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Phenotypic Detection and Antimicrobial Resistance Profiles of *Staphylococcus aureus* and ESBL-Producing *E. coli* Isolated from Poultry Litter in Pokhara, Nepal

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Abstract

Poultry litter can act as a reservoir of diverse pathogens including resistant bacteria, posing potential threats to animal health, public health and the environment. This cross-sectional study, conducted from December 2025 to April 2026, aimed to determine the prevalence and antimicrobial resistance profiles of methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL) producing *E. Coli* isolated from poultry litter from commercial farms of Pokhara, Nepal. The bacteria were isolated and identified performed using microbial culture, gram staining and biochemical tests. Antimicrobial susceptibility was performed using the Kirby–Bauer disk diffusion method, while MRSA identification and ESBL production were determined phenotypically. Of the total 100 poultry litter samples, 49 yielded bacterial growth, out of which *S. aureus* was isolated from 38 % of the litter samples. MRSA was detected in 15 of 38 (39.4%) *S. aureus* isolates with all *S. aureus* isolates exhibiting resistance to penicillin. Phenotypic ESBL production was confirmed in 9 of 11 (81.8%) E. coli isolates with 5 (45.4%) exhibiting multidrug resistance (MDR), with no statistically significant association between ESBL production and MDR phenotype ($p > 0.05$). The findings demonstrate the co-existence of *S. aureus* and ESBL-producing *E. coli* in poultry litter, highlighting its potential role as an environmental reservoir of antimicrobial resistance, suggesting the urgent need for farm level biosecurity, antimicrobial stewardship and integrated One Health approach.

Keywords: Antimicrobial Resistance, Methicillin-resistant *Staphylococcus aureus* (MRSA), Beta-lactamase, One Health, Poultry, Nepal

Introduction

Poultry litter consists of bedding material, feces, feathers and other organic residues, which can harbor several bacterial pathogens [1]. Multiple microorganisms like *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Pseudomonas* spp., *Streptococcus* spp., *Campylobacter* spp. have been isolated from poultry litter [1,2]. Therefore, poultry litter may act as a reservoir that can lead to transmission of antimicrobial resistant plasmid among pathogens [3]. The detection of several antibiotic resistance phenotypes and resistance genes in poultry litter sparks potential One Health concerns [3,4].

Antimicrobial resistance (AMR) is a growing burden across the globe. An estimated 1.27 million deaths was

attributable to bacterial AMR in 2019 [5]. According to the estimates of the Centers for Disease Control and Prevention (CDC), 11 million pounds of antimicrobials are used in the poultry sector, which can lead to emergence of antimicrobial resistance among pathogens and transfer between animal, human and environmental sources [6,7].

The extensive usage of antimicrobials in the poultry industry has enhanced the ability of *S. aureus* to acquire many resistance genes and thus become more virulent [8]. Methicillin-resistant Staphylococcus aureus (MRSA) has acquired resistance to methicillin and many other β-lactam antibiotics, which has made infections difficult to treat [9]. MRSA reported higher antimicrobial resistance to β-lactam antibiotics, especially penicillin and oxacillin, among isolates of poultry origin compared to bovine origin [10]. In addition, MRSA is a known nosocomial infectant and possess zoonotic risks leading to conditions like inter alia, skin and soft tissue infections, pneumonia or sepsis [11,12].

Extended-Spectrum Beta-Lactamase (ESBL) producing bacteria, specially belonging to Enterobacteriaceae family are another major issue because ESBL enzymes are resistant to many β-lactam antibiotics, including extended spectrum cephalosporins and monobactams [13]. ESBL producing Enterobacteriaceae have been recovered from the rectal swabs of healthy chickens, suggesting that poultry can serve as a reservoir of these organisms with potential of pathogenic transmission to humans [14]. A study conducted among food producing animals reported that the litter of chicken were highly contaminated with ESBL producing *E. coli* [15]. Among several ESBL genes, CTX-M-15 producing *E. coli* sequence type 131 (ST131) is known to cause severe extraintestinal human infections [16]. This microorganism is a threat to animal production and have significant food safety and public health which may contribute to the circulation of antimicrobial resistance between poultry, farm workers, and the wider environment implications [17,18].

