust was inoculated into 90 mL of buffered peptone water (BPW; BD Biosciences, San Jose, CA). After incubation for 18-24 h at 37°C, 1 mL of pre-enriched BPW was inoculated into 9 mL of mEC broth (Merck, Darmstadt, Germany), then streaked onto MacConkey agar (BD Biosciences, Sparks, MD). At least three presumptive *E. coli* colonies were selected from each sample and confirmed using PCR[14]. When isolates from the same origin showed the same antimicrobial susceptibility patterns, only one isolate was randomly selected and characterized.

Antimicrobial susceptibility

As per the guidelines of the Clinical and Laboratory Standards Institute[15], antimicrobial susceptibility was measured by disk diffusion using disks (BD Biosciences, Sparks, MD, USA) containing ampicillin (10 µg), amoxicillin/clavulanate (20/10 µg), cefazolin (30 µg), cefoxitin (30 µg), cefotaxime (30 µg), cefepime (30 µg), tetracycline (30 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), gentamicin (10 µg), and chloramphenicol (30 µg).

Statistical analysis

Statistical Package for the Social Sciences (SPSS) v.26 (IBM Corp., Armonk, NY, USA) was used for all analyses. Pearson's chi-square test with Bonferroni correction was performed. Differences were considered statistically significant at $p < 0.05$.

Results

Productivity as a function of production environment in the 86 farms that partially or fully implemented biosecurity practices is shown in Fig. 1. Those in which bedding was not reused or was reused once had increased than in those in which bedding was reused more than twice ($p < 0.05$). Conversely, low stocking density and short downtime between flocks did not necessarily lead to a mortality rate of <2% within one week of age, survival rate of > 98%, and production index of > 350. Additionally, productivity showed no significant differences with farm type, number of birds per cycle, number of houses, house system, ventilation system, or water-supply system (data not shown).

Productivity as a function of the application of farm management guidelines in the 86 farms with partially or fully implemented biosecurity practices is shown in Fig. 2. Farms that received regular advice and biosecurity training from poultry veterinarians, strictly enforced the distinction between clean and dirty areas at all times, and regularly educated farm workers on farm biosecurity manuals had significantly decreased mortality within 1 week of age, increased survival, and increased production than those that did not implement such guidelines ($p < 0.05$). Additionally, productivity was not significantly different in terms of feed or poultry transport vehicle control; carcass disposal; accessibility for rodents, wild birds, cats, and dogs; farm access rules for visitors, including veterinarians; and farm fencing (data

