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Evaluation of Broiler Chicken Farmers' Knowledge and Antibiotic Usage Practices and the Resistance of *Staphylococcus* spp. in Bali, Indonesia

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ABSTRACT

Antimicrobial resistance (AMR) is a global threat that seriously affects public health, animal welfare, and the sustainability of food production systems. In the poultry sector, irrational use of antibiotics is a key factor contributing to the emergence of resistance. This study investigated the association between broiler farmers' knowledge and their antibiotic usage practices with the resistance of *Staphylococcus* spp. in broiler chickens. The study employed a cross-sectional design and included 20 broiler farms located in Tabanan Regency, Indonesia. Knowledge and practices were assessed using structured questionnaires, while five tracheal swab samples were collected from randomly selected chickens on each farm. These samples were pooled and tested in the laboratory to identify *Staphylococcus* spp. and determine their susceptibility to amoxicillin, ciprofloxacin, erythromycin, and doxycycline. The antibiotic susceptibility test followed the guidelines of the Clinical and Laboratory Standards Institute (CLSI), and the associations between variables were examined using cubic regression analysis. Based on the scores obtained from the structured questionnaires, the majority of farmers demonstrated a moderate level of knowledge (65%) and a moderate level of antibiotic usage practices (60%). Laboratory testing revealed that *Staphylococcus* spp. isolates showed the highest resistance to amoxicillin (75%), followed by erythromycin (60%), doxycycline (55%), and ciprofloxacin (30%). The results showed that most farmers had moderate knowledge (65%) and practices (60%), with the highest resistance observed against amoxicillin (75%), followed by erythromycin (60%), doxycycline (55%), and ciprofloxacin (30%). A significant positive correlation was found between farmers' knowledge scores and their antibiotic usage practice scores ($r = 0.683$, $R^2 = 0.467$), indicating that higher knowledge was associated with better practices; however, practices were not significantly associated with inhibition zone diameters. These findings suggested that low antibiotic literacy may lead to improper use, while bacterial resistance is also influenced by external factors such as environmental contamination, horizontal gene transfer, and centralized medication protocols in contract farming.

Keywords: Antimicrobial resistance, Broiler, Knowledge, Practice, *Staphylococcus* spp.

INTRODUCTION

Antimicrobial resistance (AMR) represents an urgent global issue, threatening the health of humans, animals, and the environment, as well as undermining the stability of food supplies worldwide. Within the One Health perspective, the World Health Organization (WHO) places AMR among its highest priorities, emphasizing the concern over the rising number of bacterial infections that no longer respond to common medical treatments (WHO, 2023). In the poultry industry, the widespread use of antibiotics without adequate understanding or regulatory oversight is considered a major driver of AMR emergence (Van Boeckel et al., 2015).

In broiler production systems, antibiotics are often administered not only for therapeutic purposes but also for disease prevention and growth promotion (Chowdhury et al., 2021; El-Fateh et al., 2024). However, irrational use, including administration without clinical indications, inappropriate dosage and duration, and poor compliance with withdrawal periods, can exert selective pressure that

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promotes the development of resistant bacterial strains (McEwen and Collignon, 2018). Among the pathogens commonly exhibiting resistance in poultry is *Staphylococcus* spp., particularly *S. aureus*, which is known to carry various resistance genes such as *blaZ*, *erm*, and *tet* (Mak et al., 2022).

