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Identification of Bacterial Pathogens Causing Arthritis in Broiler Chickens, and Assessment of their Antibiotic Resistance Patterns

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Abstract | Antibiotic resistance in poultry bacterial pathogens poses a significant threat to the poultry industry and public health and requires ongoing monitoring. This study investigated the frequency of isolation, virulence-related genes, and antibiotic resistance profile of bacterial pathogens associated with lameness, and joint swelling in broiler flocks in Damietta, Egypt. Therefore, a total of 172 aseptic swab samples were collected from joints of 27 broiler flocks aged 5 to 48 days, exhibiting clinical signs and mortality rates up to 8%. Microbiological analysis, identified *Staphylococcus aureus* as the most prevalent pathogen (30.2%), followed by *E. coli* (21.5%), with *Enterococcus spp*. and *Pseudomonas spp.* each accounting for 9.9%. The highest detection rates for *E. coli*, *Enterococcus spp.*, and *Pseudomonas spp*. occurred in flocks aged 11-20 days, while *S. aureus* was most common in flocks aged 21-40 days. Antibiotic susceptibility testing showed that over 78.1% (25/32) of *S. aureus* isolates exhibited high-level of resistance to penicillin, erythromycin, chloramphenicol, levofloxacin, and tetracycline; however, all isolates remained sensitive to linezolid, minocycline, rifampin, vancomycin, and daptomycin. A subset of 8 isolates demonstrated multidrug (MRSA) resistant to macrolides, clindamycin, and beta-lactams. *E. coli* isolates were generally resistant to ampicillin 95% (19/20), chloramphenicol 75% (15/20), and ciprofloxacin 50% (10/20), Molecular analysis showed that *S. aureus* contained virulence-related genes, with 2/4 isolates containing *fnbA* and 1/4 carrying *tsst*, while 3/3 of the *E. coli* carried *iss* and *tsh* in 2/3 of the tested isolates. Overall, the results underscore the prominence of *S. aureus* and *E. coli* in broiler joint bacterial infections and reveal the concerning prevalence of multidrug-resistant and virulent strains. These insights emphasize the urgent need for effective control strategies in poultry health management.

Keywords | Antibiotic resistance, Arthritis, Broiler, *E. coli*, *S. aureus*, Virulence genes

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INTRODUCTION

Broiler meat provides an accessible and high-quality source of protein essential for feeding the expanding global population. The short production cycle of broiler chickens reduces operating costs and ensures a reliable and stable supply of poultry meat, making it an important and accessible source of efficient and economical animal protein (Ayugustin *et al.*, 2022; Susanti, 2023). However, the rapid growth rate and heavy body weight reached over

a short time increase susceptibility to the development of bone abnormalities and lameness (Kierończyk *et al*., 2017). Poultry lameness has a complex array of causes including genetic, environmental, nutritional and infectious factors that together lead to reduced activity, decreased feed intake, delayed market readiness, reduced productivity, increased treatment costs and culling rates (Cook, 2000; Granquist *et al*., 2019). Among the etiological factors, bacterial infections affecting joints and skeletal tissues are predominant, with pathogens such as *Staphylococcus aureus (S. aureus), Escherichia coli* (*E. coli*), *Enterococcus faecalis*, and *Pseudomonas aeruginosa* frequently implicated (Wideman Jr, 2016; Wijesurendra *et al*., 2017).

S. aureus, a Gram-positive pathogen, is known for causing septic arthritis and osteomyelitis in poultry, facilitated by its numerous virulence factors, *fnb A*, and *clf A* are the most important bacterial adhesions, which thought to be an important step in the beginning of the infections (Arciola *et al*., 2005). In addition, *E. coli* frequently causes arthritis-osteomyelitis syndrome in broiler chickens that is a sequelae of septicemia, and may be associated with intestinal or respiratory diseases (Dale and Woodford, 2015). The most observed lesions are joint edema and inflammation, which are responsible for large financial losses in poultry production (Braga *et al*., 2016). Other opportunistic pathogens such as *Enterococcus* spp. and *Pseudomonas* spp. are increasingly recognized in poultry, with *Enterococcus* spp. associated with secondary skeletal infections, often displaying high-level aminoglycoside resistance, further challenging treatment protocols (Alzahrani et al., 2022).

