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# The Prevalence of Extended-Spectrum Beta-Lactamase (ESBL) – Producing Escherichia Coli in Sandal Mats at Sukabumi Farms

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**ABSTRACT**

*Antimicrobial resistance (AMR), particularly Extended-Spectrum Beta-Lactamase–producing Escherichia coli (ESBL-E. coli), poses a global threat to human, animal, and environmental health. Intensive broiler farms serve as important reservoirs of ESBL-E. coli, exposing workers through ingestion during routine activities. This study aimed to assess ESBL-E. coli prevalence and estimate ingestion exposure among workers in closed-house broiler farms in Sukabumi City. Laboratory and field analyses were conducted using spread plate methods, MCA-CTX selective media, and ESBL confirmation by Double Disk Synergy Test (DDST) on samples from workers' footwear. The concentration of E. coli bacteria on the surface of the sandals in the dry season was* $(1.37 \pm 0.02) \times 10^3$ *CFU/cm², while in the rainy season it was* $(1.16 \pm 0.05) \times 10^3$ *CFU/cm². The confirmed concentration of ESBL-E. coli bacterial colonies in the dry season was* $(0.42 \pm 0.02) \times 10^3$ *CFU/cm². Meanwhile, for the rainy season, the ESBL-E. coli concentration obtained was* $(0.14 \pm 0.03) \times 10^3$ *CFU/cm². The results of this study confirm that workers' footwear in closed-house broiler farms is a real reservoir for the spread of ESBL-E. coli bacteria. The high concentrations of bacteria found, both in the dry and rainy seasons, indicate the need for stricter biosecurity protocols and routine sanitation of work equipment to mitigate the risk of transmitting antimicrobial resistance from the farm environment to humans.*



## INTRODUCTION

Antimicrobial resistance (AMR) is now recognized as one of the biggest threats to public health worldwide in the 21st century, including in Indonesia. The impact of these resistant bacteria, from local to global levels, can occur vertically through contact with humans, animals, and the environment (Pazra et al., 2023). In 2019, there were 1.27 million deaths among individuals suffering from treatment-resistant infections (Murray et al., 2022). By 2030, livestock productivity in developing countries is expected to decline by around 7% (WHO, 2021). This decline stems from less effective efforts to halt the spread of resistance. Consequently, rising resistance cases will disrupt nutritional fulfillment for women and children worldwide. Additionally, this condition is predicted to burden the global economy. According to Jim O'Neill (2014), the impact of AMR could cost up to $100 trillion by 2050. For this reason, joint efforts are needed to slow the rate of antimicrobial resistance through the One Health approach (WHO, 2021).

The One Health approach has spurred research to understand the epidemiological complexity of AMR. A recent study on *E. coli* bacteria that spread rapidly in the environment linked them to pathogenic strains in humans, posing a threat to public health (Loayza et al., 2020). This is closely tied to the health of animals in surrounding ecosystems. These conditions allow resistance genes to transmit horizontally through contact with AMR sources (Liu et al., 2022; Widodo et al., 2024; Mahmood et al., 2025). The prevalence of AMR so far includes clinically pathogenic bacteria, such as *Escherichia coli* that produce extended-spectrum beta-lactamase (ESBL) enzymes (Ceccarelli et al., 2020). The circulation of ESBL-producing *E. coli* in different animal populations requires an integrated One Health approach to better understand, predict, and prevent its spread (Jamborova et al., 2018). One sector drawing attention in livestock farming is its potential to accelerate resistance through antibiotic-contaminated bacteria in animal products. The use of antibiotics on farms—both for treating diseases and reducing mortality—can impact these products if prolonged use or misuse increases resistance in livestock (Ariningsih et al., 2024). Irrational and prolonged antibiotic use in livestock farming selects for resistant bacteria. These bacteria can contaminate animal products such as meat, milk, and eggs, increasing the risk of human exposure to AMR through the food chain (Akil et al., 2024; Apostolakos et al., 2024; Blaak et al., 2015).

