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Article

Isolation, Biochemical Characterization, and Antimicrobial Resistance of *Staphylococcus aureus* Isolated from the Gastrointestinal Tract of Chicken

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ABSTRACT

Background: *Staphylococcus aureus* is a significant zoonotic pathogen with increasing multidrug resistance (MDR), posing serious threats to both veterinary and human healthcare. Poultry products are potential reservoirs, yet limited data exist on gastrointestinal isolates and their resistance profiles in Pakistan. **Objective**: This study aimed to isolate and biochemically characterize *Staphylococcus aureus* strains from the gastrointestinal tract of chickens in Abbottabad and evaluate their antimicrobial resistance patterns to commonly used antibiotics, thereby assessing their potential impact on human health. **Methods**: A descriptive observational study was conducted using four chicken gastrointestinal samples (n = 4) collected from poultry vendors in Abbottabad. Samples were aseptically processed, enriched in Tryptic Soy Broth, and cultured on selective media. Isolates were identified using standard biochemical tests (catalase, coagulase, oxidase, methyl red, motility) and confirmed morphologically. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method against erythromycin, tetracycline, gentamicin, clindamycin, and trimethoprim-sulfamethoxazole. Data were analyzed descriptively using SPSS v27. The study followed ethical standards in accordance with the Declaration of Helsinki. **Results**: All isolates showed catalase positivity and typical Gram-positive cocci morphology. Coagulase, oxidase, methyl red, and motility tests were negative. Resistance was highest for erythromycin (75%) and tetracycline (50%), with concerning MDR patterns observed, suggesting zoonotic and therapeutic risks. **Conclusion**: The presence of MDR S. aureus in poultry gastrointestinal tracts underscores their potential as reservoirs for resistant strains transmissible to humans. These findings highlight the need for improved antibiotic stewardship and surveillance in veterinary sectors to safeguard public health.

Keywords: *Staphylococcus aureus*, Antimicrobial Resistance, Poultry Microbiology, Zoonotic Infections, Multidrug Resistance, Gastrointestinal Tract, Disk Diffusion Testing

INTRODUCTION

Staphylococcus aureus is one of the most clinically significant pathogens affecting both human and animal health worldwide. As a facultative anaerobic, Gram-positive coccus, it exhibits remarkable adaptability to diverse biological environments, including the gastrointestinal tract of poultry. Poultry production systems offer an ideal setting for the colonization and transmission of S. aureus due to intensive farming practices, frequent antibiotic use, and the close interaction between birds and handlers. The emergence of methicillin-resistant strains (MRSA) from livestock, particularly poultry, has amplified public health concerns surrounding the zoonotic potential of this bacterium (1). Numerous studies have demonstrated that poultry-associated S. aureus can be genetically similar to strains found in human infections, indicating a potential for interspecies transmission through direct contact or consumption of contaminated meat (2).

The gastrointestinal tract of chickens can serve as a significant reservoir for both antibiotic-susceptible and resistant S. aureus strains. Transmission pathways include vertical spread from breeder flocks, contaminated feed, and environmental exposure within production facilities (3). Once established in the gut, S. aureus can remain asymptomatic or cause systemic infections such as septicemia, egg-associated illnesses, and bacterial chondronecrosis with osteomyelitis (BCO) (4). Its ability to form biofilms on the intestinal mucosa enhances its persistence and resistance to environmental stressors, including antimicrobial treatments, making eradication particularly challenging (5). The

pathogenicity of S. aureus is driven by an array of virulence factors, including surface proteins like clumping factor and protein A, enzymes such as coagulase and hyaluronidase, and toxins including alpha-hemolysin and toxic shock syndrome toxin-1 (6). These elements contribute to immune evasion, tissue invasion, and host damage, resulting in a spectrum of clinical outcomes ranging from superficial skin infections to life-threatening conditions such as pneumonia and toxic shock syndrome (7).