Sustainable surveillance programs that simultaneously monitor the antimicrobial resistance profile of *S. aureus* and healthcare and veterinary settings are necessary to adequately improve control measures (Mahros *et al*., 2021; González-Machado *et al*., 2024). The indiscriminate use of antibiotics as a preventive measure, rather than solely as a treatment, has exacerbated the emergence of drug-resistant strains (Rahman and Hollis, 2023). Resistant bacteria survive and multiply, while sensitive bacteria die (Van Boeckel *et al*., 2015).

The rising incidence of multidrug-resistant strains poses a significant challenge to the poultry industry, complicates control efforts and amplifies public health concerns (Abdel-Tawab *et al*., 2016; Wallinga *et al*., 2022), emphasizing the necessity for molecular characterization studies that elucidate virulence profiles and resistance mechanisms. Such insights are crucial for developing targeted interventions, improving antimicrobial stewardship, and safeguarding both poultry health and public safety. Therefore, the aim of the current study is to investigate the prevalence of bacterial agents linked to lameness, confirmed by molecular characterization of virulence genes, and detect antimicrobial resistance strains.

MATERIALS AND METHODS

Animal ethics

The birds were handled with great care in this study to avoid or minimize suffering and pain. Birds experiencing severe or chronic pain that cannot be relieved will be euthanized. This protocol has been reviewed and accepted by the Scientific Research and Bioethics Committee of the Faculty of Veterinary Medicine, Suez University, Ismailia, Egypt, with approval number (2020092).

Sampling and clinical investigation

One hundred and seventy-two swabs were collected from joints of 27 commercial broiler chicken flocks (4-10 swabs/flock) in Damietta, Egypt, under aseptic conditions. The broiler flocks were (5-48) days old and exhibited a variety of clinical signs suggestive of lameness, as well as general symptoms of illness with mortality rate ranged from 0.1% to 8%. A thorough clinical and necropsy examination was performed on clinically diseased and recently dead birds.

Isolation and identification of *S. aureus* and other bacteria

The swabs were aseptically inoculated into 5-10 ml of buffered peptone water or nutrient broth (Oxoid, UK), then incubated aerobically at 37°C for 18-24 hours. A loopful from each broth was subculture on blood, MacConkey, and nutrient agar (Oxoid, UK) and incubated at 37°C for 24 hours. Pure colonies were transferred to selective media such as mannitol salts, Cled, or EMB agar. Bacterial identification involved Gram's staining, motility testing, colony observation, hemolytic activity on blood agar, and biochemical analysis as described by (Quinn *et al*., 2011).

Slide coagulase test (bound coagulase test)

A loop of staphylococcal culture was mixed into a drop of saline, then rabbit plasma was added and mixed thoroughly on a microscope slide. Gently shake the slide, clumping within one minute indicates positive for *S. aureus* (Koneman *et al*., 1997).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed on the isolated bacterial strains using an automated turbidity method with the ID and AST System MA120 (Render Biotech Co., China), following the manufacturer's instructions. The system quantitatively assessed bacterial growth in the presence of various antibiotics to determine susceptibility profiles. The following antimicrobial agents were involved azithromycin, clarithromycin, clindamycin, trimethoprim/sulfamethoxazole, penicillin, erythromycin, oxacillin, doxycycline, tetracycline, linezolid, minocycline,

rifampicin, vancomycin, daptomycin, ciprofloxacin, chloramphenicol, gentamicin, and levofloxacin. The classification of resistance or susceptibility was interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2017).

Molecular typing of virulence-determining genes

This study conducted molecular analysis on four *S. aureus* and three *E. coli* isolates to detect key virulence genes (*fnbA, tsst, iss,* and *tsh*), selected for their roles in disease development. Genomic DNA was extracted using a QIAamp DNA Mini Kit, following the manufacturer's instructions. PCR amplification was performed in 25 µl reactions containing specific primers (obtained from Metabion, Germany, listed in Table 1), PCR master mix, DNA template, and water, using an Applied Biosystems 2720 thermal cycler. The PCR products were analyzed via 1% agarose gel electrophoresis stained with ethidium bromide and visualized with a gel documentation system. Fragment sizes were compared against a 100-bp DNA ladder to confirm the presence of target genes.