Previous research has mostly focused on livestock health and resistance related to antibiotic concentrations used. Meanwhile, the prevalence of ESBL-producing *E. coli* in livestock environments has not been widely studied. Inadequate sanitation infrastructure and low personal hygiene awareness can facilitate AMR spread, as pathogen concentrations entering the human body through various exposures can cause disease (Diyasti et al., 2021). Therefore, this research aimed to examine the prevalence of ESBL-producing *E. coli* in sandal mats at Sukabumi farms. This research provides significant practical and scientific benefits. Practically, the findings serve as a basis for developing more effective biosecurity protocols on farms, especially regarding sanitation of workers' footwear, thereby reducing the risk of spreading resistant bacteria. In terms of public health, these results raise awareness about transmission routes of ESBL-producing *E. coli* through the work environment, supporting worker education and prevention programs. At the policy level, the generated data can serve as scientific evidence to strengthen AMR control regulations in line with the One Health approach. Academically, this study contributes to understanding the dynamics of resistant bacterial contamination in farm environments in relation to seasonal variation and the role of fomites, while opening avenues for further research on sanitation interventions and resistance gene spread mechanisms.

## METHOD

This quantitative study was conducted to detect ESBL-*E. coli* in closed house broiler chicken farms in Sukabumi using two main approaches. The first approach involved taking swab samples from workers' slippers. Samples were enumerated using the spread plate method on Cefotaxime-supplemented MacConkey Agar (MCA-CTX), followed by confirmatory ESBL testing using the Double Disk Synergy Test (DDST) (Paramitadevi,2025). DDST is a phenotypic method for detecting ESBL enzyme production based on the synergistic effect between β-lactam antibiotics (third-generation cephalosporins, such as cefotaxime) and β-lactamase inhibitors (clavulanic acid). Isolates were considered ESBL-positive when an expansion of the inhibition zone toward the clavulanic acid disk was observed, indicating inhibition of ESBL activity by clavulanate. Microbiological analysis was conducted for 18 samples consisting of 8 samples of workers' sandals with sample codes (Boots 2, SAK 2, SAK 4, AL1, AL2) and 10 samples of the feed area with sample codes (TP1, TP 2, TP3, TP 4, TP5)

**Table 1. Data Collection Schedule**

| Activity | Time | Season | Method | Observed Parameters |
|---|---|---|---|---|
| **Worker slipper swab** | April 23, 2024<br>Juni 20 2024 | Rainy (n=5)<br>Dry (n=5) | Swab sampling<br>Swab sampling | ESBL-*E. coli* concentration (CFU/cm$^2$<br>ESBL-*E. coli* concentration (CFU/cm$^2$) |
| **Feed Area** | April 23, 2024<br>Juni 20 2024 | Rainy (n=5)<br>Dry (n=5) | Swab sampling<br>Swab sampling | ESBL-*E. coli* concentration (CFU/cm$^2$<br>ESBL-*E. coli* concentration (CFU/cm$^2$) |

Source: Primary research data, 2024

The table above shows a longitudinal study design that compares two different seasons (rainy and dry) to understand seasonal variations in ESBL-*E. coli* contamination. Swab sampling was performed on two different matrices (slipper pads and feed trays) to identify contamination hotspots.

## RESULT AND DISCUSSION

### Characteristics of Location and Condition of Farm

This research was carried out at a closed house broiler chicken farm strategically located on Jalan Sarasa No 45, Babakan Village, Sibeleum District, Sukabumi City, West Java. This farm is located at the geographical coordinates of 106° 57' 20" E and 06° 56' 29" S, located between Cibeureum Hilir Village and Cikaret Village. This farm facility is a modern facility equipped with high-standard cages, feed processing areas, and adequate sanitation systems to support efficient and sustainable poultry production.

Despite implementing a closed house system with controlled ventilation technology, some challenges remain found in biosecurity management. The microenvironmental conditions in the cage show significant temperature variation between the rainy and dry seasons, with an average temperature of 29°C in the rainy season and 23°C in the dry season. The wind speed in the cage is consistent at 0.4 m/s, but the air humidity fluctuates considerably depending on external weather conditions. These environmental factors have a direct influence on the condition of poultry feces and the level of bacterial contamination in the farm environment.