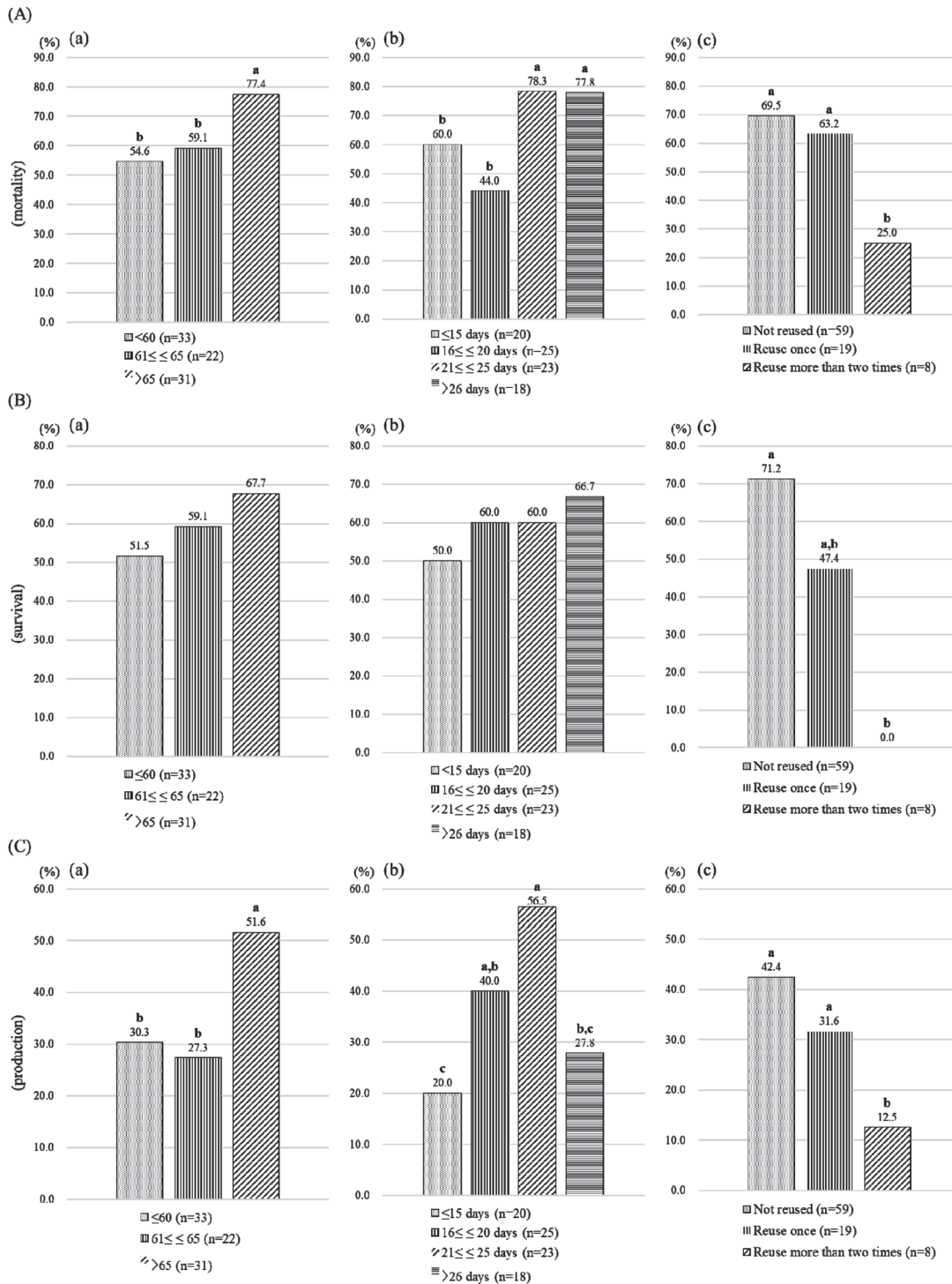


Fig. 1. Distribution of commercial broiler farms with mortality rates of <2% within one week of age (A), survival rates of >98% (B), and production index of >350 (C) according to stocking density per 3.3 m² (a), period of downtime between flocks (b), and number of times bedding was reused (c). n is the number of the 86 commercial broiler farms that partially or fully implemented biosecurity practices. Values with different lowercase letters indicate significant differences ($p < 0.05$).