RESULTS AND DISCUSSION

Clinical and post-mortem findings

The examined broiler chickens exhibited clinical signs indicative of systemic illness, including dullness, ruffled feathers, reduced activity, wing dropping, decreased feed intake, weight loss, and diarrhea. The most frequently observed clinical manifestations were lameness, loss of mobility, and joint swelling (Figure 1). Post-mortem examinations revealed persistent lesions such as arthritis and erosions of joint surfaces, along with other pathological changes including pneumonia, fibrinous pericarditis, perihepatitis, air sacculitis, splenomegaly, unabsorbed yolk sacs, distended gall bladders, nephritis, and enteritis (Figure 1).

Microbiological identification of pathogens

Microbiological analysis of 172 samples collected from various anatomical sites identified *S. aureus* as the most prevalent pathogen, with a detection rate of 30.2%. Other pathogenic bacteria included *E. coli* (21.5%), *Enterococcus spp.* (9.9%), and *Pseudomonas spp.* (9.9%) (Table 2). *S. aureus* isolates were confirmed based on characteristic morphological and biochemical features: colonies on Mannitol Salt Agar displayed yellow to golden coloration, with beta-hemolysis on blood agar; no growth was observed on MacConkey and EMB agars. Microscopic examination revealed Gram-positive cocci arranged in grape-like clusters and non-motile, consistent with typical *S. aureus* morphology. The highest prevalence of *E. coli* was observed in flocks aged 11–20 days, accounting for 57.1% of *E. coli* isolates. *Enterococcus spp*. and *Pseudomonas* spp. were most frequently detected in the same age group, with detection rates of 100% and 50%, respectively. *S. aureus* was predominantly isolated from flocks aged 21-40 days, with a detection rate of 77%.

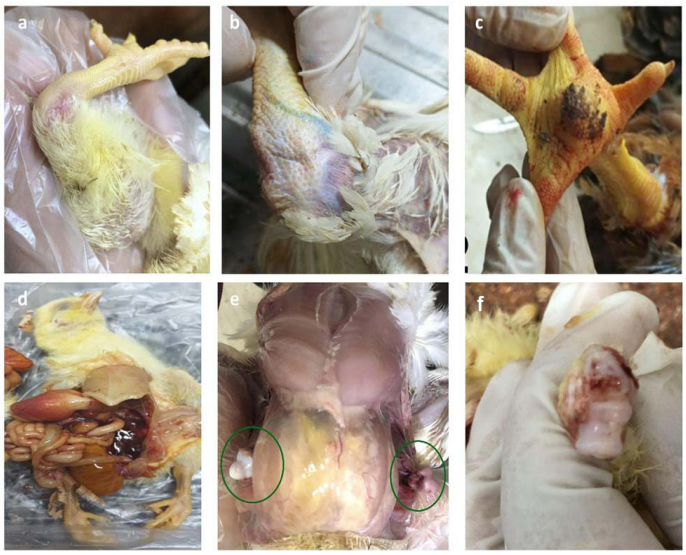

Figure 1: Clinical signs of swollen joints were seen in 14- and 26-day-old Cobb broilers (a and b), respectively, and severe footpad dermatitis was seen in 48-day-old Sasso broilers reared on wood shaving litter (c). Post-mortem lesions of unabsorbed yolk sac and dehydration in 5-day-old Cobb broiler (d), ascites and hip joint (hip-femoral joint) erosion in 35-day-old Cobb broiler (e) and erosions of the joint surfaces (f).

Table 1: Oligonucleotide primers sequences.