ESBL producing *E. coli* and *S. aureus* have been detected from poultry farms and feed shops with both showcasing high antimicrobial resistance [19]. While there are studies that have detected *S. aureus* and ESBL producing bacteria in Nepal, reports on their prevalence and antimicrobial resistance profiles in poultry litter are limited. This study is aimed at investigating *S. aureus* and ESBL producing *E. coli* from poultry litter by determining their prevalence and assessing their antimicrobial resistance profiles. The findings are targeted to provide locally relevant baseline data to support antimicrobial resistance surveillance, farm level control and prevention measures and One Health interventions in Nepal.

Materials and Methods

Study design, area and duration

A cross-sectional study was conducted in Pokhara Metropolitan City, Kaski district (28°12′30″N 83°59′20″E) from December 2025 to April 2026. Pokhara metro is home to many small and large-scale poultry farmers with significant contribution to country's overall poultry meat production.

Sample collection

A total of 100 poultry litter samples (5g) were collected aseptically in a labeled sterile plastic bag from different commercial and non-commercial farms by random sampling strategy. Samples with no visual evidence of contamination met the inclusion criteria for the study. Samples were then transported to the laboratory of the Department of Microbiology, Janapriya Multiple Campus, Tribhuvan University, and Pokhara in cold chain and analyzed within 12 hours.

Isolation and identification of the isolates

First, 1g of sample was pre-enriched in Nutrient broth (NB) and incubated at 37°C for 24 hours. A loopful of broth was then subsequently cultured on Mannitol salt agar (MSA) (HiMedia, India) and incubated aerobically at 37°C for 24 hours. Yellow colonies on MSA agar were presumptively considered positive for *S. aureus*. However, for the isolation of *E. coli*, the broth was streaked on MacConkey Agar (MCA) and incubated aerobically at 37°C for 24 hours. The isolates that produced pink colonies on MCA agar were subsequently sub-cultured on Eosin Methylene Blue (EMB) agar. The colonies that produced metallic-green sheen on EMB agar were considered positive for *E. coli*. To obtain pure cultures of the isolates, distinct individual colonies were selected and sub-cultured on Nutrient agar (NA) plates. Further confirmation of bacterial isolates was based on biochemical tests like IMViC tests (indole, methyl red, Voges–Proskauer, and citrate utilization), urease and triple sugar iron (TSI) agar test, along with oxidase test as described by Quinn [20].

Antimicrobial susceptibility testing

Antimicrobial susceptibility was evaluated via the Kirby–Bauer disk diffusion method, following Clinical and Laboratory Standards Institute guidelines [21]. Antibiotic discs included cefoxitin (30 µg), oxacillin (1 µg), penicillin (10 IU), amikacin (30 µg), amoxicillin–clavulanate (30+10 µg), nitrofurantoin (30 µg), doxycycline (30 µg), sulphamethoxazole-trimethoprim (25 µg) and cefotaxime (30 µg), all obtained from Hi-media, India. A 24-hour culture, which was suspended in sterile normal saline to match the 0.5 McFarland standard (1.5×10^8 CFU/ml), was swabbed onto Mueller–Hinton agar (MHA), and the discs were placed

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aseptically before incubating the MHA plates at 37°C for 24 hours. Antimicrobials were chosen based on their clinical importance, bacterial group, anticipated susceptibility profiles and the guidance provided by recognized antimicrobial susceptibility testing standards. The zone of inhibition was measured and interpreted based on Clinical and Laboratory Standard Institute (CLSI) guidelines. The isolates that were resistant to at least three antimicrobial classes were categorized as multidrug resistant (MDR).