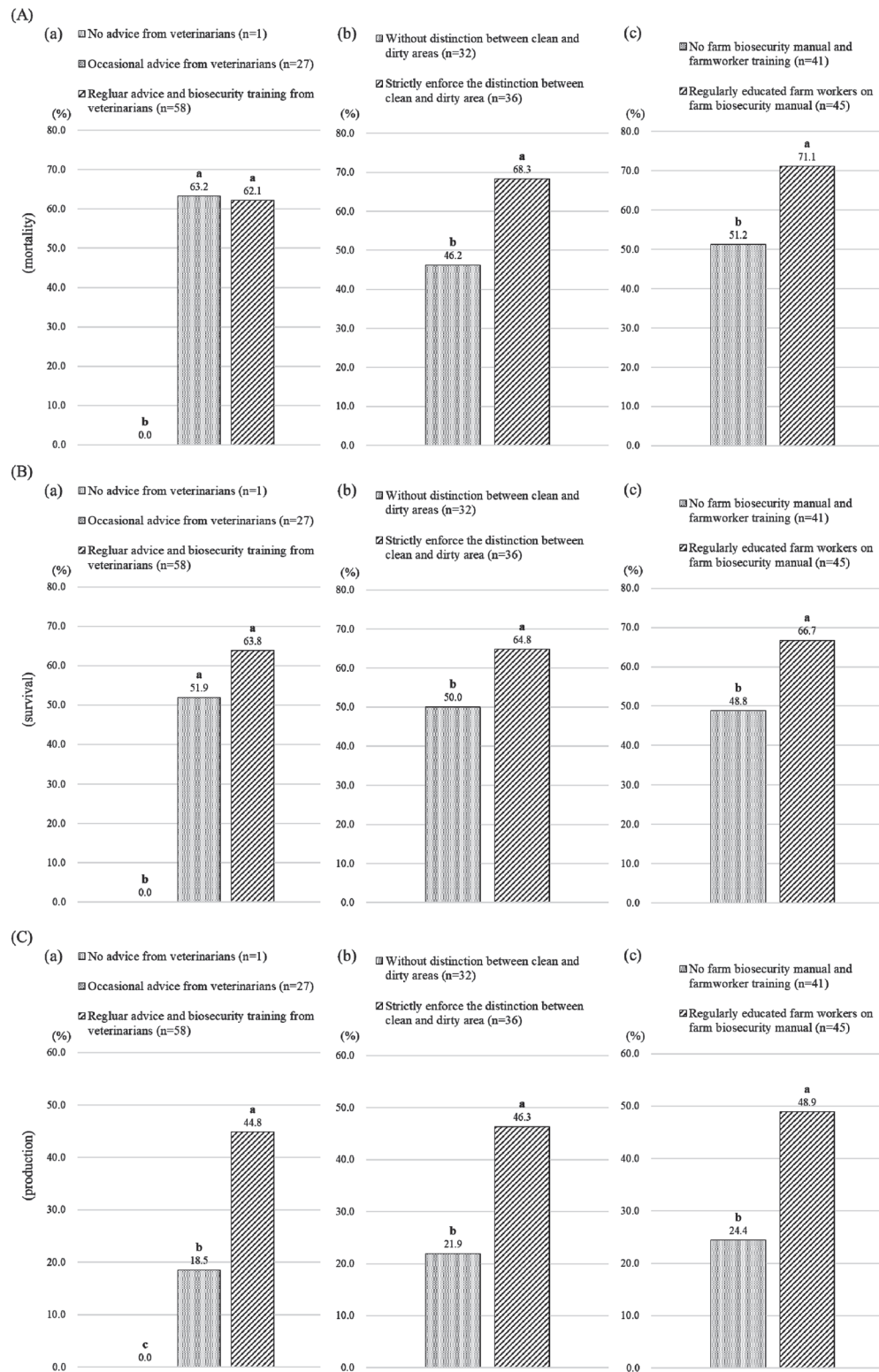


Fig. 2. Distribution of commercial broiler farms with mortality rates of < 2% within one week of age (A), survival rates of >98% (B), and production index of >350 (C) according to regular visits and advice from poultry veterinarians (a), strict enforcement of division into clean and dirty areas (b), and regular training of farm workers in farm biosecurity manuals (c). n is the number of farms included among the 86 commercial broiler farms that partially or fully implemented biosecurity practices. Values with different lowercase letters indicate significant differences ($p < 0.05$).

not shown).

A comparison of productivity according to the application of disinfection guidelines in 86 commercial broiler farms with partially or fully implemented biosecurity practices is shown in Fig. 3. The farms that fully implemented disinfection guidelines, including cleaning the farms before introducing new flocks and disinfecting them daily throughout the growing period, had significantly decreased mortality within 1 week of age, increased survival, and increased production compared to farms that did not disinfect at all ($p < 0.05$). However, the farms that implemented only footpath disinfection also showed improvements in all three parameters compared to the farms that did not disinfect at all ($p < 0.05$).