Reference	Amplified segment	Primer sequence (5'-3')	Gene	Bacteria
Delicato *et al.*, 2003	620 bp	GGT GGT GCA CTG GAG TGG	Tsh	*E. coli*
		AGT CCA GCG TGA TAG TGG		
Yaguchi *et al.*, 2007	266 bp	ATGTTATTTTCTGCCGCTCTG	Iss	
		CTATTGTGAGCAATATACCC		
Vancraeynest *et al.*, 2004	127 bp	CATAAATTGGGAGCAGCATCA	fnbA	*S. aureus*
		ATCAGCAGCTGAATTCCCATT		
Mehrotra *et al.*, 2000	326 bp	ACCCCTGTTCCCTTATCATC	tsst	
		TTTTCAGTATTTGTAACGCC		

Table 2: Frequency of *S. aureus* and other bacterial pathogens isolated from broiler chickens.

Bacterial species	Total number of examined samples	Positive flocks	
		No	%
S. aureus	172	52	(30.2%)
E. coli		37	(21.5%)
Enterococcus spp.		17	(9.9%)
Pseudomonas spp.		17	(9.9%)

Environmental sampling indicated that wood shavings litter served as a significant bacterial reservoir, supporting pathogen persistence and transmission. Co-infections of *S. aureus* and *E. coli* were found on two farms, with mortality rates of 5.3% and 5.8%, respectively, which were higher than those on other farms, as well as coinfections of *S. aureus* and *Pseudomonas* spp. was observed in one farm.

Antimicrobial susceptibility profiles

Antimicrobial susceptibility testing was conducted on 40 *S. aureus* and 20 *E. coli isolates* to assess their response to various antibiotics. *S. aureus* exhibited high resistance over 78.1% to penicillin, erythromycin, chloramphenicol, levofloxacin, and tetracycline. All *S. aureus* isolates remained sensitive to linezolid, minocycline, rifampin, vancomycin, and daptomycin. A subset of 8 isolates (20%) demonstrated MRSA resistance, resistant to macrolides (azithromycin, clarithromycin, erythromycin), clindamycin, and beta-lactams (penicillin, oxacillin) (Table 3). *E. coli* isolates were generally resistant to ampicillin 95% (19/20), chloramphenicol 75% (15/20), and ciprofloxacin 50% (10/20) (Table 4).

Virulence-determining genes

PCR detection of virulence genes in selected isolates demonstrated that *S. aureus* harbored *fnbA* in 50% (2/4) and *tsst* in 25% (1/4) of isolates (Figure 2). Among *E. coli* isolates (n=3), the *iss* gene was present in all isolates (100%), while *tsh* was detected in 66.6% (2/3) (Figure 3). The presence of these virulence factors suggests their potential role in disease severity and pathogen persistence.

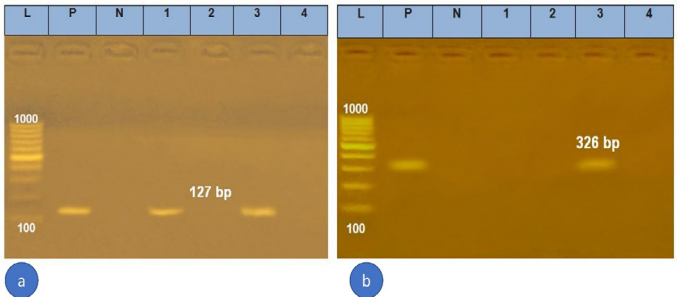

Figure 2: PCR gel pattern for four isolates of *S. aureus* virulence genes (*fnbA, tsst*). Lane L: 100bp DNA ladder, Lane 1:4: tested samples, Lane P: positive control, Lane N: negative control.

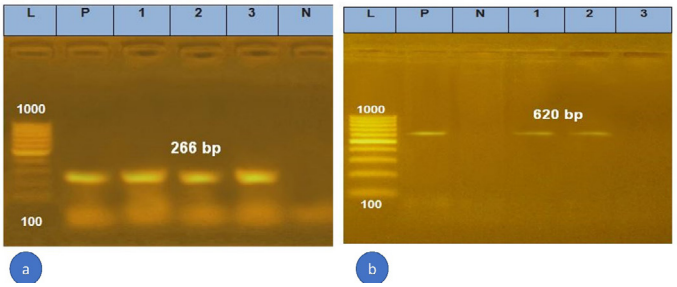

Figure 3: PCR gel pattern for three isolates of *E. coli* virulence genes (*iss, tsh*). Lane L: 100bp DNA ladder, Lane 1:3: tested samples, Lane P: positive control, Lane N: negative control.