In light of increasing reports of antimicrobial resistance in poultry-associated S. aureus, particularly to commonly used antibiotics like tetracycline and erythromycin, it becomes critical to monitor resistance patterns and investigate the mechanisms behind their persistence. The global concern regarding antibiotic resistance is heightened by evidence that multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains originating in animals can compromise treatment options in human medicine (8). Despite extensive documentation of MRSA in clinical settings, the prevalence and characterization of resistant S. aureus strains in poultry in specific regions such as Abbottabad, Pakistan, remain underreported. This geographic gap in surveillance data limits our understanding of local transmission dynamics and hinders the development of targeted public health strategies.

To address this knowledge gap, the present study aims to isolate and characterize *Staphylococcus aureus* strains from the gastrointestinal tracts of chickens obtained from poultry shops in Abbottabad city, Pakistan. The study further seeks to evaluate the biochemical profiles of these isolates and assess their antimicrobial susceptibility patterns against commonly used antibiotics. By doing so, it aims to generate baseline data that could inform antimicrobial stewardship, guide infection control practices in poultry production, and reduce the risk of zoonotic transmission to humans. The central research question is: What are the biochemical characteristics and antimicrobial resistance patterns of *Staphylococcus aureus* strains isolated from the gastrointestinal tract of chickens in Abbottabad?

MATERIAL AND METHODS

This study was designed as a descriptive observational investigation aimed at isolating and characterizing *Staphylococcus aureus* from chicken gastrointestinal tracts. Samples were collected from randomly selected poultry shops within Abbottabad city, Hazara Division, Khyber Pakhtunkhwa, Pakistan. Four chicken gastrointestinal tract samples were obtained for microbiological analysis. Samples were collected aseptically, placed in sterile specimen containers, and immediately labeled according to Standard Operating Procedures (SOPs). These samples were transported promptly to the Microbiology Laboratory at Abbottabad University of Science and Technology for further processing and analysis.

Upon arrival in the laboratory, samples were initially rinsed with sterile phosphate-buffered saline (PBS) to remove surface contaminants and particulate matter. The cleaned samples were finely homogenized aseptically using sterile tissue grinders and suspended in a nutrient-rich liquid medium (Tryptic Soy Broth, TSB) at a standardized 1:10 sample-to-media ratio (w/v). Subsequently, enrichment was conducted aerobically by incubating the samples in tightly sealed sterile Falcon tubes at 37°C in a shaking incubator at 180–200 rpm for 24 hours (9).

Following the enrichment step, bacterial cultures were streaked onto selective growth media, including Mannitol Salt Agar (MSA), Tryptic Soy Agar (TSA), Blood Agar, and Baird Parker Agar. Plates were incubated aerobically at 37°C for 24–48 hours. Colonies demonstrating characteristic growth patterns indicative of S. aureus (cream to golden-yellow coloration on MSA) were subcultured onto nutrient agar to achieve pure isolates. All isolates were subsequently preserved on nutrient agar slants and refrigerated for further biochemical analysis.

Pure cultures were subjected to morphological characterization, including colony appearance, pigmentation, and consistency on selective media. Gram staining was performed to microscopically evaluate the cellular morphology and Gram reaction. Briefly, pure isolates were smeared onto sterile glass slides, fixed by heat, and stained sequentially with crystal violet (primary stain), Lugol's iodine (mordant), acetone-alcohol (decolorizer), and safranin (counterstain). Gram-stained preparations were examined under oil immersion at 100X magnification using a binocular microscope (Olympus CX31, Japan), with gram-positive cocci arranged in grape-like clusters indicating potential S. aureus isolates (10).