Phenotypic screening and confirmation for MRSA and ESBL producing *E. coli*

S. aureus isolates were tested for methicillin susceptibility using 30 µg cefoxitin discs on MHA. *S. aureus* showing a zone of inhibition of ≤21 mm with cefoxitin (30 µg) after incubation at 37°C for 24 hours, were considered positive for MRSA as per CLSI guidelines. The isolates showing zone of inhibition ≤27 mm for cefotaxime were considered positive on ESBL screening and processed for phenotypic confirmation. Screen-positive isolates were subjected to the combined disc (CD) test where 30 µg cefotaxime and cefotaxime–clavulanic acid (30+10 µg) were placed 20 mm apart. An isolate was confirmed as an ESBL producer if adding clavulanate increased the zone of inhibition by at least 5 mm compared to the antibiotic tested on its own [21].

Data analysis

Data obtained from the laboratory analysis was entered into MS Excel v2016. Descriptive statistics were used for analysis of prevalence of bacterial isolates, mean zone of inhibition and its standard deviation. Antibiotic Sensitivity Test (AST) was interpreted according to CLSI guidelines for the organism [21]. Chi-squared test was carried out to assess the association where $p \leq 0.05$ was considered statistically significant. 95% confidence interval was calculated using Clopper-Pearson exact method.

Results

Prevalence of *S. aureus* and *E. coli*

Of 100 poultry litter samples, 49 (49%) yielded bacterial growth. Among the positive isolates, 38 (38%) were *S. aureus* and 11 (11%) were *E. coli.*

Antimicrobial susceptibility test of the isolates

The isolates were subjected to antimicrobial susceptibility test, and the result is shown in Table 1 & 2.

Prevalence and antimicrobial susceptibility of MRSA isolates

Of the 38 *S. aureus* isolates, 15 (39.4%) were categorized as methicillin-resistant *S. aureus* (MRSA) and 23 (60.5%) as methicillin-sensitive *S. aureus* (MSSA). All MSSA and MRSA isolates were 100% resistant to penicillin.

Prevalence and Multidrug resistance of ESBL producers

Out of 11 E. coli isolates, 9 (81.8%) were phenotypically detected as ESBL producers with an overall prevalence of 9%. Among the isolates, only 5(45.4%) exhibited multidrug resistance, which were all phenotypic ESBL producers. However, Chi-squared test revealed no statistically significant association between ESBL production and the MDR phenotype ($p > 0.05$).

Antimicrobial agents	Class	Potency (µg)	No. of sensitive isolates (%) [95% CI]	No. of Intermediate isolates (%) [95% CI]	No. of resistant isolates (%) [95% CI]
Oxacillin	Penicillin	1	23(60.5) [43.3-75.9]	5(13.1) [4.4-28.1]	10(26.3) [13.4-43.1]
Cefoxitin	2nd gen. Cephalosporin	30	23(60.5) [43.3-75.9]	5(13.1) [4.4-28.1]	10(26.3) [13.4-43.1]
Penicillin	Penicillin	10	0 [0-9.2]	9(23.6) [11.4-40.2]	29(76.3) [59.7-88.5]
Amoxicillin-clavulanate	Penicillin	30+10	18(47.3) [30.9-64.1]	7(18.4) [7.7-34.3]	13(34.2) [19.6-51.3]
Amikacin	Aminoglycoside	30	32(84.2) [68.7-93.9]	2(5.2) [0.6-17.7]	4(10.5) [2.9-24.8]
Sulphamethoxazole-Trimethoprim	Sulfonamide	25	12(31.5) [17.5-48.6]	7(18.4) [7.7-34.3]	19(50) [33.3-66.6]
Doxycycline	Tetracycline	30	16(42.1) [26.3-59.1]	8(21.1) [9.5-37.3]	14(36.8) [21.8-54.1]

Table 1: Antimicrobial susceptibility profile of *S. aureus* (n=38) isolated from poultry litter.