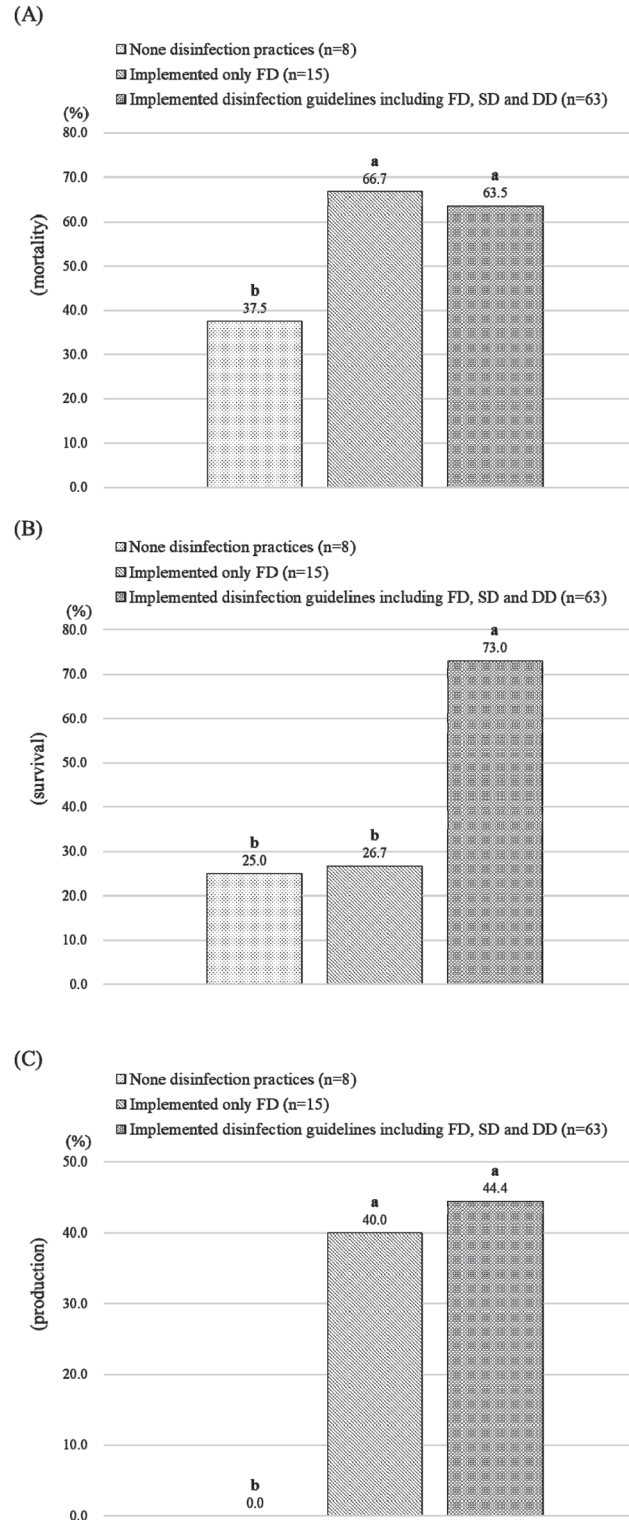
A comparison of frequency of antimicrobial administration and prevalence of antimicrobial-resistant *E. coli* from environmental dust between the 86 farms that implemented biosecurity practices and 74 farms that did not is shown in Fig. 4. Antimicrobial use in farms that did not implement the regulations was significantly higher: once (25.7%), twice (39.2%), and thrice (25.7%) ($p < 0.05$). For farms that implemented the regulations, the antimicrobial use was significantly higher in two times (44.2%; $p < 0.05$), and only 17.4% of farms administered antimicrobials up to three times. Although *E. coli* resistance to ampicillin, cefotaxime, tetracycline, sulfamethoxazole, ciprofloxacin, and gentamicin was not significantly different with respect to implementation of regulations, *E. coli* resistance to amoxicillin, ceftazolin, cefoxitin, cefepime, and chloramphenicol was significantly lower in farms that implemented regulations than in farms that did not ($p < 0.05$).

Discussion

Although farm biosecurity is a promising tool for reducing antimicrobial usage while maintaining animal health, production, and welfare, its relationship with antimicrobial usage cannot be conclusively demonstrated due to multiple factors[5,16,17]. Chowdhury *et al.*[18] reported that both morbidity and farm location were significantly associated with increased antimicrobial use. Imam *et al.*[19] found that the separation of sick from

healthy poultry was significantly related to a reduction in antimicrobial usage. However, Luiken *et al.*[20] reported that increased internal biosecurity correlated with increased oxazolidinone resistance, although it is not used in broiler production; therefore,

Fig. 3. Distribution of commercial broiler farms with mortality rates < 2% within 1 week of age (A), survival rates of >98% (B), and production index >350 (C) according to the application of disinfection guidelines throughout the growing period. These guidelines for commercial broiler farms include thorough cleaning with removal of feathers, droppings, and litter, disinfecting water pipelines before introducing new flocks, and imposing general requirements such as footpath disinfection (FD), spray disinfection between poultry houses (SD), and drinking water disinfection (DD) daily during the growing period. n is the number of farms included among the 86 commercial broiler farms that partially or fully implemented biosecurity practices. Values with different lowercase letters indicate significant differences ($p < 0.05$).