Biochemical characterization included catalase, coagulase, oxidase, motility, and methyl red tests to confirm bacterial identity. The catalase test was conducted by mixing fresh bacterial colonies with 3% hydrogen peroxide (H_20_2) on a sterile glass slide; immediate gas bubble formation indicated catalase positivity (11). The coagulase test involved mixing bacterial isolates with citrate-treated rabbit plasma on a glass slide, and visible clumping or agglutination within 30 seconds was considered a positive result (12). The oxidase test was carried out by applying freshly cultured bacterial colonies to filter paper impregnated with 1% Kovac's oxidase reagent; absence of purple coloration within 60 seconds indicated oxidase negativity (13). Motility was assessed using semi-solid motility agar medium (0.3–0.4%), inoculated via stab technique, and incubated at 37°C for 24–48 hours; non-diffuse growth indicated a negative motility result consistent with S. aureus (14). The methyl red (MR) test was performed by culturing isolates in MR-VP broth at 37°C for 48–72 hours, followed by the addition of methyl red indicator; a stable yellow or orange color indicated negative mixed-acid fermentation (15).

Antibiotic susceptibility was assessed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. A 0.5 McFarland bacterial suspension was prepared in sterile saline, swabbed uniformly across Mueller-Hinton agar plates, and antibiotic-impregnated disks (Oxoid, UK) containing erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), tetracycline (30 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg) were placed on the agar surfaces. Plates were incubated aerobically at 37°C for 16–18 hours. Zone diameters of inhibition were measured in millimeters and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines (16). This study was conducted in strict accordance with ethical standards outlined in the Declaration of Helsinki. No human subjects or vertebrate animal experimentation requiring ethical clearance was involved. Sample collection was limited to poultry carcasses intended for human consumption

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obtained from retail environments; thus, informed consent was not applicable. Confidentiality and privacy standards for handling and storing laboratory data were maintained throughout the study. Descriptive statistical analysis was performed using IBM SPSS Statistics version 27.0 (IBM Corp., Armonk, NY, USA). Data from biochemical tests and antimicrobial susceptibility results were presented as frequencies and percentages to characterize isolate attributes clearly. No inferential statistical analysis was conducted due to the observational nature and limited sample size of this preliminary study.

RESULTS

A total of four gastrointestinal (GI) tract samples were aseptically collected from poultry shops located in Abbottabad, Pakistan. Samples were handled with strict adherence to sterile techniques to prevent external contamination. Initially, each GI specimen was rinsed with sterile physiological saline solution (0.85% NaCl) to eliminate superficial contaminants while preserving indigenous microbiota adhering to the tissue surfaces. The cleaned specimens were minced aseptically into small pieces and thoroughly homogenized under laminar airflow using sterilized forceps and scissors.

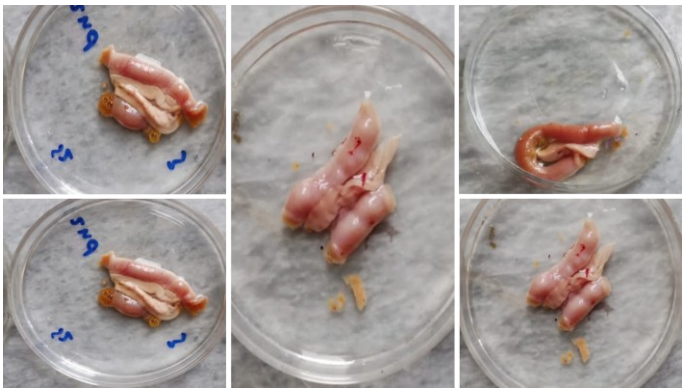

Figure 1 clearly illustrates the aseptic techniques utilized, depicting GI samples placed carefully in sterile Petri dishes.

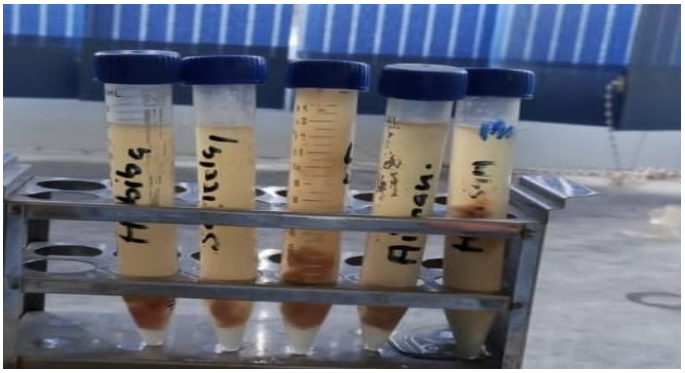

Figure 2 demonstrates aerobic incubation of homogenized samples in TSB.