Staphylococcus species are normally present within the poultry microbiota but can become opportunistic pathogens responsible for diseases such as dermatitis, arthritis, and omphalitis (Szafraniec et al., 2022). Continuous and repeated use of antimicrobial agents in poultry production contributes to the development of resistant strains, including those resistant to multiple drugs. Thus, surveillance of resistance trends in *Staphylococcus* spp. is vital to understand how antibiotic use by farmers affects animal health and poses potential risks to public health. Previous studies conducted in Indonesian broiler farms have highlighted considerable resistance of *Staphylococcus* spp. to frequently administered antibiotics (Khusnan et al., 2016; Hermana et al., 2021). However, most of these studies have focused solely on resistance patterns, without examining human behavioral factors such as farmers' knowledge and antibiotic use practices. In fact, farmer literacy is a critical determinant of on-farm antimicrobial usage (Rware et al., 2024). Despite this, specific data linking farmer behavior to bacterial resistance are still lacking, especially in contract farming regions such as Tabanan Regency in Bali, which is a major broiler production center with a population exceeding 2 million broilers (BPS Bali, 2023). Therefore, this study aimed to evaluate the relationship between broiler chicken farmers' knowledge and practices regarding antibiotic use and the resistance patterns of *Staphylococcus* spp. isolated from broiler chickens in Tabanan, Bali, Indonesia.

MATERIALS AND METHODS

Ethical approval

This study was approved by the Animal Ethics Committee of the Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia, under approval number: B/81/UN14.2.9/PT.01.04/2025.

Study area and sample size

A cross-sectional study was conducted from April to May 2025 on 20 broiler chicken farms located in Tabanan Regency, Bali Province, Indonesia (coordinates: 8.4596°S, 115.0466°E). Farms were randomly selected based on inclusion criteria: having a minimum population of 5,000 broiler chickens and having been operational for at least one year. According to the available population data, 21 farms met the inclusion criteria. The required number of farms was determined using the Slovin formula (Riyanto and Hatmawan, 2020), resulting in a sample size of 20 farms. The selected farms varied in size and management systems, ranging from small-scale traditional operations to larger commercial enterprises. In terms of ownership, the farms included both privately owned operations and contract farms collaborating as plasma farms with major companies, reflecting the diversity of broiler production in the region. Tracheal swab samples were collected from five randomly selected live broiler chickens at each farm. The chickens were carefully handled without sedation to minimize stress during sampling. The five swab samples were then pooled and analyzed as a single representative sample per farm, considering the observed homogeneity of results among individuals within each location.

Farmer knowledge and practice survey

Data on farmers' knowledge and practices regarding antibiotic usage were collected using a structured questionnaire employing a four-point Likert scale, which was administered directly to the respondents. Educational levels were categorized as primary school, junior high school, senior high school, and university from primary school to university. Total knowledge and practice scores were classified into five categories: very poor, poor, moderate, good, and excellent. The influence of education level on knowledge and practices was analyzed using the Kruskal-Wallis test.

Bacterial sampling and isolation

Samples were collected only from broilers exhibiting clinical symptoms such as swollen head syndrome or bumblefoot to increase the likelihood of isolating *Staphylococcus* spp. This targeted sampling approach was chosen because symptomatic broilers are more likely to carry pathogenic strains, facilitating the study of antibiotic resistance profiles. Sampling asymptomatic broilers was not conducted due to the lower probability of isolating clinically relevant *Staphylococcus* strains from healthy broilers. Samples were aseptically collected and transported to the Veterinary Microbiology Laboratory, Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia. Swabs were inoculated onto Mannitol Salt Agar (MSA; Merck KGaA, Darmstadt, Germany; Cat. No. 1.05404.0500) and incubated at 37°C for 24 hours. Grown colonies were identified as *Staphylococcus* spp. based on Gram staining and

biochemical tests (Catalase and oxidase; Musliu et al., 2021).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was conducted using the Kirby-Bauer disk diffusion method. Bacterial suspensions were prepared by diluting cultures in 0.9% sodium chloride solution and standardized to a turbidity equivalent to 0.5 McFarland standard (Approximately 1.5×10^8 CFU/ml; Rubin and Damborg, 2024). Then, 0.2 ml of each suspension was uniformly spread onto Mueller-Hinton Agar plates (MHA; Merck KgaA, Darmstadt, Germany; Cat. No. 1.05437.0500). Disks impregnated with antibiotics are frequently applied in broiler production, including amoxicillin (AML, 25 µg; Oxoid Ltd., Hampshire, UK; Cat. No. CT0061B), ciprofloxacin (CIP, 5 µg; Oxoid Ltd.; Cat. No. CT0425B), erythromycin (E, 15 µg; Oxoid Ltd.; Cat. No. CT0020B), and doxycycline (DO, 30 µg; Oxoid Ltd.; Cat. No. CT0018B). Plates were incubated at 37°C for 24 hours. The diameter of the inhibition zones was measured and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2021).