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Antimicrobial agents	Class	Potency (µg)	No. of sensitive isolates (%) [95% CI]	No. of Intermediate isolates (%) [95% CI]	No. of resistant isolates (%) [95% CI]
Oxacillin	Penicillin	1	0 [0-28.4]	3(27.2) [6.1-60.9]	8(72.7) [39-93.9]
Cefoxitin	2nd gen. Cephalosporin	30	0 [0-28.4]	3(27.2) [6.1-60.9]	8(72.7) [39-93.9]
Penicillin	Penicillin	10	0 [0-28.4]	2(18.1) [2.2-51.7]	9(81.8) [48.2-97.7]
Amoxicillin-clavulanate	Penicillin	30+10	0 [0-28.4]	4(36.3) [10.9-69.2]	7(63.6) [30.7-89.1]
Amikacin	Aminoglycoside	30	11(100) [71.5-100]	0 [0-28.4]	0 [0-28.4]
Sulphamethoxazole-Trimethoprim	Sulfonamide	25	2(18.1) [2.2-51.7]	3(27.2) [6-60.9]	6(54.5) [23.3-83.2]
Doxycycline	Tetracycline	30	4(36.3) [10.9-69.2]	3(27.2) [6-60.9]	4(36.3) [10.9-69.2]
Cefotaxime	3rd gen. Cephalosporin	30	2(18.1) [2.2-51.7]	6(54.5) [23.3-83.2]	3(27.2) [6-60.9]
Nitrofurantoin	Nitrofuran	25	7(63.6) [30.7-89.1]	5(45.4) [16.7-76.6]	4(36.3) [10.9-69.2]
Cefoxitin	2nd gen. Cephalosporin	30	23(60.5) [43.3-75.9]	5(13.1) [4.4-28.1]	10(26.3) [13.4-43.1]
Penicillin	Penicillin	10	0 [0-9.2]	9(23.6) [11.4-40.2]	29(76.3) [59.7-88.5]
Amoxicillin-clavulanate	Penicillin	30+10	18(47.3) [30.9-64.1]	7(18.4) [7.7-34.3]	13(34.2) [19.6-51.3]
Amikacin	Aminoglycoside	30	32(84.2) [68.7-93.9]	2(5.2) [0.6-17.7]	4(10.5) [2.9-24.8]
Sulphamethoxazole-Trimethoprim	Sulfonamide	25	12(31.5) [17.5-48.6]	7(18.4) [7.7-34.3]	19(50) [33.3-66.6]
Doxycycline	Tetracycline	30	16(42.1) [26.3-59.1]	8(21.1) [9.5-37.3]	14(36.8) [21.8-54.1]

Table 2: Antimicrobial susceptibility profile of *E. coli* (n=11) isolated from poultry litter.

Discussion

The present study shows that poultry litter from commercial farms in Pokhara can harbor bacterial population with high antimicrobial resistance among *S. aureus* and ESBL-producing *E. coli*. Among the 100 poultry litter samples, 49% of them recorded bacterial growth, with *Staphylococcus aureus* being the predominant isolate (38%) overall, comparable to the findings of Paudyal [22]. The high prevalence of bacteria is consistent with recent studies that have demonstrated poultry litter and farm environments can act as reservoirs for antimicrobial resistant pathogens and favor their dissemination [23-25].

In our study, 39.4% (15/38) of the *S. aureus* isolates were Methicillin resistant. A study conducted across poultry farms of Punjab, India reported MRSA occurrence in 32.5% in deep-litter farms [26]. Similarly, a recent study from Kathmandu Valley reported multidrug resistant (MDR) *S. aureus* in poultry litter, with 65% of isolates showcasing MDR with widespread carriage of resistance genes [27]. Therefore, the relatively high MRSA occurrence in our study adds evidence to the fact that resistant *S. aureus* is established in poultry associated environments across South Asian countries. This explanation is further supported by the regional systematic review conducted among four countries of South Asia, which highlighted MRSA as an important livestock and poultry associated resistance problem [28]. These results suggest that poultry litter can provide a persistent environmental matrix for the resistant staphylococci.