**Prevalence and Distribution of *E. coli* in Livestock Samples**

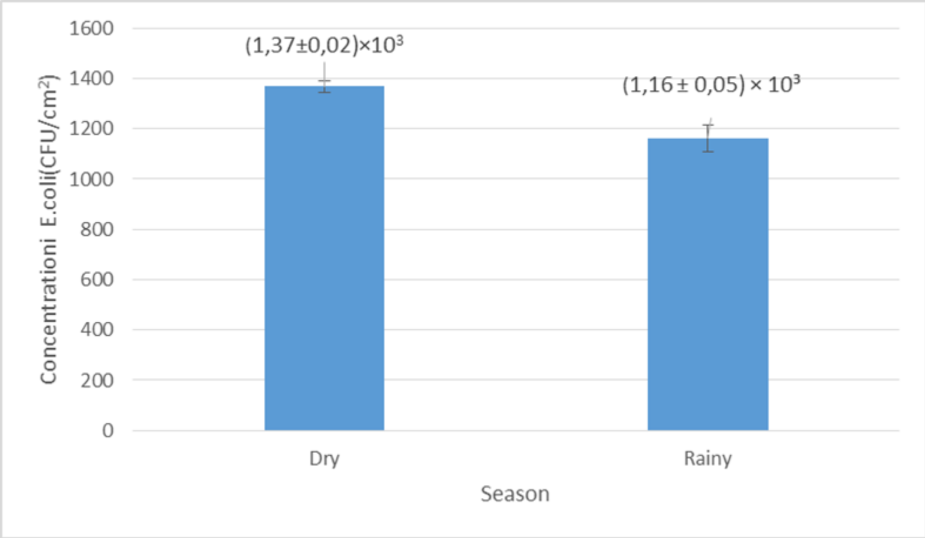


**Figure 1 Concentration of *E. coli* in slippersSoles in the Dry and Rainy Seasons**
Source: Laboratory analysis results, 2024

The concentration of *E. coli* on the soles of workers' sandals on broiler chicken farms was higher in the dry season (1.37±0.02)×$10^3$ CFU/ $cm^2$. compared to the rainy season (1.16±0.05)×$10^3$ CFU/ $cm^2$.compared to the rainy season. This indicated that bacterial growth and accumulation tend to be higher in the dry season, which is related to higher worker activity intensity and increased contact with dry feces and dust that still contain viable bacteria. This finding aligns with Pandey et al. (2013) and Jamal et al. (2020), which reported increased ESBL-*E. coli* concentrations in the dry season due to reduced water runoff and fecal accumulation. Conversely, during the rainy season, although rain can reduce surface contamination, bacteria can still persist in porous materials such as soil, rice husks, or organic litter (Díaz-Nevado et al., 2023). The absence of *E. coli* in the feeding area suggests that the primary source of contamination is direct contact of footwear with the contaminated environment. This finding underscores the importance of worker mobility in the spread of bacteria and the need for strict biosecurity measures on farms.

**ESBL-Ec concentration confirmed by DDST test**

To determine the number of Escherichia coli colonies that produce ESBL (ESBL-Ec), confirmation is required through antimicrobial susceptibility testing (AST) using specific antibiotics, Double Disk Synergy Test (DDST) method to test specific such as ceftazidime or cefotaxime, to identify bacterial resistance to β-lactam antibiotics and ensure the presence of ESBL enzymes. The following are the results in the Figure Confirmed Concentration of ESBL-*E. coli* on Sandal Soles in the Dry Season and Rainy Season

