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RESEARCH ARTICLE

Antibiotic Profiles of Bacteria Associated with Chicken Omphalitis Isolated in Northern Palestine

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ABSTRACT

The most common reason of chick death is omphalitis, which can be economically significant and usually manifests in the first week following hatching. The current study aimed to isolate and identify the bacterial species associated with omphalitis from dead broilers to evaluate the occurrence of omphalitis and the antibiotic susceptibility toward these bacterial isolates using the standard Kirby-Bauer test. Results of this study showed that the overall prevalence of omphalitis was 52.6% and the frequency of isolation of Gram-negative bacteria was 58.15%. Also, results demonstrated that 117 distinct bacterial strains were identified in 60 dead birds that were positive for omphalitis and mixed infections. The frequency of isolation of bacterial species was *Staphylococcus aureus* (78.3%), *Escherichia coli* (76.7%), *Klebsiella* spp., (20.0%), *Pseudomonas* spp., (6.7%), *Shigella* spp., (5%); *Proteus* spp., (5%) and *Staphylococcus epidermidis* (3.3%). *S. aureus*, *E. coli* and *Klebsiella* spp. exhibited high resistance rate to several antimicrobial agents with high rate of multi-drug resistant. In addition, the average of multiple antibiotic resistance index was 0.75, 0.59 and 0.62 for *S. aureus*, *E. coli* and *Klebsiella* spp., respectively. In summary, omphalitis is quite prevalent, and so are the fatalities linked to it. The occurrence of multi drug resistant pathogens in chicks suffering from omphalitis is a problem as these pathogens can spread to humans and animals, complicating the treatment of bacterial infections. To control and prevent such infections, greater efforts must be made to create strict hygiene standards. This would result in a general reduction in the use of antimicrobials in chicken farms.

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INTRODUCTION

The yolk sac, which functions comparable to the placenta in mammals, serves a significant role in managing nutrient absorption, digestion, and delivery by acting as a conduit from the yolk to the developing embryo. In addition, it is the site where hematopoiesis and the production of serum proteins occur. It also serves as an anatomical barrier that assists in protection against microbial pathogens (Rezaee *et al*., 2021).

Infectious illnesses are one of the main causes of chicken mortality globally and are believed to be the primary economic obstacles to poultry production. Microorganisms such as protozoa, mycoplasma, fungi, parasites, bacteria and viruses are the cause of these diseases. One of the major causes of the early chick death in the initial days following hatching is omphalitis, the inflammation of the navel (EL-Sawah *et al*., 2016; Oliveira *et al*., 2024). The term, as it is widely used, describes an incorrect navel closure followed by a bacterial infection (Oliveira *et al*., 2024). Since it happens in the initial few days of life, it is unlikely to be spread from one bird to another. Newly born birds with unhealed navels might acquire it via contaminated hatchery equipment. It ranks among the primary causes of chick mortality in the first week globally and imposes a considerable, although mostly preventable, financial strain on poultry producers in Palestine. Omphalitis is the leading cause of chick mortality, often showing economic implications and typically occurring within the first week

after hatching (Yassin *et al*., 2009). Significant economic losses would occur due to mortality rates ranging from 1.7 to 8.6% (Awobajo *et al*., 2007). Bacteria linked to omphalitis have been associated in the degradation and disintegration of vital residual yolk sac nutrients that were intended to serve as an energy source post-hatch (Shahjada *et al*., 2017).

There are many studies carried out to isolate and identify the bacteria that associated with omphalitis and the resistant of these isolates against different classes of antimicrobial agents (Ahmed *et al.*, 2009; Nasrin *et al*., 2012; Amare *et al*., 2013; Jalob *et al*., 2015; Abdel-Tawab *et al*., 2016; Amer *et al*., 2017; Melese *et al*., 2017; Saad *et al*., 2017; Shahjada *et al*., 2017; Kaboudi *et al*., 2021; Stępień-Pyśniak *et al*., 2021; Mohibbullah *et al*., 2022; Hasan *et al*., 2024). Numerous bacteria, including *Escherichia coli* (*E. coli*), *Proteus* spp., *Salmonella* spp., *Enterobacter* spp., *Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., *Enterococcus* spp., *Streptococcus* spp., *Bacillus* spp., and *Clostridium* spp., which have been recovered from infections in the yolk sac of poultry and the most frequent cause of death for chicks in the first week following the hatching process (Shahjada *et al*., 2017; Kaboudi *et al*., 2021; Stępień-Pyśniak *et al*., 2021; Crespo and El-Gazzar, 2024). *E. coli* is considered the most prevalent cause of omphalitis in chicks (Ahmed *et al*., 2009). *Salmonella* infections in hatcheries are known to be mostly spread via egg shells (Cox *et al*., 1990). *Salmonella* in the viable fertile eggs may cause abnormal hatching and embryonic deaths.

