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Research Article

Prevalence and Antimicrobial Resistance of *E.coli* and Salmonella isolated from Poultry litter in District Umerkot, Sindh

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ABSTRACT

The present study was conducted to determine the prevalence and antimicrobial resistance patterns of Escherichia coli and Salmonella spp. isolated from poultry litter in District Umerkot, Sindh, Pakistan. A total of 100 poultry litter samples were collected from four talukas, namely Umerkot, Pithoro, Samaro, and Kunri, with 25 samples obtained from each taluka. Standard bacteriological methods were employed for the isolation and identification of *E. coli* and *Salmonella* spp. The prevalence results revealed that *E. coli* was detected in 58% of the samples, while *Salmonella* spp. were isolated from 26% of the samples, indicating a higher level of *E. coli* contamination in the study area. Taluka-wise variation in bacterial prevalence was observed, reflecting differences in farm hygiene and management practices. Antimicrobial susceptibility testing was performed using the agar disk diffusion method following Clinical and Laboratory Standards Institute guidelines. The *E. coli* isolates exhibited high resistance to several commonly used antibiotics, including oxacillin, streptomycin, ciprofloxacin, tetracycline, and sulfonamides. However, notable sensitivity was observed against amikacin, gentamycin, and erythromycin. Similarly, Salmonella isolates showed resistance to nalidixic acid, ciprofloxacin, tetracycline, doxycycline, and sulfonamide antibiotics, while sensitivity was primarily observed to amoxicillin, gentamycin, and erythromycin. The presence of multidrug-resistant *E. coli* and Salmonella spp. in poultry litter highlights a potential risk to animal and public health. The findings emphasize the need for improved biosecurity measures, hygienic farm practices, and rational use of antimicrobials in poultry production systems to minimize the spread of resistant pathogens.

Keywords: *Escherichia coli*; *Salmonella* spp.; Poultry litter; Antimicrobial resistance; District Umerkot.

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INTRODUCTION

Poultry plays a crucial role in supplying animal-derived protein for human consumption through meat and egg production. Poultry production systems and markets vary globally depending on geographic location, consumer demand, supply chains, and consumption patterns. Poultry consumption is rapidly increasing worldwide in response to population growth, with a particularly notable rise in Asian countries compared to other regions (Ullah et al., 2022; Putri et al., 2018).

In recent years, countries such as China, the United States, Brazil, Russia, Mexico, India, and Pakistan have emerged as leading poultry producers worldwide (Kaleri et al., 2025; Akbar et al., 2020). However, poultry birds are susceptible to a wide range of diseases, including bacterial infections such as colibacillosis and pullorum disease, viral diseases like Newcastle disease and avian influenza, and parasitic infestations including coccidiosis and Heterakis gallinarum. These diseases result in substantial economic losses to the poultry industry due to increased mortality, reduced productivity, and elevated management costs (Kaleri et al., 2024; Akbar et al., 2026).

Disease outbreaks frequently arise due to inadequate management practices. The flooring of poultry farms is a critical but often overlooked source of infection, as it is routinely covered with bedding material known as poultry litter, typically composed of rice husk or wood straw. This litter accumulates considerable waste material, including feathers, urine, feces, and spoiled feed (Kaleri et al., 2023; Jaehjo et al., 2016; Jayathilakan et al., 2012). Poultry litter serves as a reservoir for numerous pathogenic microorganisms, among which Escherichia coli and Salmonella spp. are most prevalent, contributing to severe economic losses through increased morbidity and mortality in poultry flocks (Alali et al., 2010). Despite this, limited data are available on the microbial burden of poultry litter in semi-arid and under-resourced poultry-producing regions of Pakistan, including District Umerkot.

Antimicrobial drugs are extensively used in intensive poultry farming systems to control microbial infections for both therapeutic and prophylactic purposes (Buriero et al., 2017). Consequently, antimicrobial resistance has become increasingly prevalent in poultry production, rendering many previously effective antibiotics less efficacious (Ullah et al., 2019). Monitoring bacterial resistance patterns is particularly important for poultry destined for human consumption, as it enables assessment of the transmission potential of resistant bacteria along the food chain (Collignon et al., 2016). The World Organisation for Animal Health has reported rising resistance to critically important antibiotic classes such as Quinolones, Cephalosporins, and Macrolides in bacterial pathogens including E. coli and Salmonella spp. (Davies and Wales, 2019; Sellah and Drissi, 2015). In response, the World Health Organization has emphasized the prudent use of antimicrobials in food-producing animals to mitigate the spread of resistance (Belanger et al., 2011).