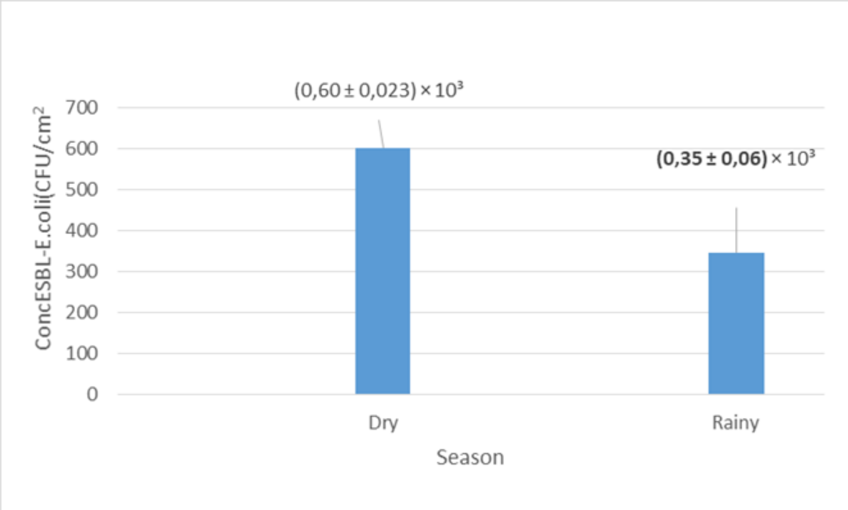


**Figure Confirmed Concentration of ESBL-*E. coli* in slippers Soles in the Dry Season and Rainy Season**
Source: DDST test results, 2024

The results of antibiotic susceptibility tests showed that the concentration of ESBL-*E. coli* was higher in the dry season ($(0.6\pm0.0{,}023)\times10^3$ CFU/ $cm^2$.) compared to the rainy season ($(0.35\pm0.06)\times10^3$ CFU/ $cm^2$.). These results indicated that the load of resistant bacteria tends to be greater in dry conditions, where bacteria accumulate on surfaces and are not easily eliminated by water. Conversely, in the rainy season, rainfall and humidity cause the distribution of bacteria to be more uneven. This finding confirms that the season plays an important role in influencing the concentration and variability of ESBL-*E. coli* exposure in the farm environment. These results are in line with Blak et al. (2015), who reported high concentrations of ESBL-*E. coli* in broiler farms due to the accumulation of dust and dry feces and minimal natural rinsing processes by water.

## ESBL-*E. coli* to Total *E. coli* ratio

Analysis of the ratio of ESBL-*E. coli* to total *E. coli* population yielded findings that are important for understanding the level of antimicrobial resistance in farm environments.

**Table 2. Ratio of ESBL-Ec to *E. coli***

| Season | Sample | Ratio of ESBL-*E. coli* to *E. coli* |
|---|---|---|
| Dry | Boots $_2$ | 47% |
| | Boots $_1$ | 35% |
| Rain | $AL_1$ | 34% |
| | $AL_2$ | 18% |

Source: Laboratory analysis results, 2024

The table shows that the ratio of ESBL-Ec samples was higher in the dry season than in the rainy season. In the dry season, samples from boots combined with CTX showed the highest ratios, at 47% in $Boots_2$ + CTX and 35% in $Boots_1$ + CTX, indicating high levels of contamination on surfaces frequently in direct contact with the cage environment. Conversely, in the rainy season, the positive ratios were lower, at 34% in $AL_1$ + CTX and 18% in $AL_2$ +

CTX, indicating strong selective pressure, which could be caused by intensive or inappropriate antibiotic use in the location, either as disease preventatives or growth promoters. This variation between samples confirms that local factors such as cage hygiene, antimicrobial history, and environmental conditions play a key role in determining levels of contamination and bacterial resistance. This contrasts with previous studies.is in line with reports by Yunindika et al. (2022) reported that the lowest ESBL-*E. coli* ratio percentage in slaughterhouse effluent was 10.45% while the highest value was 39.52%. Meanwhile, Suswati et al. (2025) found that approximately 27% of chicken farm environmental samples in Blitar and Surabaya were contaminated with ESBL-*E. coli*. The range of values obtained in this study not only strengthens the evidence that ESBL *E. coli* contamination in livestock environments in Indonesia does vary, but also confirms that local factors such as hygiene, livestock density, and antibiotic use patterns play an important role in determining resistance levels.

**Implications of Seasonal Variation on Bacterial Contamination**

The findings of this study reveal the significant influence of seasonal variation on the prevalence and distribution of *E. coli*, including strains that produce ESBL, in closed house farm environments. The difference in bacterial concentrations between the rainy and dry seasons reflects the complex interactions between micro and macro environmental factors that affect the survival of microorganisms. In the rainy season with 258 mm of rainfall, high humidity conditions create an environment conducive to the growth and spread of bacteria, although paradoxically the number of lower concentration than in the dry season.