Data analysis

Data on farmers' knowledge and practices were analyzed descriptively and statistically using the Kruskal-Wallis test to compare differences across groups, with p-values reported accordingly. The resistance profile of *Staphylococcus* spp. isolates were also analyzed descriptively based on the antibiotic susceptibility test results (Besung et al., 2024). Furthermore, linear regression analysis was performed to assess the associations between farmers' knowledge and practices and the correlation between antibiotic use practices and the inhibition zone diameters of bacterial isolates. All statistical analyses were conducted using IBM SPSS Statistics version 25, with significance set at $p < 0.05$.

RESULTS

Out of the 20 broiler farmers surveyed in Tabanan Regency, 65% demonstrated a moderate level of knowledge regarding antibiotics, 30% exhibited low knowledge, and only 5% showed good knowledge. A similar trend was observed in antibiotic use practices, where 60% of farmers fell into the moderate category, 30% were classified as low, and only 10% reported good practices. The results showed a significant difference ($p < 0.05$) in knowledge and practices associated with the farmers' educational background. The detailed results are presented in Table 1.

Table 1. Differences in knowledge and practices related to antibiotic use based on the educational background in broiler chicken farmers in Tabanan Regency, Bali Province, Indonesia

Education Level	Knowledge score (Mean ± SD)	Practice score (Mean ± SD)
Primary School	51.500 ± 8.0234[a]	51.500 ± 5.1841[a]
Junior High School	61.250 ± 1.7678[ab]	60.000 ± 7.0711[ab]
Senior High School	61.250 ± 10.000[ab]	58.438 ± 4.9888[ab]
University	71.500 ± 2.8504[b]	68.000 ± 8.9093[c]

[abc]Different superscript letters in the same column indicate significant differences ($p < 0.05$). SD: Standard deviation

The lowest levels of knowledge and practice were recorded among farmers with only primary school education, which significantly differed from those with secondary school, high school, and university-level education. Farmers with university-level education had the highest average scores in both knowledge and practice compared to the farmers with a primary level of education ($p < 0.05$). Amoxicillin was the most frequently used antibiotic among respondents (40%), followed by ciprofloxacin, erythromycin, doxycycline, and enrofloxacin (each used by 25% of farmers). Other antibiotics reported included norfloxacin, oxytetracycline, and a combination of sulfadiazine-trimethoprim (20% each), while Tylosin tartrate and neomycin sulfate were used by only 10% and 5% of farmers, respectively.

Antibiotic susceptibility testing of 20 *Staphylococcus* spp. isolates revealed that resistance was highest to amoxicillin (75%), followed by erythromycin (60%), doxycycline (55%), and ciprofloxacin (30%). Ciprofloxacin showed 40% sensitivity, while 30% of isolates exhibited intermediate response and 30% were resistant. Sensitivity to erythromycin and doxycycline was observed in 35% and 45% of isolates, respectively (Figure 1).

The pattern of multiple resistance showed that 40% of isolates were resistant to a single antibiotic (amoxicillin), 15% to two antibiotics, 20% to three, and 20% were resistant to all four antibiotics tested. None of the isolates were fully sensitive to all antibiotics. Regression analysis results regarding knowledge, practices, and antibiotic resistance are summarized in Table 2. A significant correlation was found between farmers' knowledge and their practices in antibiotic use ($r = 0.683$; $R^2 = 0.467$; $p < 0.05$). However, no significant difference was found between antibiotic use practices and the inhibition zone diameters of *Staphylococcus* spp. against the four antibiotics tested ($p > 0.05$).