In this context, the present study is novel in providing region-specific data on the prevalence and antimicrobial resistance profiles of E. coli and Salmonella isolated from poultry litter in District Umerkot, an area where systematic surveillance remains scarce. The findings are expected to contribute valuable baseline information for improving biosecurity, antimicrobial stewardship, and public health risk assessment in poultry production systems of southern Pakistan.

MATERIALS AND METHODS

Study area and design

The present study was conducted at the Central Veterinary Diagnostic Laboratory (CVDL), Tandojam, to determine the prevalence and antimicrobial resistance patterns of *Escherichia coli* and *Salmonella* spp. isolated from poultry litter. The study was designed as a cross-sectional survey and carried out in District Umerkot, Sindh, Pakistan.

Sample collection

A total of 100 poultry litter samples were collected from commercial poultry farms located in four talukas of District Umerkot, namely Umerkot, Pithoro, Samaro, and Kunri. From each taluka, 25 litter samples were randomly collected from different farms. Samples were aseptically collected using sterile spatulas and transferred into sterile polythene bags following standard sampling procedures. All samples were properly labeled and transported to the laboratory under chilled conditions for further microbiological analysis.

Isolation and Identification of Bacteria

The collected poultry litter samples were subjected to bacteriological examination following standardized laboratory procedures. Briefly, representative portions of each litter sample were aseptically homogenized in buffered peptone water and incubated at 37 °C for 18–24 h for pre-enrichment.

Isolation and identification of *Salmonella* spp. were performed in accordance with ISO 6579 (2002) and ISO 6579-1 (2017). After pre-enrichment, aliquots were selectively enriched in Rappaport–Vassiliadis and Muller–Kauffmann tetrathionate broths, followed by incubation at 37–42 °C for 24 h. Enriched cultures were then streaked onto selective agar media, including Xylose Lysine Deoxycholate (XLD) and Brilliant Green Agar, and incubated at 37 °C for 24 h. Presumptive *Salmonella* colonies were selected based on typical colony morphology and subjected to biochemical confirmation.

For *Escherichia coli* isolation, pre-enriched samples were streaked onto selective and differential media, including

MacConkey agar and Eosin Methylene Blue (EMB) agar, and incubated at 37 °C for 24 h. Presumptive *E. coli* colonies were identified based on characteristic lactose fermentation and metallic sheen on EMB agar, followed by Gram staining and biochemical characterization as described by Lie et al. (2008).

Confirmatory identification of both *Salmonella* spp. and *E. coli* isolates was carried out using standard biochemical tests, including Triple Sugar Iron (TSI) agar, Indole, Methyl Red, Voges–Proskauer, Citrate utilization, and Urease tests. Only isolates exhibiting characteristic biochemical profiles were considered confirmed and included in subsequent analyses.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of confirmed *E. coli* and *Salmonella* isolates was determined using the agar disk diffusion method. The procedure was conducted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) as described by Humphries et al. (2018).

For this purpose, Mueller–Hinton agar was prepared by dissolving 3.8 g of media in 100 mL of double-distilled water, followed by sterilization in an autoclave at 121°C for 15 minutes at 15 lb pressure. After cooling, the media was poured into sterile Petri plates and allowed to solidify. Bacterial suspensions were evenly inoculated on the agar surface using sterile cotton swabs.

A total of 14 antibiotic discs were used, including oxacillin, streptomycin, polymyxin-B, amoxicillin, nalidixic acid, amikacin, ciprofloxacin, sulphafurazole, doxycycline, sulfamethoxazole, gentamycin, tetracycline, erythromycin, and cefixime. The antibiotic discs were placed on the inoculated agar plates using a disc dispenser and gently pressed with sterile forceps to ensure proper contact. Plates were incubated at 37°C for 24 hours, after which the zones of inhibition were measured in millimeters and interpreted as sensitive, intermediate, or resistant according to CLSI standards.

RESULTS

The results presented in table 1 show taluka-wise variation in the prevalence of *E. coli* and *Salmonella* contamination in District Umerkot. Overall, E. coli was detected in 58% of the samples, indicating a higher level of contamination compared to *Salmonella* (26%).

The highest *E. coli* prevalence was observed in Umerkot taluka (64%), while Kunri showed the lowest (52%). Salmonella contamination was most frequent in Samaro (32%) and least in Kunri (20%), reflecting differences in hygienic and environmental conditions among the talukas.

Table 1. Taluka-wise distribution, number and percentage of *E. coli* and *Salmonella* contaminated samples in District Umerkot.