The resistance profile further highlights the clinical importance of these isolates. Penicillin was 100% resistant to all *S. aureus* isolates. The extensive β-lactam resistance in this is consistent with the findings of other studies where penicillin was found to be highly resistant towards *S. aureus*

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[29,30,8]. These findings reflect the indiscriminate and irrational use of antimicrobials in poultry sector that may have created intense selective pressure that has contributed to the growing burden of anti-microbial resistance [31]. A high prevalence of MRSA increases the risk of zoonotic transmission to farm workers, veterinarians and poultry handlers [32,33].

Phenotypic ESBL-producing *E. coli* was reported in 9 (9%) of the litter sample. Comparable to our findings, a study in Malaysia detected phenotypic ESBL production in 14.7% of *E. coli* isolates from broilers and their farm environments [34]. In contrast, a Lithuanian study reported an higher overall prevalence of 29.1% ESBL producing *E. coli* [35]. An alarming high frequency (65.7%) of ESBL producing *E. coli* isolates from chicken farms in Egypt has also been reported in a study [36]. These variations might be due to the influence of geographic location, production system, sampling strategy, antimicrobial exposure and laboratory methodology. These findings are in accordance with the increasing recognition of poultry environment as reservoirs of ESBL-producing *E. coli*.

Although our study reported only 9% prevalence of ESBL producing *E. coli*, this finding is still concerning because this organism can serve not only as a poultry associated pathogen but also as a vehicle for dissemination of resistance determinants, with a potential for zoonotic transmission [37]. Recent studies have demonstrated substantial ESBL burdens among *E. coli* isolates in poultry settings, including detection of bla_{CTX-M}, bla_{TEM} and other ESBL genes [38,35,39,34]. All in all, these observations together with our findings indicate that ESBL-positive *E. coli* represents a geographically distributed AMR concern within poultry production system.

55.5% of the ESBL *E. coli* isolates exhibited multi-drug resistance which demonstrates that β-lactam resistance generally occurs alongside resistance to other antimicrobial classes. Studies have shown that 100% of the ESBL isolates were multi-drug resistant [38,34]. As there was no statistically significant association between ESBL production and MDR in the present study, the result should be interpreted carefully. This can be because ESBL production represents a specific resistance mechanism, whereas MDR is a phenotypic classification of antimicrobial resistance. Therefore, the two features may not occur in every isolate.

Poultry litter may also play an important role in environmental dissemination of resistance. The application of poultry litter increased antibiotic resistant *E. coli* in agricultural soil, while all ESBL-producing Enterobacterales isolated carried bla_{CTX-M} [40] A study demonstrated that resistant bacteria and resistance genes originating from poultry litter can cycle between soil, crops and water [41]. Reports in Nepal have also detected ESBL-positive *E. coli* among human, poultry and environmental sources in Kathmandu with high antimicrobial resistance [42]. The coexistence of MRSA and ESBL-positive organisms in the present study therefore highlights poultry litter as an important component of farm level AMR surveillance and One Health.

Overall, the detection of MRSA in 39.4% of *S. aureus* isolates and a high prevalence of ESBL-positive *E. coli* isolates highlights poultry litter as an important environmental reservoir of antimicrobial resistant bacteria in commercial farms of Pokhara. However, molecular detection of *mecA/mecC* and ESBL genes, together with the assessment of biofilm formation is required to establish the underlying resistance mechanisms.

Conclusion

This study demonstrated that poultry litter from commercial farms in the Pokhara Valley harbors resistant pathogens, such as MRSA and ESBL producing bacteria. MDR was observed in 55.5% of the ESBL isolates. However, no significant association was found between ESBL production and MDR in the present study. The detection of these organisms highlights poultry litter as a potential environmental reservoir and possible source of antimicrobial resistant bacteria, with implications for poultry health, environmental contamination and public health. These findings suggest the urgent need for farm level biosecurity, hygiene, antimicrobial stewardship and integrated One Health approach to further analyze the persistence, transmission and environmental dissemination of resistant bacteria throughout poultry production systems in the regions.