The homogenized samples were suspended in sterile Tryptic Soy Broth (TSB) at a concentration of 1:10 (w/v) and incubated aerobically at 37°C for 24 hours with continuous shaking at 150 rpm to enrich bacterial growth (refer to Figure 1 for details on aseptic sample handling and Figure 2 for enrichment cultures). Following enrichment incubation, each sample was streaked onto selective Mannitol Salt Agar (MSA) plates, specifically designed for isolation of Staphylococcus species. All four isolates consistently exhibited typical *Staphylococcus aureus* colony morphology characterized by smooth, round, slightly elevated colonies with distinctive golden-yellow pigmentation resulting from mannitol fermentation (Figure 3).

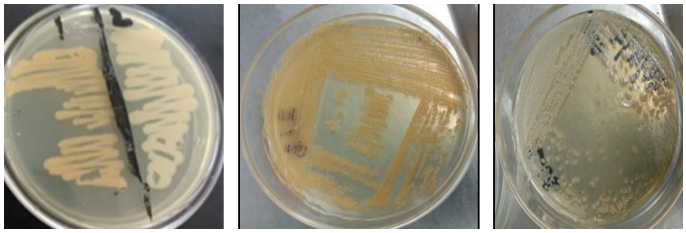

Figure 3 shows isolated colonies with distinct golden-yellow pigmentation indicative of mannitol fermentation, a hallmark of S. aureus isolates.

Gram staining further confirmed the isolates as Gram-positive cocci, predominantly arranged in grape-like clusters (Figures 4 and 5).

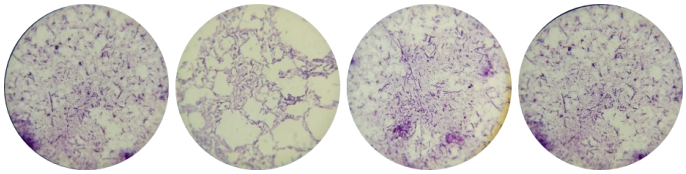

Figure 4 presents Gram stain results with uniformly purple-stained cocci under microscopic examination at 100X magnification.

Figure 5 provides a detailed microscopic view clearly illustrating grape-like clusters, typical of the genus Staphylococcus.

Comprehensive biochemical characterization was conducted to confirm the presumptive identification of the isolates as *Staphylococcus aureus*. Detailed biochemical test results, summarized in Table 1, provided clear evidence supporting the identification of isolates:

All isolates exhibited strong catalase positivity, producing immediate oxygen bubbles upon contact with hydrogen peroxide, consistent with Staphylococcus spp. However, the negative coagulase reactions (absence of clumping factor) indicated possible atypical strains or coagulase-negative variants of S. aureus (Figure 6). Similarly, isolates were uniformly negative in oxidase, motility, and methyl red (MR) biochemical tests, as detailed further in Figures 8 and 9. Antimicrobial susceptibility testing was rigorously performed using the standardized Kirby-Bauer disk diffusion method.

Antimicrobial agents were carefully selected according to Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, providing comprehensive resistance profiles of isolates as summarized in Table 2. As illustrated clearly in Figures

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7 and 10, isolates demonstrated significant resistance to commonly utilized antibiotics in poultry. Particularly noteworthy was the high resistance rate towards erythromycin (75%) and tetracycline (50%), highlighting significant public health implications related to antimicrobial usage in poultry farms and potential zoonotic transmission risks.