Lameness remains a significant welfare concern and a major economic challenge within the global broiler industry, primarily due to its association with high culling rates and diminished performance metrics (Abd El-Naser *et al*., 1994; Olkowski *et al*., 2011; Szafraniec *et al*., 2022). There is limited local information about it in Egypt, especially in Damietta. This makes it difficult to determine the best ways to address the issue. Since Damietta is heavily involved in poultry farming, our study aims to learn more about this problem. The pathogenesis of lameness is often linked to the mechanical stress exerted on rapidly growing bones, such as the femur and tibia, which may predispose these structures to bacterial infections (Wideman Jr, 2016). Notably, there is an increasing trend in lameness cases attributable to infectious pathogens like *S. aureus*, which raises public health concerns due to its zoonotic potential (Marcon *et al*., 2019). The present study was designed to elucidate the prevalence of bacterial pathogens associated with lameness in broiler chickens, alongside their antimicrobial resistance profiles and the molecular characterization of key virulence determinants.

In this investigation, 172 samples were subjected to comprehensive microbiological analyses to determine the spectrum and prevalence of pathogenic bacteria. The results demonstrated that *S. aureus* was the most frequently isolated pathogen, accounting for 30.2% of the cases, underscoring its prominent role in bacterial lameness. *E. coli* emerged as the second most common isolate, representing 21.5% of the total, with other opportunistic pathogens such as *Enterococcus* spp. *and Pseudomonas* spp. each constituting 9.9%. These findings reinforce the notion that *S. aureus* is a primary contributor to bacterial lameness in broiler populations, with *E. coli* also playing a significant role-aligning with previous reports (Abd El-Naser *et al*., 1994).

The prevalence of *S. aureus* identified in this study was 30.2%, a figure that aligns with prior research recognizing *S. aureus* as a predominant pathogen implicated in arthritis and lameness among broilers (Abd El-Naser *et al*., 1994).

Table 3: Antibiotic susceptibility profile of 8 isolates *S. aureus* (MRSA).

Antibiotic	Sensitive			Intermediate			Resistant		
	MIC (µg/mL)	No.	%	MIC (µg/mL)	No.	%	MIC (µg/mL)	No.	%
Trimethoprim/ Sulfa	<=2/38	1/8	12.5%	-	0	0%	>4/76	7/8	87.5%
Azithromycin	<=2	0	0%	=4	0	0%	>=8	8/8	100%
Clarithromycin	<=2<	0	0%	=4	0	0%	>=8	8/8	100%
Clindamycin	<=0.5	0	0%	=1-2	0	0%	>4	8/8	100%
Penicillin	<=0.12	0	0%	-	0	0%	> 0.25	8/8	100%
Erythromycin	<=0.5	0	0%	1-4	0	0%	>=8	8/8	100%
Oxacillin	<=2	0/8	0%	-		0%	>4	8/8	100%
Doxycycline	<=4	1/8	12.5%	=8	2/8	25%	>=16	5/8	62.5%
Tetracycline	<=4	0	0%	=8	0	0%	>=16	8/8	100%
Linezolid	<=4	8/8	100%	-	0	0%	>=8	0	0%
Minocycline	=4	8/8	100%	=8	0	0%	>=8	0	0%
Rifampin	=0.5	8/8	100%	=2	0	0%	>=4	0	0%
Vancomycin	< 2	8/8	100%	4–8	0	0%	>=16	0	0%
Daptomycin	<=1	8/8	100%	-	0	0%	-	0	0%
Ciprofloxacin	<=1	1/8	12.5%	=2	0	0%	>=4	7/8	87.5%
Chloramphenicol	<=8	0	0%	=16	0	0%	>=32	8/8	100%
Gentamicin	<= 4	6/8	75%	=8	2/8	25%	>=16	0	0%
Levofloxacin	<=1	0	0%	=2	0	0%	>=4	8/8	100%

Interpretive criteria of sensitivity according to CLSI, 2017. MIC: minimum inhibitory concentration

Table 4: Antibiotic sensitivity test of 20 isolates of *E. coli*.