Table 2. Regression analysis between knowledge, practices, and antibiotic resistance variables in Tabanan Regency, Bali Province, Indonesia

Relationship tested	r	R^2	p-value
Knowledge versus antibiotic use practices	0.683	0.467*	0.001
Practices versus inhibition zone diameter (Amoxicillin)	0.156	0.024^{ns}	0.858
Practices versus inhibition zone diameter (Ciprofloxacin)	0.257	0.066^{ns}	0.366
Practices versus inhibition zone diameter (Erythromycin)	0.267	0.071^{ns}	0.249
Practices versus inhibition zone diameter (Doxycycline)	0.365	0.133^{ns}	0.086

r: Correlation coefficient; R^2: Coefficient of determination. Vs: Versus, *: Significant, ns: Not significant

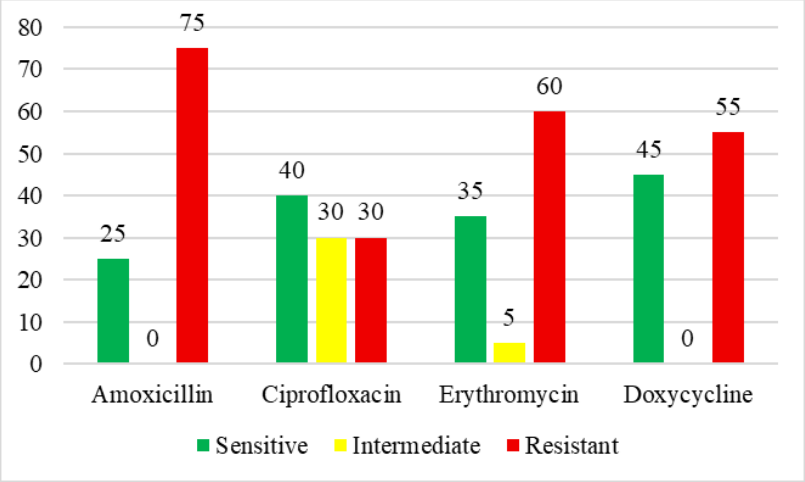

Figure 1. Percentage of antibiotic susceptibility test results for *Staphylococcus* spp. isolates collected from broiler farms in Tabanan Regency, Bali Province, Indonesia, between April and May 2025.

DISCUSSION

The findings of this study revealed that the majority of broiler farmers in Tabanan Regency possess only moderate knowledge and practices regarding antibiotic use. This finding indicated a limited level of veterinary health literacy, which may contribute to the irrational use of antibiotics in poultry production. Inadequate understanding of appropriate antibiotic use has been previously linked to poor on-farm practices in various studies (McEwen and Collignon, 2018; Rware et al., 2024). Low awareness of withdrawal periods, differentiation between bacterial and viral infections, and the broader implications of antimicrobial resistance (AMR) for human and environmental health are key issues that require urgent attention.

Ongoing education and the active involvement of veterinarians in treatment decisions are essential to improving antibiotic stewardship on farms (FAO, 2021; WHO, 2022). This is supported by the regression analysis in the present study, which demonstrated a significant relationship between farmers' knowledge and their antibiotic use practices with a correlation coefficient of 0.683 and a determination coefficient (R^2) of 0.467. These results suggest that improving farmers' understanding may positively influence their practices, including the selection, dosage, and duration of antibiotic administration (Sadiq et al., 2018).

Questionnaire results showed that amoxicillin was the most frequently used antibiotic among respondents. Consistently, amoxicillin exhibited the highest resistance rate (75%) among the isolates of *Staphylococcus* spp. in this study. This correlation between frequent use and resistance development aligned with previous reports (Van Boeckel et al., 2019). Similarly, high levels of resistance to erythromycin and doxycycline may reflect repeated use without accurate diagnosis or laboratory confirmation. Furthermore, knowledge of antibiotic use among poultry farmers was also reported in Kwara State, Nigeria, where farmers' knowledge levels were significantly associated with their perceptions but showed no significant correlation with actual antibiotic usage practices (Al-Mustapha et al., 2020).