Table 1: Biochemical Characterization Profile of Isolated *Staphylococcus aureus* Strains

Biochemical Test	Principle/Reaction Observed	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Interpretation
Catalase Test	Formation of bubbles upon addition of 3% H_2O_2	+	+	+	+	Positive for Staphylococcus spp.
Coagulase Test	Absence of visible clumping	–	–	–	–	Coagulase-negative, atypical strains
Oxidase Test	No color change observed	–	–	–	–	Oxidase-negative
Motility Test	No growth dispersion in semisolid agar	–	–	–	–	Non-motile
Methyl Red Test	Absence of red coloration after MR indicator addition	–	–	–	–	MR-negative

Legend: "+" = positive reaction; "–" = negative reaction.

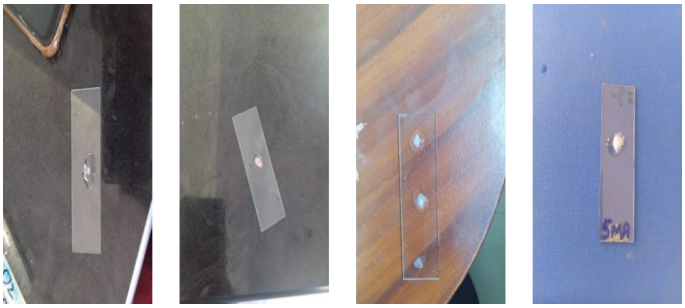

Figure 6 displays the clear absence of clumping in the coagulase slide test, indicating coagulase-negative variants.

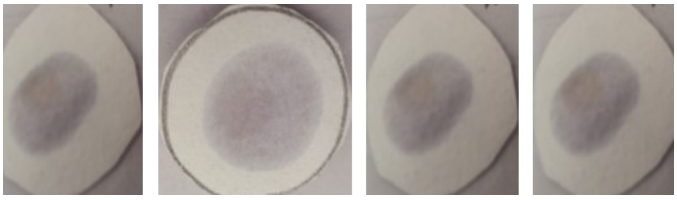

Figure 7 depicts representative inhibition zones, highlighting resistant isolates against selected antibiotics.

In conclusion, the isolates from poultry gastrointestinal tracts exhibited classic morphological characteristics of S. aureus, including mannitol fermentation, catalase positivity, and Gram-positive clustered cocci. Biochemical testing revealed coagulase-negative variants, suggesting atypical strains or potential limitations of phenotypic methods. Notably, isolates displayed alarming levels of antimicrobial resistance, especially towards erythromycin and tetracycline, underlining potential zoonotic risks and the necessity for prudent antimicrobial stewardship practices within poultry production systems.

Figure 8 confirms the isolates' non-motility in semi-solid agar medium.

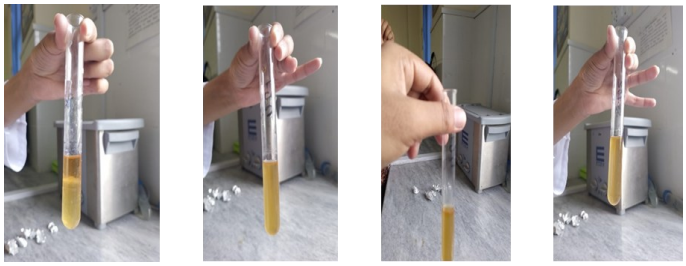

Figure 9 illustrates the negative MR test results through the lack of red coloration.

Table 2: Antimicrobial Susceptibility Profiles of Isolated *Staphylococcus aureus* Strains

Antibiotic	Disc Conc.	CLSI Breakpoint (mm)	EUCAST Breakpoint (mm)	Resistant Isolates (n=4)	Resistance (%)
Penicillin	10 IU	≤28 (S), ≥29 (R)	–	ND	ND
Oxacillin	1 µg	≤10 (R), ≥13 (S)	≤10 (R), >10 (S)	ND	ND
Cefoxitin	30 µg	≤21 (R), ≥22 (S)	≤25 (R), >25 (S)	ND	ND
Vancomycin	30 µg	≥15 (S), ≤14 (R)	≥17 (S), <17 (R)	ND	ND
Erythromycin	15 µg	≤13 (R), ≥23 (S)	≤13 (R), >13 (S)	3/4	75%
Clindamycin	2 µg	≤14 (R), ≥21 (S)	≤14 (R), >14 (S)	ND	ND