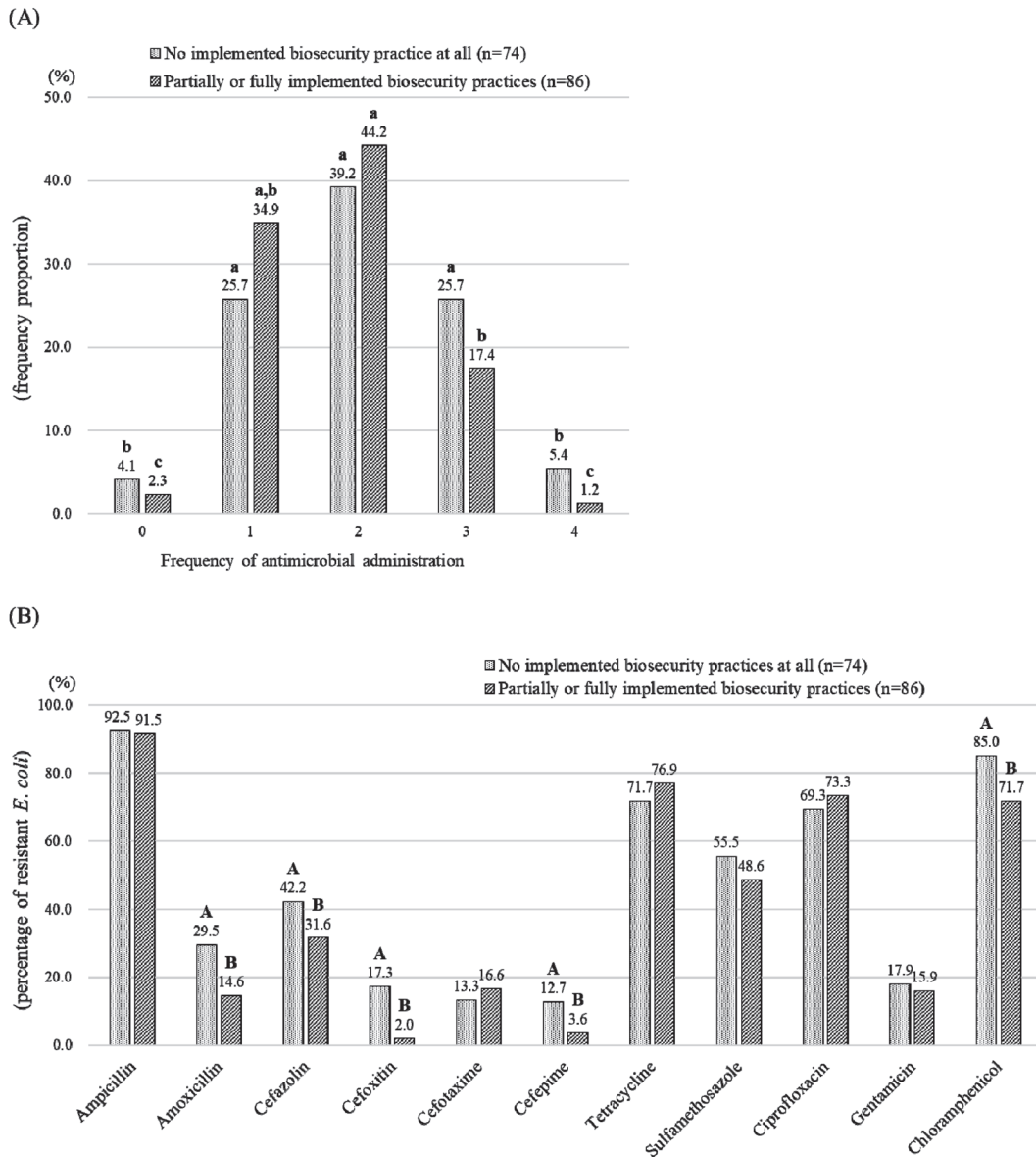


Fig. 4. Distribution of antimicrobial administration frequency (A) and antimicrobial-resistant *Escherichia coli* isolated from environmental dust (B) in 86 farms that partially or fully implemented biosecurity practices and 74 farms that did not implement biosecurity practices, where n is the number of farms. Lowercase letters (a, b) represent significant differences in the frequency of antimicrobial administration by group, whereas uppercase letters (A, B) represent significant differences between groups for the individual antimicrobial agents ($p < 0.05$).