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Conflict of interest statement

The authors declare no conflict of interest.

Authorship contribution statement

Amrit Poudel: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Formal analysis, conceptualization, Validation, Project administration.

Ashesh Kafle: Writing – review & editing, Writing – original draft, Methodology, Data curation, Investigation,

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Visualization, Validation, Supervision.

Mamata Poudel Subedi: Writing – review & editing, Writing – original draft, Methodology, Data curation, Investigation, Visualization, Validation, Supervision.

Data availability

The data are included in the article. Further inquiries can be directed to the corresponding author.

Ethics statement

Litter samples were collected non-invasively, ensuring no harm or stress to Poultry. No ethical approval was required for it.

Consent for publication

Not applicable.

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References

1. Lawrence JR, Cudnik D & Oladeinde A (2022) Bacterial Detection and Recovery From Poultry Litter. Front Microbiol 12: 803150.

2. Sule IO, Olorunfemi AA, Otori AO (20190 Mycological and Bacteriological Assessment of Poultry Droppings from Poultry Pens within Ilorin, Kwara, Nigeria. Sci World J 14(4): 11-16.

3. Oxendine A, Walsh AA, Young T, Dixon B, Hoke A, et al. (2023) Conditions Necessary for the Transfer of Antimicrobial Resistance in Poultry Litter. Antibiotics 12(6): 1006.

4. Tripathi A, Jaiswal A, Kumar D, Pandit R, Blake D, et al. (2025) Whole genome sequencing revealed high occurrence of antimicrobial resistance genes in bacteria isolated from poultry manure. J Ijantimicag 65(3): 107452.

5. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, et al. (2022) Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. Lancet 399: 629-655.

6. Abate TA, Birhanu AG (2025) Antibiotic Use in Livestock and Environmental Antibiotic Resistance: A Narrative Review. Environ. Health Insights 19: 11786302251357775.

7. Alagawany M, Elgammal M, Saeed M, Khan M, Ali A, et al. (2022) Hazards of Using Antibiotic Growth Promoters in the Poultry Industry. Bentham Science Publishers: 1-18.

8. Otalu O, Junaidu K, Chukwudi OE, Jarlath UV (2011) Multi-drug Resistant Coagulase Positive Staphylococcus aureus from Live and Slaughtered Chickens in Zaria, Nigeria. Int J Poult Sci 10(11): 871-875.

9. El-Ghany WA (2021) Staphylococcus aureus in poultry, with special emphasis on methicillin-resistant strain infection: A comprehensive review from one health perspective. Int. J. One Health 7(2): 257-267.

10. Kechih S, Aggad H, Meguenni N, Hamdi MT, Cantekin Z (2018) Carriage Methicillin-Resistant Staphylococcus aureus in Poultry and Cattle in Northern Algeria. Vet Me Int 2018: 4636121.

11. Dahms C, Hübner NO, Wilke F, Kramer A (2014) Mini-review: Epidemiology and zoonotic potential of multiresistant bacteria and Clostridium difficile in livestock and food. GMS Hyg Infect Control 9(3): Doc21.

12. Kinross P, Petersen A, Skov R, Van Hauwermeiren E, Pantost, A, et al. (2017) Livestock-associated meticillin-resistant Staphylococcus aureus (MRSA) among human MRSA isolates, European Union/European Economic Area countries 2013. Euro Surveill 22(44): 16-00696.

13. Castanheira M, Simner PJ, Bradford PA (2021) Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. JAC Antimicrob Resist 3(3): dlab092.

14. Subramanya SH, Bairy I, Nayak N, Amberpet R, Padukone S et al. (2020) Detection and characterization of ESBL-producing Enterobacteriaceae from the gut of healthy chickens, Gallus gallus domesticus in rural Nepal: Dominance of CTX-M-15-non-ST131 Escherichia coli clones. PLoS One 15(5): e0227725.