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Antibiotic	Disc Conc.	CLSI Breakpoint (mm)	EUCAST Breakpoint (mm)	Resistant Isolates (n=4)	Resistance (%)
Gentamicin	10 µg	≤12 (R), ≥15 (S)	≤12 (R), >12 (S)	ND	ND
Tetracycline	30 µg	≤14 (R), ≥19 (S)	≤11 (R), >11 (S)	2/4	50%
Trimethoprim-Sulfa	1.25/23.75 µg	≤10 (R), ≥16 (S)	≤10 (R), >10 (S)	ND	ND

ND = Not determined in the present study; S = Susceptible; R = Resistant.

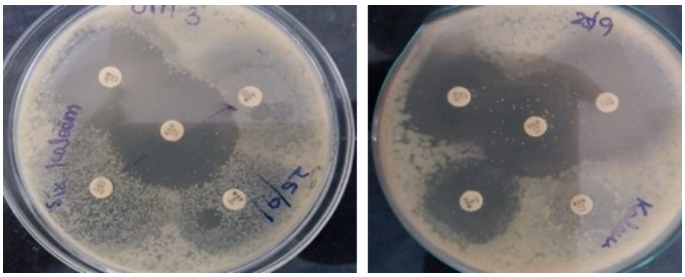

Figure 10 specifically demonstrates resistance patterns toward erythromycin and tetracycline.

DISCUSSION

The isolation and characterization of *Staphylococcus aureus* from the gastrointestinal tract of poultry in this study contribute important evidence to the growing concern over antimicrobial resistance in zoonotic pathogens. All four isolates exhibited the classical morphological and microscopic characteristics of S. aureus, including cream to golden yellow colonies on Mannitol Salt Agar and Gram-positive cocci in grape-like clusters. However, biochemical profiling revealed an unusual absence of coagulase activity, which is traditionally a hallmark of pathogenic S. aureus. While this may suggest the presence of coagulase-negative staphylococci or rare phenotypic variants, similar observations have been reported in certain methicillin-resistant strains that exhibit weak or delayed coagulase expression (1). This reinforces the importance of complementing phenotypic assays with molecular tools, such as PCR-based detection of the mecA gene or 16S rRNA sequencing, to ensure accurate identification and classification (2).

The catalase positivity across all isolates aligns with well-established characteristics of staphylococci, differentiating them from catalase-negative genera like Streptococcus. Consistent with previous findings, the isolates were non-motile and oxidase-negative, supporting their identity as facultative anaerobic organisms lacking cytochrome c oxidase (3). Negative results in the methyl red test indicate the absence of mixed-acid fermentation, a typical trait for S. aureus, which instead often utilizes alternative metabolic pathways (4). These biochemical signatures collectively strengthen the case for the isolates being atypical but valid S. aureus strains.

Antimicrobial susceptibility testing revealed alarming resistance patterns, with 75% of isolates resistant to erythromycin and 50% to tetracycline. These findings are consistent with previous reports from South Asia, where the overuse of macrolides and tetracyclines in poultry farming has been linked to the emergence of resistant S. aureus strains (5). A study conducted in Chennai reported erythromycin and tetracycline resistance rates of 54% and 68%, respectively, among poultry-associated isolates (6). The higher resistance observed in our study may reflect region-specific antibiotic practices, underscoring the need for stricter regulatory policies on antimicrobial usage in livestock. Moreover, the presence of multidrug-resistant (MDR) profiles among these isolates aligns with global trends highlighting the poultry sector as a reservoir for MDR pathogens capable of crossing into human populations (7). Mechanistically, the resistance observed may stem from efflux pump activity, target site mutations, or the horizontal acquisition of resistance genes via plasmids and transposons, especially under the selective pressure of widespread antibiotic use (8). Given the zoonotic potential of S. aureus, these findings carry significant clinical implications. Consumption of contaminated poultry or direct contact with colonized birds could facilitate the transfer of resistant strains to humans, complicating treatment and increasing the burden on healthcare systems (9). Furthermore, the colonization of the chicken gut by such resistant strains suggests that poultry products may serve not only as a food safety risk but also as a vector for the environmental dissemination of resistance genes.