Antibiotic	Sensitive			Intermediate			Resistant		
	MIC (µg/mL)	No.	%	MIC (µg/mL)	No.	%	MIC (µg/mL)	No.	%
Trimethoprim/ Sulfa	<=2/38	15/20	75%	-	0	0%	>4/76	5/20	25%
Ciprofloxacin	<1	6/20	30%	=2	4/20	20%	>=4	10/20	50%
Chloramphenicol	<8	5/20	25%	=16	0	0%	>=32	15/20	75%
Gentamicin	<=4	12/20	60%	=8	0	0%	>=16	8/20	40%
Ampicillin	<=8	0	0%	=16	1/20	5%	>=32	19/20	95%
Cefazolin	<=16	10/20	50%	-	0	0%	>=32	10/20	50%
Tobramycin	<=4	15/20	75%	=8	0	0%	>=16	5/20	25%
Amoxicillin/CA	<=8/4	0	0%	=16/8	6/20	30%	>=32/16	14/20	70%
Ampicillin/ Sulbactam	<=8/4	2/20	10%	=16/8	9/20	45%	=32/16	9/20	45%
Cefepime	<=8	9/20	45%	=16	0	0%	>=32	11/20	55%
Cefoxitin	<=8	15/20	75%	=16/8	0	0%	>=32	5/20	25%
Meropenem	<=1	20/20	100%	=2	0	0%	>=4	0	0%
Piperacillin/ Tazobactam	=16/4	20/20	100%	32/4–64/4	0	0%	>=128/4	0	0%
Cefuroxime	<=8	10/20	50%	=16	0	0%	>=32	10/20	50%
Ertapenem	<=0.5	15/20	75%	=1	0	0%	>=2	5/20	25%
Cefotaxime	<=8	12/20	60%	16–32	4/20	20%	>64	4/20	20%
Amikacin	<=16	20/20	100%	=32	0	0%	>=64	0	0%
Ceftazidime	<=4	15/20	75%	=8	0	0%	>=16	5/20	25%
Aztreonam	<=0.25	16/20	80%	=2	1	5%	>=16	3/20	15%
Levofloxacin	<=2	3/20	15%	=2	15/20	75%	>=8	2/20	10%

Interpretive criteria of sensitivity according to CLSI, 2020. MIC: minimum inhibitory concentration.

Nonetheless, notable discrepancies exist when comparing our findings with other studies, which have reported *S. aureus* prevalence rates spanning from as low as 7.4% to as high as 62.8% (El-Jakee *et al*., 2008; Lebdah *et al*., 2015; Bakheet *et al*., 2018; Younis *et al*., 2021). These variations are likely influenced by numerous factors, including geographical location, differences in management and husbandry practices, sample sizes, and the diagnostic techniques employed in different investigations (Mamza *et al*., 2019; Younis *et al*., 2021). Recognizing and understanding these epidemiological disparities are critical steps toward designing effective, targeted intervention strategies aimed at controlling bacterial lameness and improving overall poultry health.

The current study detected *Enterococcus* spp. in 9.9% of the samples from arthritic broilers. While this incidence is comparatively lower than that of *S. aureus* and *E. coli,* its detection underscores the emerging significance of such potential pathogen associated with lameness, arthritis, and osteomyelitis in poultry. Historically, *enterococci* have been considered primarily as commensal organisms of the gastrointestinal tract, with limited pathogenic potential; however, recent evidence suggests their role in systemic infections warrants further attention (Devriese *et al*., 1995).