15. Geser N, Stephan R, Hächler H (2012) Occurrence and characteristics of extended-spectrum β-lactamase (ESBL) producing Enterobacteriaceae in food producing animals, minced meat and raw milk. BMC Vet Res 8: 21.

16. Chanoine MH, Bertrand X & Madec JY. 2014. Escherichia coli ST131, an Intriguing Clonal Group. Clin Microbiol Rev 27(3): 543-574.

17. Subramanya SH, Bairy I, Metok Y, Baral BP, Gautam D (2021) Detection and characterization of ESBL-producing Enterobacteriaceae from the gut of subsistence farmers, their livestock, and the surrounding environment in rural Nepal. Sci Rep 11(1): 2091.

Copyright© Kafle A, et al.

18. Aliyu AB, Jalila A, Saleha AA, Zunita Z (2024) ESBL Producing E. coli in Chickens and Poultry Farms Environment in Selangor, Malaysia: A Cross-Sectional Study on Their Occurrence and Associated Risk Factors with Environment and Public Health Importance. Zoonoses Public Health 71(8): 962-971.

19. Afegbua S, Akuson S, Musa M, Muhammad H, Hamajam M, et al. (2026) Detection of ESBL-Producing Escherichia coli and Methicillin-resistant Staphylococcus aureus from Poultry Farms and Feed Shops in Zaria. UJMR 11: 46-56.

20. Quinn P, Markey B, Leonard F, FitzPatrick E, Fanning S (2011) Veterinary Microbiology and Microbial Disease. Second edition: 912. Oxford, Wiley-Blackwell.

21. CLSI M100 (2026) Performance Standards for Antimicrobial Susceptibility Testing. 36th edition.

22. Paudyal S, Joshi LR, Tiwari A, Devkota SP, katiwada S, et al. (2014) Prevalence of Methicillin-Resistant Staphylococcus aureus (MRSA) in Dairy Farms of Pokhara, Nepal. Inter J Vet Sci 3(2): 87-90.

23. Cortés P, Pokrant E, Yévenes K, Maddaleno A, Flores A, et al. (2025) Antimicrobial Residues in Poultry Litter: Assessing the Association of Antimicrobial Persistence with Resistant Escherichia coli Strains. Antibiotics 14(1): 89.

24. Singh S, Kriti M, Anamika KS, Sharma P, Pal N, et al. (2025) A one health approach addressing poultry-associated antimicrobial resistance: Human, animal and environmental perspectives. J Microb 7: 100309.

25. Subhasinghe I, Bandara HMHN, Karunarathna HMTK, Kodithuwakku SP, Gallage HC, et al. (2025) Antimicrobial resistance patterns and biofilms resurgence ability of Escherichia coli associated with commercial layer chicken farms in Sri Lanka. J Vetmic 302: 110422.

26. Taskeen S, Singh R, Bedi JS, Arora AK, Aulakh RS, et al. (20240 Occurrence, Multidrug Resistance, SCCmec Typing of Methicillin-Resistant Staphylococcus aureus from Farmed Eggs and Environment. Curr Microbiol 82: 47(2025).

27. Shrestha P, Bajracharya B, Shrestha D, Karna AK, Shah P, et al. (2026) Molecular detection of antibiotic resistance genes in Staphylococcus aureus isolated from poultry farms of Kathmandu Valley, Nepal. BMC Microbiol 26(1): 291.

28. Barua N, Rahman N, Tin M, Yang L, Alim A, et al. (2025) Prevalence of MRSA in Livestock, Including Cattle, Farm Animals, and Poultry, in Mainland China, Hong Kong Special Administrative Region, Sri Lanka, and Bangladesh: A Systematic Review and Meta-Analysis. Microorganisms 13: 704.