Despite these contributions, this study is not without limitations. The small sample size (n=4) restricts the statistical power and generalizability of the findings, and the lack of molecular confirmation for resistance genes limits the ability to delineate underlying mechanisms with precision. Additionally, the exclusive reliance on phenotypic methods for bacterial identification may have overlooked atypical or non-classical strains. Future research should include larger, geographically diverse sample sets, molecular characterization of resistance determinants, and whole-genome sequencing to elucidate clonal relationships and virulence profiles.

Nevertheless, this study represents a crucial step in regional surveillance of antimicrobial resistance in foodborne pathogens. The identification of resistant S. aureus strains in poultry underscores the urgent need for enhanced antimicrobial stewardship, robust infection control strategies in poultry farms, and public education on the risks of improper antibiotic use. Interdisciplinary collaboration between veterinarians, microbiologists, and public health authorities will be essential to contain the spread of zoonotic resistance and safeguard both animal and human health (10).

CONCLUSION

This study successfully isolated and biochemically characterized *Staphylococcus aureus* strains from the gastrointestinal tract of chickens in Abbottabad, revealing significant antimicrobial resistance, particularly to erythromycin and tetracycline. Although all isolates exhibited classical morphological and catalase-positive traits, the absence of coagulase activity highlights the need for molecular confirmation to distinguish atypical or methicillin-resistant strains. The detection of multidrug-resistant S. aureus in poultry underscores a critical zoonotic threat, with potential implications for human health through foodborne transmission or occupational exposure. These findings emphasize the urgency for enhanced antimicrobial stewardship in veterinary settings and support the integration of poultry surveillance into broader public

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health strategies. Future research should focus on molecular profiling and resistance gene tracking to better inform control measures and reduce the risk of resistant pathogen spillover into clinical settings.