Taluka	No. of samples (N)	*E. coli* positive n (%)	*Salmonella* positive n (%)
Umerkot	25	16 (64%)	07 (28%)
Pithoro	25	14 (56%)	06 (24%)
Samaro	25	15 (60%)	08 (32%)
Kunri	25	13 (52%)	05 (20%)
Total	100	58 (58%)	26 (26%)

Note: N = Total number of samples

The antibiotic susceptibility results revealed that *E. coli* isolates exhibited a high level of resistance to most commonly used antibiotics. Strong resistance was observed against oxacillin, streptomycin, tetracycline, ciprofloxacin, and sulfonamides, indicating widespread antimicrobial resistance. In contrast, *E. coli* isolates showed good sensitivity to amikacin, gentamycin, and erythromycin, with larger zones of inhibition. Amoxicillin showed an intermediate response, suggesting partial effectiveness. Overall, the findings highlight the presence of multidrug-resistant *E. coli* strains in the study area.

The antibiotic susceptibility pattern of *Salmonella* spp. revealed varying responses to commonly used antibiotics. The isolates showed sensitivity to amoxicillin, gentamycin, and erythromycin, indicating their potential effectiveness against *Salmonella* infections. Intermediate responses were observed for oxacillin, streptomycin, amikacin, and cefixime, while high resistance was noted against nalidixic acid, ciprofloxacin, tetracycline, doxycycline, and sulfonamides. These findings suggest the presence of partially resistant *Salmonella* strains circulating in the study area.

Table 2. Antibiotic susceptibility pattern of *E. coli* spp. isolated from various farms of four talukas of District Umerkot.

Antibiotics	Zone of inhibition (mm)**	Resistance level
Oxacillin	03	Resistant
Streptomycin	05	Resistant
Polymyxin-B	03	Resistant
Amoxicillin	14	Intermediate
Nalidixic acid	03	Resistant
Amikacin	21	Sensitive
Ciprofloxacin	04	Resistant
Sulfafurazole	05	Resistant
Doxycycline	04	Resistant
Sulfamethoxazole	03.0	Resistant
Gentamycin	19	Sensitive
Tetracycline	03.1	Resistant
Erythromycin	22	Sensitive
Cefixime	02.0	Resistant

**According to CLSI Standards, antibiotics sensitivity is shown in millimeter (mm) Sensitive >20, Intermediate 15-19, Resistant <14.

Table 3. Antibiotic susceptibility pattern of *Salmonella* spp. isolated from various farms of four talukas of District Umerkot.

Antibiotics	Zone of inhibition (mm)**	Resistance level
Oxacillin	15	Intermediate
Streptomycin	17	Intermediate
Polymyxin-B	02.0	Resistant
Amoxicillin	22	Sensitive
Nalidixic acid	02.0	Resistant
Amikacin	18	Intermediate
Ciprofloxacin	03.0	Resistant
Sulphafurazole	05.0	Resistant
Doxycycline	06.5	Resistant
Sulfamethoxazole	05.8	Resistant
Gentamycin	19	Sensitive
Tetracycline	02.5	Resistant
Erythromycin	24	Sensitive
Cefixime	17	Intermediate

**According to CLSI Standards, antibiotics sensitivity is shown in millimeter (mm) Sensitive >20, Intermediate 15-19, Resistant <14.

DISCUSSION

The present study investigated the prevalence of *Escherichia coli* and *Salmonella* contamination in different talukas of District Umerkot and evaluated the antimicrobial susceptibility patterns of the isolated organisms. The findings revealed a relatively high prevalence of *E. coli* (58%) compared to *Salmonella* (26%), indicating widespread fecal contamination and poor hygienic practices in livestock farms of the study area. Similar trends have been reported in previous studies from Pakistan and other developing countries, where *E. coli* was more frequently isolated than *Salmonella* due to its ubiquitous nature and ability to survive under diverse environmental conditions (Biswas et al. (2004).

Taluka-wise variation in contamination levels observed in the present study may be attributed to differences in farm management practices, water quality, sanitation, and biosecurity measures. Higher *E. coli* prevalence in Umerkot and Samaro talukas suggests inadequate waste disposal systems and the use of contaminated water sources for animal drinking and cleaning purposes. Comparable findings were documented by Ghanbarpour et al, (2011), who reported significant spatial variation in bacterial contamination linked to farm hygiene and environmental exposure.