The observed multiple resistance patterns, where isolates were resistant to up to four antibiotics, suggest a high selective pressure resulting from prolonged or inappropriate antibiotic exposure (Sunartatie et al., 2024). This result further emphasizes the possibility of resistance genes being horizontally transferred through plasmids, transposons, and various other mobile genetic elements (Michael and Schwarz, 2016). Although this study did not include molecular detection of resistance genes, genes such as *blaZ*, *erm*, *mecA*, *msr(A)*, and *tet(K/L)* have been widely reported to mediate resistance to the tested antibiotics (Hooper and Jacoby, 2015; Mak et al., 2022).

Interestingly, no significant correlation was found between knowledge of antibiotic use and the inhibition zone diameters of *Staphylococcus* spp. against the tested antibiotics. This finding suggests that resistance is not solely influenced by individual behaviors but also by external factors such as centralized treatment protocols in contract farming systems, environmental antibiotic exposure, and the broader circulation of resistance genes (Carrique-Mas et al., 2015; Nhung et al., 2016). In many nucleus-plasma systems, treatment regimens are determined by the integrator company, which often administers antibiotic packages en masse without considering specific on-farm conditions, consequently limiting farmers' control over actual antibiotic exposure and overlooking the role of their knowledge (Adam et al., 2020).

Additional contributing factors such as subtherapeutic dosing, cross-contamination from the environment, and lack of antibiotic rotation further intensify selection pressure and promote resistance (Tang et al., 2017). Therefore, the control of AMR in the poultry sector must be systemic and cross-sectoral, rather than relying solely on changes in individual farmer behavior.

Overall, the results of this study underscore the urgent need to adopt the One Health approach in addressing AMR in poultry production. The significant association between antibiotic usage practices at the farm level and the potential risks to public health underscores the need for comprehensive, multidisciplinary strategies incorporating educational initiatives, improvements in farm management, and enhanced regulatory oversight of antibiotic application. An effective approach to AMR mitigation, therefore, requires a comprehensive strategy that combines farmer education, strict policy implementation, and enhanced farm management practices within the One Health perspective. Building farmers' capacity refers to enhancing their knowledge, skills, and practices related to responsible antibiotic use and biosecurity measures. Fostering cross-sector collaboration involves coordinated efforts among stakeholders from agriculture, veterinary, public health, and regulatory sectors to effectively manage and reduce antimicrobial resistance risks along the entire food production chain (FAO, 2021; WHO, 2022).

CONCLUSION

Most broiler chicken farmers in Tabanan exhibited limited knowledge and suboptimal practices regarding antibiotic use, contributing to the high resistance levels of *Staphylococcus* spp. While knowledge significantly influenced practice, practices did not show a direct effect on bacterial resistance, indicating that AMR is a multifactorial issue. A comprehensive control strategy based on the One Health approach is essential to effectively address antimicrobial resistance in the poultry farming sector.

DECLARATIONS

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Authors' contributions

I Gusti Bagus Aryanta Kusuma Putra designed the study, collected the samples, and analyzed the data. Kadek Karang Agustina, I Wayan Suardana, and Ni Ketut Suwiti collected the samples and provided media for the study. I Wayan Masa Tenaya and Putu Henrywaesa Sudipa conducted the study process in the laboratory. Yeocelin Meida Utami and I Nengah Kerta Besung analyzed the data and wrote the manuscript. All authors reviewed the analyzed data and gave their approval to the final draft of the manuscript.

Competing interests

The authors declare that there are no conflicts of interest related to this publication.

Ethical considerations

All ethical issues, including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy, have been carefully reviewed and addressed by all authors.

Availability of data and materials

The original data presented in this study are included in the article. For inquiries, please contact the corresponding author.

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