Furthermore, *Pseudomonas* spp. was isolated at a rate of 9.9%, emphasizing their role as opportunistic pathogens within poultry production systems (Walker *et al*., 2002; Joh *et al*., 2005). Although the occurrence was relatively low in our study, *Pseudomonas* spp. remain significant due to their association with conditions such as omphalitis, septicemia, arthritis, and elevated mortality rates, particularly in young chicks (Walker *et al*., 2002). Interestingly, the highest detection rates of *E. coli* (57.1%), *Enterococcus* spp. (100%), and *Pseudomonas* spp. (50%) were observed in broiler chickens aged 11 to 20 days, suggesting that younger birds might be more susceptible to these infections. This increased susceptibility could be attributed to the immaturity of their immune systems (Morishita, 2023), as well as potential hatchery-related infection sources (Abd El-Ghany, 2021). Conversely, *S. aureus* was most frequently isolated from broiler flocks aged between 21 and 40 days, with most cases occurring around 35 days, consistent with previous reports (McNamee and Smyth, 2000; Hamed and Youssef, 2013). The observed age-dependent increase in infection rates underscores the critical role of age-specific management strategies and biosecurity protocols in mitigating bacterial infections within broiler flocks. Broiler chickens at this age are demonstrably vulnerable to lameness and associated bacterial infections, including *S. aureus*, with prevalence increasing with both age and weight (Kierończyk *et al*., 2017). This heightened susceptibility likely stems from the increasing biomechanical stress placed on developing skeletal systems as the birds mature.

Litter quality clearly emerges as a significant risk factor for leg health and bacterial infections in broiler chickens. Wet or poor-quality litter, such as observed in some farms, appears to increase the incidence of hock burns and footpad dermatitis, providing opportunistic entry points for *S. aureus* (Shepherd and Fairchild, 2010; Boussaada *et al*., 2022). Interestingly, our findings show a correlation between wood shavings litter and higher bacterial isolation rates compared to straw litter, suggesting a direct influence of litter type and management on the environmental microbial load (Eichner *et al*., 2007; Fisher and Phillips, 2009) (Figure 2).

The observed co-infections, particularly of *S. aureus* and *E. coli* in 2 farms, *Pseudomonas* spp. and *S. aureus* in one farm, highlight the complex interplay of bacterial species and their potential synergistic effects on disease development. The prevalence of multiple bacterial pathogens at similar frequencies further emphasizes the need for comprehensive disease control strategies. These observations align with previous reports by (Sid *et al*., 2015).

Birds exhibited general signs of illness such as dullness, inactivity, and reduced feed intake, alongside specific signs of lameness, loss of mobility, and swollen joints. The postmortem observations of arthritis and erosions of joint surfaces, along with other pathological changes including pneumonia, fibrinous pericarditis, perhepatitis, air sacculitis, splenomegaly, unabsorbed yolk sacs, and enteritis align with previous research reports by (Awan and Matsumoto, 1998; Amen *et al*., 2019), which established a connection between staphylococcal infections and systemic issues like osteomyelitis, synovitis, and bumble-foot. Furthermore, the presence of colibacillosis likely contributed to a weakened bird immune system, increasing their vulnerability to concurrent staphylococcal infections. These findings suggest a multifactorial disease process impacting multiple organ systems, consistent with bacterial, viral, or parasitic infections common in poultry.

Molecular analysis of *E. coli* virulence genes revealed the consistent presence of the (*iss)* gene in 3/3, and the (*tsh*) gene in 2/3 of isolates. These findings corroborate those of (Oliveira *et al*., 2019), who also reported 100% *(iss)* gene detection. The high prevalence of (*iss*) suggests its crucial role in *E. coli* pathogenesis. The (*iss*) gene's ability to facilitate evasion of host defenses, multiplication, and spread likely contributes significantly to disease progression, as previously hypothesized (López *et al*., 2017). Staphylococcal infections are often associated with the presence of virulence genes. Fifty percent (2/4) of the tested isolates carried *fnbA* and 25% (1/4) of the tested isolates carried (*tsst)*. The presence of (*fnbA*) in our isolates is noteworthy, as this staphylococcal adhesion factor is crucial for the initial stages of infection (Arciola *et al*., 2005). However, The current results contrast with a prior