The antibiotic susceptibility pattern of *E. coli* isolates demonstrated high resistance to commonly used antibiotics such as oxacillin, streptomycin, tetracycline, ciprofloxacin, and sulfonamides. Sensitivity was mainly observed against

amikacin, gentamycin, and erythromycin. These results are consistent with earlier reports indicating increasing multidrug resistance (MDR) in *E. coli* isolates from livestock, largely due to indiscriminate and prolonged use of antibiotics for therapeutic and prophylactic purposes (Iqbal et al., 2016; World Health Organization, 2017). The intermediate response of *E. coli* to amoxicillin suggests partial loss of efficacy, which may worsen if irrational antibiotic use continues.

Similarly, *Salmonella* isolates showed resistance to nalidixic acid, ciprofloxacin, tetracycline, doxycycline, and sulfonamides, while maintaining sensitivity to amoxicillin, gentamycin, and erythromycin. Intermediate susceptibility to oxacillin, streptomycin, amikacin, and cefixime indicates evolving resistance patterns. These findings align with studies by Dhanarani, et al., (2009); Islam et al., (2014) and Rahman et al. (2019), who reported rising resistance of *Salmonella* spp. to fluoroquinolones and tetracyclines in food-producing animals. The relatively lower prevalence of *Salmonella* compared to *E. coli* in the present study may be due to its more specific growth requirements and intermittent shedding by infected animals.

The occurrence of MDR bacteria in livestock poses a serious public health concern, as resistant pathogens can be transmitted to humans through direct contact, contaminated animal products, or the environment. The present findings emphasize the urgent need for improved farm hygiene, regular microbial surveillance, and strict regulation of antibiotic usage in livestock production systems. Implementation of antimicrobial stewardship programs and awareness campaigns among farmers could play a vital role in reducing the emergence and spread of resistant bacterial strains

CONCLUSION

The highest production performance was observed in the HF breed at fifth parity, indicating greater environmental adaptability and suitability for farming in Pakistan's local conditions. Daily milk yield was highly correlated with peak milk yield and standard 305-day milk yield. Lactation length was found to be highly correlated with lactation yield. Negative correlations were found between fat and lactose, and between protein and total solids; however, other correlations among milk components were positive. Traits of economic importance were found to be better in the Holstein Friesian breed of cattle, so it is recommended that this type of breed should be raised for future breeding purposes. Also, advanced parity animals, such as (fourth and fifth), should be maintained on the farm and avoid culling if not diseased. Production of the animals increased with advancing parities, which can be helpful for selection indices in exotic breeds. Daily milk yield should be given higher priority and better managed to improve an animal's performance record.

AUTHOR CONTRIBUTIONS

All authors contributed equally.

COMPETING OF INTEREST

The authors declare no competing interests.

REFERENCES

Akbar, L., Rashid, A., Soomro, Z. P., Jabar, A., Kaleri, R. R., Ahmed, I., ... & Hussain, M. (2026). Evaluation of Serum Albumin, Urea, and Creatinine in Broilers Fed Yacon Powder–Supplemented Diets. Planta Animalia, 5(1), 31-36.

Akber, I., Rajput, N., Ali, S., Naeem, M., Mumtaz, M., Rajput, L. and Kaleri, R.R. (2020) 'Comparative study on the efficiency of various feed additives on growth performance of broilers', Pure and Applied Biology, 9(1), pp. 240–248.

Alali, W.Q., S. Thakur, R.D. Berghaus, M.P. Martin, and W.A. Gebreye., (2010). Prevalence and distribution of Salmonella in organic and conventional broiler poultry farms. Food borne Pathogens and Disease, 7(11), 1363-1371.

Bélanger, L, (2011). "*E.coli* from animal reservoirs as a potential source of human extra intestinal pathogenic *E.coli. FEMS* Immunology and Medical Microbiology, 62.1: 1-10.

Biswas, S., Biswas, I., & Hasegawa, H. (2004). Prevalence of *Escherichia coli* and *Salmonella* in poultry farms and their antimicrobial resistance patterns. International Journal of Poultry Science, 3(9), 592–596.

Buriro, R. S., Habib, F., Rind, R., Shah, M. A., Kaleri, R. R., Samoon, A. K., ... & Goswami, N. (2017). Antibiotic sensitivity tests of various micro-organisms from poultry eggs. Pure and Applied Biology (PAB), *6*(4), 1417-1426.

Collignon, P. C., Conly, J. M., Andremont, A., McEwen, S. A., Aidara-Kane, A., World Health Organization Advisory Group, Bogotá Meeting on Integrated Surveillance of Antimicrobial Resistance (WHO-AGISAR), ... & Woo, G. J. (2016). World Health Organization ranking of antimicrobials according to their importance in human

medicine: a critical step for developing risk management strategies to control antimicrobial resistance from food animal production. Clinical infectious diseases, 63(8), 1087-1093.