This phenomenon can be explained through the natural washing mechanism that occurs during the rainy season, where rainwater helps reduce the concentration of bacteria through dilution and transfer of contaminants from one location to another. In contrast, dry season conditions with lower rainfall (152 mm) allow bacteria to accumulate on surfaces without a washing effect, thereby increasing the concentration of contaminants at certain points such as workers' footwear. These findings are in line with research by Ogden et al. (2001) and Brooks et al. (2009) which suggests that dry conditions can lead to higher concentrations of bacteria on certain surfaces due to the absence of natural washing.

The practical implications of these findings are crucial for the management of livestock biosecurity. Risk mitigation strategies need to be adapted to seasonal conditions, where in the dry season more intensive cleaning protocols are needed to prevent the accumulation of contaminants, while in the rainy season the focus should be on moisture control and ventilation improvement to prevent conditions that support the growth of pathogenic bacteria (Mediouni et al., 2025; Veldkamp et al., 2025).

**Clinical and Epidemiological Significance of Antimicrobial Resistance**

The identification of ESBL-producing *E. coli* bacteria showed a ratio of sample sources, with the highest value found in the soles of workers' sandals (Boots 2 + CTX) in the dry season (47%), followed by Boots 1 + CTX (35%). In the rainy season, the ESBL-*E. coli* ratio was lower, at 34% in AL1 + CTX and 18% in AL2 + CTX, respectively. Although the overall ESBL-*E. coli* ratio is relatively lower( In general, ESBL-*E. coli* ratios varied between sample sources and seasons, with the highest values identified in workers' footwear during the dry season) compared to several previous studies, this finding still indicates the presence of resistance hotspots, especially in workers' footwear, which are potentially related to antibiotic use, worker mobility, and environmental conditions that support the selection and persistence of resistant bacteria. Variations in the ratio between seasons and sample sources confirm that local factors play an important role in resistance dynamics, so that the presence of ESBL-*E. coli* remains a serious threat from a public health perspective and the One Health approach.

The mechanism of spread of antimicrobial resistance in livestock occurs not only through vertical transmission from mother to offspring, but also horizontally through gene transfer between bacterial species. Farm environments with high animal density, intensive use of antibiotics, and suboptimal sanitary conditions are ideal conditions for horizontal gene transfer (Gang Liu et al. 2022) This reinforces the argument that controlling antimicrobial resistance requires a holistic approach that involves not only managing the use of antibiotics, but also improving sanitation conditions, implementing good farming practices, and raising workers' awareness of the risks of antimicrobial resistance..

### Implications of Risk Mitigation Policies and Strategies

The findings of this study have important implications for the development of antimicrobial resistance control policies in Indonesia's livestock sector. The identification of contamination hotspots in workers' footwear indicates the need for the implementation of stricter footbath disinfection protocols, the use of footwear specifically for different areas, and the implementation of a more rigid zoning system in the livestock environment. This protocol must be adapted to seasonal conditions, where in the rainy season a higher frequency of disinfection is needed to compensate for humidity conditions that support the survival of bacteria.

## CONCLUSION

This study found higher *E. coli* concentrations on workers' sandal surfaces in closed-house broiler farms during the dry season $(1.37 \pm 0.02) \times 10^3$CFU/cm² compared to the rainy season $(1.16 \pm 0.05) \times 10^3$CFU/cm², with confirmed ESBL-producing *E. coli* (ESBL-*Ec*) levels at $(0.6 \pm 0.023) \times 10^3$CFU/cm² in the dry season and $(0.35 \pm 0.06) \times 10^3$CFU/cm² in the rainy season. These elevated bacterial loads across both seasons confirm that workers' footwear serves as a significant reservoir for ESBL-*Ec* dissemination, underscoring the need for stricter biosecurity protocols and routine sanitation of work equipment to curb antimicrobial resistance transmission from farm environments to humans. For future research, longitudinal studies could investigate the efficacy of targeted footwear disinfection interventions (e.g., antimicrobial mats or UV treatments) in reducing seasonal ESBL-*Ec* loads and track horizontal gene transfer dynamics via molecular genotyping.

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