The poultry sector in Palestine contributes significantly to the country's economic growth. There are around 6 commercial hatcheries in North Palestine. According to our knowledge, there is no information about morbidity, mortality rates due to omphalitis, and no information about the pathogens that cause omphalitis in broilers of 1 to 6 days old chicks in Palestine and their susceptibility to antimicrobial agents. This study research aimed to isolate and recognize the bacterial species associated with omphalitis from dead broilers chicks with an age 1 to 6 days in North Palestine and to evaluate the occurrence of omphalitis. In addition, to evaluate the antibiotic susceptibility toward these bacterial isolates using the standard Kirby-Bauer test.

MATERIALS AND METHODS

Ethical approval and Informed consent: According to the University's Ethics Committee, ethical permission was not necessary for this investigation. Nonetheless, during the sample collection procedure, the animals were dealt with carefully. A prior permission was given by the owner of the hatcheries.

Sample collection, necropsy examination, isolation and identification: Six commercial hatcheries and their associated broiler farms located in Northern Palestine were included in the study. The farms' capacities ranged from 8,000 to 20,000 birds per flock. The farms were selected based on their accessibility and willingness to participate in the study. No formal sample size calculation was performed. The study population consisted of 114 freshly dead broiler chicks with an age of 1 to 6 days, determined according to the hatching date of the chicks. The samples were submitted for postmortem examination with suspected omphalitis as well as for isolation and identification of the responsible pathogens. These samples were collected during the study period between July and November 2025 using a convenience sampling approach rather than random selection.

All chicks were autopsied in the avian clinic and its laboratory which belong to the hatcheries and poultry farms. The primary lesions in several organs were identified by a thorough postmortem investigation, with particular focus given to the yolk sac. Navel swabs were aseptically collected from yolk sac with gross lesions. Collection, isolation and identification of pathogens were carried out in farms' laboratory. After being collected, the navel swab samples were then incubated for 6-10 hours at 37ºC in sterile 5 mL of tryptone soy broth for enrichment. In order to isolate and investigate the cultural characteristics of the pathogenic bacteria, enriched cultures were streaked onto various culture media, including nutrient agar, blood agar, xylose lysine deoxycholate (XLD) agar, eosin methylene blue (EMB) agar, Salmonella-Shigella (SS) agar and mannitol salt agar (MS). Morphological characteristics of isolates were done by Gram staining, cultural characteristics of colonial morphology on different cultural media, detection of motility and biochemical characterization using several biochemical tests such as sugar fermentation test, catalase test, citrate utilization test, urease test, coagulase test, oxidase test, indole test, Methyl Red and Voges-Proskauer (MRVP) test.

Antimicrobial susceptibility testing: Antimicrobial susceptibility was assessed using the standard disk diffusion (Kirby-Bauer disk test) susceptibility test, which is recommended by the Clinical and Laboratory Standard Institute (CLSI, 2025). *E. coli*, *Klebsiella* spp. and *S. aureus* were subjected to 22 antimicrobial agents (Oxoid, England) to determine their antibiotic resistance. These antimicrobial agents belonged to 11 different classes: aminopenicillin (amoxicillin (AX, 30µg), Penicillin (P, 10U), ampicillin (AMP, 10µg)), cephalosporines (ceftriaxone (CRO, 30µg), cephalexin (CL, 30µg), cefoxitin (CZ, 30µg), ceftiofur (FUR, 30µg)), tetracyclines (doxycycline (DO, 30µg)), polymyxins (polymyxin B (PB, 300U), polymyxins E (colistin sulfate (CT, 10µg)), fluoroquinolones (ciprofloxacin (CIP, 5µg), norfloxacin (NOR, 10µg), levofloxacin (LEV, 5µg), enrofloxacin (ENR, 5µg)), aminoglycosides (gentamicin (CN, 10µg), neomycin sulfate (N, 30µg), kanamycin (K, 30µg)), sulfonamides (sulphamethoxazole/trimethoprim (STX, 25µg)), amphenicols (florfenicol (FFC, 30µg)), macrolides (erythromycin (E, 15µg)), aminocyclitol (spectinomycin (SH, 100µg)) and phosphonic antibiotic (fosfomycin (FO, 200µg). After being cultivated for 6 to 8 hours, the bacterial isolates were swabbed onto Mueller-Hinton agar (HMA) plates, antibiotic disks were applied on the swabbed plates. After that, the plates were incubated for 24 hours at 37°C. The CLSI protocol (CLSI, 2025) was followed in order to determine the inhibitory zones, if any. Three categories of isolates were identified: sensitive, intermediate, and resistant. In every assessment of the antimicrobial susceptibility tests, a reference bacterial strains included *Escherichia coli* (ATCC

25922), *Staphylococcus aureus* (ATCC 6538P) and *Klebsiella pneumoniae* (ATCC 13883) were utilized as a quality control strains for antimicrobial susceptibility test.