Davies, R. and A. Wales. (2019). Antimicrobial resistance on farms: a review including biosecurity and the potential role of disinfectants in resistance selection. Comprehensive Reviews in Food Science and Food Safety, 18(3), .753-774.

Dhanarani, T. S., Shankar, C., Park, J., Dexilin, M., Kumar, R. R., & Thamaraiselvi, K. (2009). Study on acquisition of bacterial antibiotic resistance determinants in poultry litter. Poultry Science, 88(7), 1381-1387.

Ghanbarpour, R., Soltani, M., & Saeedzadeh, A. (2011). Spatial variation and prevalence of bacterial contamination in poultry farms: A case study on *E. coli* and *Salmonella.* Avian Pathology*,* 40(3), 245–251.

Humphries, R. M., Kircher, S., Ferrell, A., Krause, K. M., Malherbe, R., Hsiung, A., & Burnham, C. A. D. (2018). The continued value of disk diffusion for assessing antimicrobial susceptibility in clinical laboratories: report from the clinical and laboratory standards institute methods development and standardization working group. Journal of clinical microbiology, *56*(8), 10-1128.

International Standards Organization, ISO 6579-1 (2017) (en) Microbiology of the Food Chain—Horizontal Method for the Detection, Enumeration and Serotyping of Salmonella Part 1: Detection of *Salmonellaspp,* International Standards Organization, Geneva, Switzerland.

International Organization for Standardization. (2006). Microbiology of food and animal feeding stuffs-horizontal method for detection and enumeration of Campylobacter spp. ISO. International Standards Organization, Geneva, Switzerland.

Iqbal, J., Hussain, M., & Khan, M. (2016). Antimicrobial resistance patterns of E. coli isolates from livestock in Pakistan*.* Pakistan Veterinary Journal, 36(4), 411–415.

Islam, M. A., Khan, M. M., & Rahman, M. (2014). Antibiotic resistance in Salmonella isolated from poultry: Current trends and concerns. Journal of Veterinary Medicine, 1–7.

Jahejo, A. R., Rajput, N., Rajput, N. M., Leghari, I. H., Kaleri, R. R., Mangi, R. A., & Pirzado, M. Z. (2016). Effects of heat stress on the performance of Hubbard broiler chicken. Cells, Animal and Therapeutics, *2*(1), 1-5.

Jayathilakan, K., K., Sultana, k. Radhakrishna, and A.S., Bawa. (2012). Utilization of byproducts and waste materials from meat, poultry and fish processing industries: a review. Journal of food Science and Technology,49(3), 278-293

Kaleri, R. R., Kaleri, H. A., Mangi, R. A., Solangi, G. M., Khoso, Z. A., Channa, A. A., & Solangi, I. H. (2023). Phenotypic diversity of aseel chicken in district Tando Allahyar, Sindh, Pakistan. Pure and Applied Biology (PAB), *12*(4), 1541-1547.

Kaleri, R. R., Khoso, Z. A., Mangi, R. A., Solangi, G. M., Ahmed, I., Memon, M. A., & Kumar, L. (2023). Economic Importance of Backyard Poultry in Taluka Jhando Mari, District Tando Allahyar, Sindh: Importance of Backyard Poultry in Taluka Jhando Mari. *MARKHOR (The Journal of Zoology)*, *4*(02), 74–79.

Sellah, M., & Drissi, M. (2015). Resistance to 3rd generation cephalosporin of Escherichia coli isolated from the feces of healthy broilers chickens in Algeria. Journal of Veterinary Medicine and Animal Health, *7*(8), 290-295.

Ullah, M., Chand, N., Kabir, A., Rasheed, M., Kaleri, H. A., Kakar, S., & Kaleri, R. (2022). Evaluation of the comparative effect of irradiated and controlled feed on the body growth of broiler chicken. *Pakistan Journal of Agriculture Research*, 35(2), pp. 434–441.

Ullah, M., Marri, G. M., Jogi, Q., Rasheed, M., Rizawana, H. R., Kaleri, R. R., ... & Kabir, A. (2019). 23. Dietary effect of autolysed yeast on broilers (Levabon®, Biomin Austria) on broiler growth. Pure and Applied Biology (PAB), *8*(2), 1223-1227.

World Health Organization (WHO). (2017). Antimicrobial resistance*:* Global report on surveillance*.* Geneva: WHO. https://www.who.int/publications/i/item/9789241564748