evidence is insufficient to support the hypothesis that biosecurity measures reduce antimicrobial resistance. In this study, productivity was analyzed in terms of production environments on commercial broiler farms that partially or fully comply with biosecurity practices. Interestingly, all three of our assessment criteria showed significant differences as a function of bedding reuse. Although mortality was lower and production was significantly higher at stocking densities of >65 birds per 3.3 m^2 , these dif-

ferences could also be attributed to a higher proportion of farms in which bedding was not reused or only reused once (data not shown). Jung et al.[3] found that only 58% of broiler farms did not reuse or reused bedding once, whereas 14% reused bedding more than four times. Moreover, two of the five major integrated broiler chicken operations in Korea used bedding more than four times, at (30% and 27%). Integrated broiler operations have their own biosecurity manuals; however, their biosecurity levels

or implementations of regulations are not clearly known. Therefore, this report supports the notion that bedding management of each operation is important for productivity. However, this study also showed that farm type, number of birds per cycle, number of houses, house systems, ventilation systems, water supply systems, low stocking density, and short downtime between flocks did not significantly affect productivity. However, Tsiouris *et al.*[21] reported that higher stocking densities increased mortality due to bird stress and increased horizontal pathogen transmission. Insufficient housing management, including bedding, can negatively affect poultry welfare, disease prevention, and production.

Caneschi *et al.*[22] concluded that antimicrobials should be used only with the advice of veterinarians after an accurate disease diagnosis. In the present study, productivity did not significantly differ as a function of feed or poultry transport vehicle control; carcass disposal; accessibility for rodents, wild birds, cats, and dogs; farm access rules for visitors, including veterinarians; and fencing. However, the productivity of farms that received occasional or regular advice and biosecurity training from poultry veterinarians was significantly improved for all three of our criteria than that of farms that did not implement such training. Laanen *et al.*[23] reported a clear link between biosecurity and production- and antimicrobial treatment-related criteria in pig husbandry. In the present study, the productivity of farms that strictly enforced the distinction between clean and dirty areas at all times and those that regularly educated farm workers on farm biosecurity manuals was clearly higher than that of farms that did not.

In this study, broiler farms had to follow disinfection guidelines, including thorough cleaning and removal of feathers, droppings, and litter; disinfecting water pipelines before introducing new flocks; and adhering to general requirements such as footbath disinfection, spray disinfection between poultry houses, and drinking water disinfection daily during the growing period. However, some farms limited disinfection to footpaths or not disinfect at all. Ultimately, mortality was lower and production indices were higher for farms that implemented disinfection guidelines.

Dhaka *et al.*[16] reported that farm biosecurity was positively correlated with reduced antimicrobial use in 52% of studies. In the present study, only 19% of the farms that implemented biosecurity practices administered antimicrobials more than three times, compared to 31% of farms that did not. Moreover, the prevalence of environmental *E. coli* resistant to cephalosporins and chloramphenicol, which are not used on broiler farms, decreased in farms that implemented biosecurity practices. Therefore, this study demonstrates that improved biosecurity practices further reduce antimicrobial usage, the emergence of antimicrobial-resistant bacteria, and can the elimination of resistant bacteria in farm environments. Moreover, our findings can serve as a basis for improving guidelines for broiler chicken production and reduction of antimicrobial use in Korea.

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Author Contributions

Min Beom Kim: writing – original draft, visualization, validation, methodology, investigation, formal analysis, data curation, conceptualization. **Young Ju Lee:** writing – review & editing, writing – original draft, supervision, project administration, data curation, conceptualization.

Conflicts of Interest

The authors declare no conflicts of interest.

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