study (Momtaz *et al*., 2013), which reported the absence of (*tsst*) but high prevalence of other adhesion/coagulation genes. These differing findings highlight the potential variability in the genetic makeup of staphylococcal isolates and the complex interplay of virulence factors in infection development. Further investigation is needed to understand the specific factors contributing to these discrepancies. This variation may be related to geographical differences, sample sources and strain diversity. The combination of virulence-determining genes and antibiotic resistance pattern may lead to the emergence of unexpected new bacterial strains with high mortality and morbidity rates. In this study, eight MRSA strains exhibited a multidrug-resistant phenotype, being completely resistant to macrolides (azithromycin, clarithromycin, erythromycin), clindamycin, and β-lactams (penicillin, oxacillin). *E. coli* isolates were generally resistant to ampicillin 95% (19/20), chloramphenicol 75% (15/20), and ciprofloxacin 50% (10/20). The resistance patterns highlight the emergence and prevalence of multidrug-resistant strains, underscoring the importance of rational antibiotic use and continuous resistance monitoring. Despite antibiotic restrictions, tetracyclines, macrolides, lincosamides, and β-lactams are permitted in subtherapeutic doses to promote weight gain in animals (Diaz-Sanchez *et al*., 2015; Cox, 2016). The threats from wide spreading of MRSA strains have been previously reported (Lee, 2003; Borg *et al*., 2007; Hamed and Youssef, 2013). Detection of antibiotic resistant MRSA among the broilers at marketing ages provokes a risk of contamination of chicken meat and possibly MRSA and other virulent and antimicrobial resistant strains of *S. aureus* enter and spread through the food chain by marketing chicken (Faraj *et al*., 2025). This is alarming, considering the rate at which food pathogens have developed resistance against some of these antibiotics (Chinemerem-Nwobodo *et al*., 2022).

Staphylococcal isolate and the interaction of virulence factors, as evidenced by differing results compared to previous studies, underscore the need for further investigation into the underlying determinants of these discrepancies. Potential contributing factors include geographical location, sample origin, and strain diversity. The combined effects of virulence genes and antibiotic resistance profiles, particularly the observed multidrug resistance in eight MRSA isolates (including complete resistance to macrolides such as azithromycin), may drive the emergence of novel, highly pathogenic strains with significant clinical implications.

CONCLUSION AND RECOMMENDATIONS

S. aureus and *E. coli* are highly pathogenic bacteria implicated in poultry arthritis, could be due to their virulence genes (*fnbA* and *tsst* in *S. aureus* and *iss* and *tsh* in *E. coli*). The rise of antibiotic resistance, driven by misuse and overuse of antibiotics in broiler production, has rendered conventional antimicrobial treatments largely ineffective. Therefore, implementing strict management practices, especially biosecurity measures, is essential for controlling bacterial infections. Developing effective vaccines offers a promising approach to mitigate the economic losses associated with these infections and improve poultry health. Future studies should focus on elucidating the genetic variability of these pathogens and their virulence mechanisms to develop targeted interventions.

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NOVELTY STATEMENT

Bacteriological examination of swab samples collected from joints of broiler chickens aged 5 to 48 days from Damietta governorate, Egypt, exhibiting clinical signs of lameness and mortality rates up to 8% showed that *S. aureus* is the main bacterial pathogen (30.2%), followed by *E. coli* (21.5%). Over 78.1% (25/32) of *S. aureus* isolates exhibited high-level resistance to penicillin, erythromycin, chloramphenicol, levofloxacin, and tetracycline; however, all isolates remained sensitive to linezolid, minocycline, rifampin, vancomycin, and daptomycin. A subset of 8 isolates demonstrated multidrug (MRSA) resistant to macrolides, clindamycin, and beta-lactams. *E. coli* isolates were generally resistant to ampicillin 95% (19/20), chloramphenicol 75% (15/20), and ciprofloxacin 50% (10/20). Therefore, strict management measures should be implemented, and antibiotic use should be drastically reduced to prevent the further emergence of drug-resistant strains.

AUTHOR'S CONTRIBUTION

Each author contributed equally to the writing of this article, offering their technical expertise and insights.

Ethical consideration

Ethical issues (including plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publication and/or submission, and redundancy) have been checked by all the authors.

Generative AI and AI-assisted technology statement

Authors utilize artificial intelligence and AI assistant technologies, employing language tools and grammar checkers to improve the readability and language quality of the article, and uses endnotes to organize references.

Conflict of interest

The authors have declared no conflict of interest.

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