29. Abunna F, Adugna B, Tufa TB, Ayana D, Gutema FD, et al. (2022) Detection and Antimicrobial Resistance of Staphylococcus Species from Chicken, Chicken Litter, and Humans in Addis Ababa, Ethiopia. Vet Med Int (1): 9084334.

30. Belfihadj F, Meriem E, Naouel B, Bourouba A, Mouffok CE, et al. (2025) Emergence of Multidrug-Resistant and Biofilm-Producing Staphylococcus aureus from Raw Poultry in Algeria: Implications for Public Health. Microorganisms 13(12): 2764.

31. Acharya KP, Wilson RT (2019) Antimicrobial Resistance in Nepal. Front Med 6: 105.

32. Oke AJ, Oke AA (2013) Incidence of Methicillin-Resistant Staphylococcus aureus (MRSA) In a Small Poultry in South West, Nigeria. IOSR-JAVS 5(3): 53-55.

33. Adhikari S, Khanal S, Marasini A, Panthi P, Adhikari A, et al. (2025) Antimicrobial resistance and biofilm-forming ability in Staphylococcus aureus causing clinical bovine mastitis in Chitwan, Nepal. Vet Anim Sci 30: 100508.

34. Lemlem M, Aklilu E, Mohamed M, Kamaruzzaman NF, Devan SS, et al. (2024) Prevalence and molecular characterization of ESBL-producing Escherichia coli isolated from broiler chicken and their respective farms environment in Malaysia. BMC Microbiol 24(1): 499.

35. Kasparaviciene B, Novoslavskij A, Aksomaitiene J, Stankeviciene J, Kasetiene N, et al. (2025) Prevalence and Antimicrobial Resistance of ESBL E. coli in Early Broiler Production Stage and Farm Environment in Lithuania. Microorganisms 13(2): 425.

36. Salem G, Abdelaziz ES, Kamel M, Ali R (2023) Prevalence of multidrug-resistant and extended-spectrum β–lactamase-producing Escherichia coli from chicken farms in Egypt. Vet World 16(5): 1001-1007.

37. Widodo A, Khairullah AR, Effendi MH, Moses IB, Agustin ALD (2024) Extended-spectrum β-lactamase-producing Escherichia coli from poultry: A review. Vet World 17(9): 2017-2027.

38. González J, Ríos MS, Hernandez L, Chiapparrone ML, Riccio MB, et al. (2025) Extended-spectrum beta-lactamase-producing Escherichia coli through the poultry production chain in Argentina. J. Vetmic 302: 110421.

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39. Lemlem M, Aklilu E, Mohammed M, Kamaruzzaman F, Zakaria Z, et al. (2023) Molecular detection and antimicrobial resistance profiles of Extended-Spectrum Beta-Lactamase (ESBL) producing Escherichia coli in broiler chicken farms in Malaysia. PLoS ONE 18(5): e0285743.

40. Agga G, Durso L, Sistani K (2024) Effect of poultry litter soil amendment on antibiotic-resistant Escherichia coli. J Environ Qual 53(3): 300-313.

41. Lopes ES, Santaren KC, Souza LC, Parente CET, Picão RC, et al. (2024) Cross-environmental cycling of antimicrobial resistance in agricultural areas fertilized with poultry litter: A one health approach. Environ Pollut 363(1): 125177.

42. Acharya J, Jha R, Gompo TR, Chapagain S, Shrestha L, et al. (2024) Prevalence of Extended-Spectrum Beta-Lactamase (ESBL)–Producing Escherichia coli in Humans, Food, and Environment in Kathmandu, Nepal: Findings from ESBL E. coli Tricycle Project. Int J Microbiol (1): 1094816.

43. Al-harbi H, Ranjbar S, Moore RJ, Alawneh JI (2021) Bacteria Isolated From Milk of Dairy Cows with and Without Clinical Mastitis in Different Regions of Australia and Their AMR Profiles. Front Vet Sci 8: 743725.

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