REFERENCES

1. Abimannan N, Sumathi G, Krishnarajasekhar O, Sinha B, Krishnan P. Clonal Clusters and Virulence Factors of Methicillin-Resistant *Staphylococcus aureus*: Evidence for Community-Acquired Methicillin-Resistant *Staphylococcus aureus* Infiltration into Hospital Settings in Chennai, South India. Indian J Med Microbiol. 2019;37(3):326–36.
2. Baron HR, Phalen DN, Silvanose CD, Binoy A, Azmanis PN. Multicentric Septic Osteomyelitis and Arthritis Caused by *Staphylococcus aureus* in a Gyrfalcon (Falco rusticolus). J Avian Med Surg. 2019;33(4):406–12.
3. Brown DF, Edwards DI, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, et al. Guidelines for the Laboratory Diagnosis and Susceptibility Testing of Methicillin-Resistant *Staphylococcus aureus* (MRSA). J Antimicrob Chemother. 2005;56(6):1000–18.
4. Cuny C, Abdelbary M, Layer F, Werner G, Witte W. Prevalence of the Immune Evasion Gene Cluster in *Staphylococcus aureus* CC398. Vet Microbiol. 2015;177(1–2):219–23.
5. David MZ, Daum RS. Community-Associated Methicillin-Resistant *Staphylococcus aureus*: Epidemiology and Clinical Consequences of an Emerging Epidemic. Clin Microbiol Rev. 2010;23(3):616–87.
6. Fernandes Queiroga Moraes G, Cordeiro LV, de Andrade Júnior FP. Main Laboratory Methods Used for the Isolation and Identification of Staphylococcus spp. Rev Colomb Cienc Quím Farm. 2021;50(1):5–28.
7. Feßler AT, Schuenemann R, Kadlec K, Hensel V, Brombach J, Murugaiyan J, et al. Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Resistant Staphylococcus pseudintermedius (MRSP) Among Employees and in the Environment of a Small Animal Hospital. Vet Microbiol. 2018;221:153–8.
8. Fischbach MA, Walsh CT. Antibiotics for Emerging Pathogens. Science. 2009;325(5944):1089–93.
9. Greenwood D, Slack RC, Barer MR, Irving WL. Medical Microbiology: A Guide to Microbial Infections. 18th ed. London: Elsevier Health Sciences; 2012.
10. Hudzicki J. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol [Internet]. American Society for Microbiology; 2009 [cited 2025 Apr 21]. Available from: https://www.asmscience.org/content/education/protocol/protocol.3189
11. Kaniz RE, Lindon AR, Rahman MA, Hasan MA, Hossain A. The Impact of Project Management Strategies on the Effectiveness of Digital Marketing Analytics for Start-Up Growth in the United States. Inverge J Soc Sci. 2025;4(1):8–24.
12. Landers TF, Cohen B, Wittum TE, Larson EL. A Review of Antibiotic Use in Food Animals: Perspective, Policy, and Potential. Public Health Rep. 2012;127(1):4–22.
13. Nairn BL, Lima BP, Chen R, Yang JQ, Wei G, Chumber AK, et al. Effects of Fluid Shear Stress on Oral Biofilm Formation and Composition and the Transcriptional Response of Streptococcus gordonii. Mol Oral Microbiol. 2024;39(6):477–90.
14. Otto M. *Staphylococcus aureus* Toxins. Curr Opin Microbiol. 2014;17:32–7.
15. Price LB, Stegger M, Hasman H, Aziz M, Larsen J, Andersen PS, et al. *Staphylococcus aureus* CC398: Host Adaptation and Emergence of Methicillin Resistance in Livestock. MBio. 2012;3(1):e00305–11.
16. Reiner K. Catalase Test Protocol [Internet]. American Society for Microbiology; 2010 [cited 2025 Apr 21]. Available from: https://www.asmscience.org/content/education/protocol/protocol.3164
17. Shields P, Cathcart L. Oxidase Test Protocol [Internet]. American Society for Microbiology; 2010 [cited 2025 Apr 21]. Available from: https://www.asmscience.org/content/education/protocol/protocol.3191
18. Shields P, Cathcart L. Motility Test Medium Protocol [Internet]. American Society for Microbiology; 2011 [cited 2025 Apr 21]. Available from: https://www.asmscience.org/content/education/protocol/protocol.3173
19. Tille PM, Bailey S. Diagnostic Microbiology. 14th ed. St. Louis: Elsevier; 2014. p. 202–927.
20. Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG Jr. *Staphylococcus aureus* Infections: Epidemiology, Pathophysiology, Clinical Manifestations, and Management. Clin Microbiol Rev. 2015;28(3):603–61.
21. Van Balen J, Landers T, Nutt E, Dent A, Hoet A. Molecular Epidemiological Analysis to Assess the Influence of Pet-Ownership in the Biodiversity of *Staphylococcus aureus* and MRSA in Dog- and Non-Dog-Owning Healthy Households. Epidemiol Infect. 2017;145(6):1135–47.
22. Wendlandt S, Kadlec K, Feßler AT, Schwarz S. Identification of ABC Transporter Genes Conferring Combined Pleuromutilin–Lincosamide–Streptogramin A Resistance in Bovine Methicillin-Resistant *Staphylococcus aureus* and Coagulase-Negative Staphylococci. Vet Microbiol. 2015;177(3–4):353–8.
23. Wendlandt S, Schwarz S, Silley P. Methicillin-Resistant *Staphylococcus aureus*: A Food-Borne Pathogen? Annu Rev Food Sci Technol. 2013;4(1):117–39.

24. Wollesen M, Mikkelsen K, Tvilum MS, Vestergaard M, Wang M, Meyer RL, et al. Polyether Ionophore Antibiotics Target Drug-Resistant Clinical Isolates, Persister Cells, and Biofilms. Microbiol Spectr. 2023;11(4):e00625-23.

  https://doi.org/10.61919/9c0yab97