Multiple antibiotic resistance index evaluation: The antibiotic resistance profiles of the pathogenic bacterial isolates obtained from omphalitis were assessed by computing their multiple antibiotic resistance index **(**MARI). The MARI for the bacterial isolate was calculated based on the following equation, MARI = D/E, in which D is the number of antimicrobial resistances found in the tested pathogenic isolates and E is the overall number of antibiotics that the isolate was examined for. Low-risk and high-risk contaminations were separated using a MARI of 0.20. The formula D/(E*F), where D is the total antibacterial resistance score of all isolates in the sample, E is the number of antibiotic agents analyzed for each isolate, and F is a number of isolates in the sample, is used to calculate the index for a sample containing multiple isolates (Jalil *et al*., 2023).

Statistical analyses: Since the data did not satisfy normality assumptions, the non-parametric Kruskal-Wallis test was used to compare the Multiple Antibiotic Resistance (MAR) indices amongst three bacterial groups (*E. coli*, *Klebsiella* spp., and *S. aureus*). For post-hoc pairwise comparisons, Dunn's test with Bonferroni correction was employed. The threshold for statistical significance was established at $P<0.05$. Each bacterial species' mean rank was determined. Python (v3.11) with the SciPy and scikit-posthocs packages were used for all MARI analyses. Other statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp., Armonk, NY, USA). When $P<0.05$, the findings of the Chi-square test considered statistically significant.

RESULTS

Prevalence of omphalitis in broiler chicks: Regarding age-related distribution, 60 (52.6%) of the 114 chicks tested from one to six days old were confirmed positive for omphalitis. The positivity rate on Day 1 was 38.9%, and on Day 5, it increased to 68.2%. However, Chi-square analysis showed no statistically significant correlation between positive status and age ($\chi^2 = 3.28$, df = 5, P=0.66). The prevalence of omphalitis is demonstrated in Table 1.

Table 1: Prevalence of omphalitis in chicks based on age (n = 114)

Age (days)	No. of examined chicks	No. of positive chicks	Prevalence (%)
1	18	7	38.9
2	21	9	42.9
3	17	9	52.9
4	20	11	55
5	22	15	68.2
6	16	9	56.2
Total	114	60	52.6

Gross pathological examination: The prevalent lesion found during the post-mortem examination was unabsorbed material in the yolk sac. Yolk fluid with a watery, sticky, or caseous appearance was found inside the enlarged and congested yolk sac, which also changed color to yellowish green or brown. Expansion and swelling in blood vessels in subcutaneous tissues. Infected yolk sacs were found to have a rancid and bad odor smell. Chicks older than three days had internal organ lesions, such as pericarditis, petechial hemorrhages on the serosal surface of visceral organs, enteritis, perihepatitis, and liver enlargement. Data are presented in Fig. 1. A and Fig. 1. B.

Bacteriological analysis: All chicks infected with omphalitis yielded positive cultures, with 11 birds (18.3%) showing a single type of bacterial infection, whereas 49 birds (81.7%) were co-infected with multiple types of bacterial species. Table 2a displayed the trend of co-infection in the initial six days of life.

Table 2a: Pattern of bacterial co-infection isolated during the first 6 days of life.

Bacterial Pattern	No. of isolates (%)	Total prevalence of single and mixed infections
E. coli	9 (15)	18.3%
Klebsiella spp.	2 (3.3)	
S. aureus, *Shigella* spp., *E. coli*	2 (3.3)	
S. aureus, *E. coli*	3 (50)	
E. coli, *Pseudomonas*, *S. aureus*	1 (1.7)	
S. epidermidis, *E. coli*	2 (3.3)	
Klebsiella spp., *S. aureus*	8 (13.3)	81.7%
Proteus spp., *Klebsiella* spp., *S. aureus*, *E. coli*	1 (1.7)	
S. aureus, *Klebsiella* spp., *Shigella* spp., *Proteus* spp.	1 (1.7)	
S. aureus, *Pseudomonas* spp.	3 (5.0)	
S. aureus, *E. coli*, *Proteus* spp.	1 (1.7)	
Total	60 (100)	

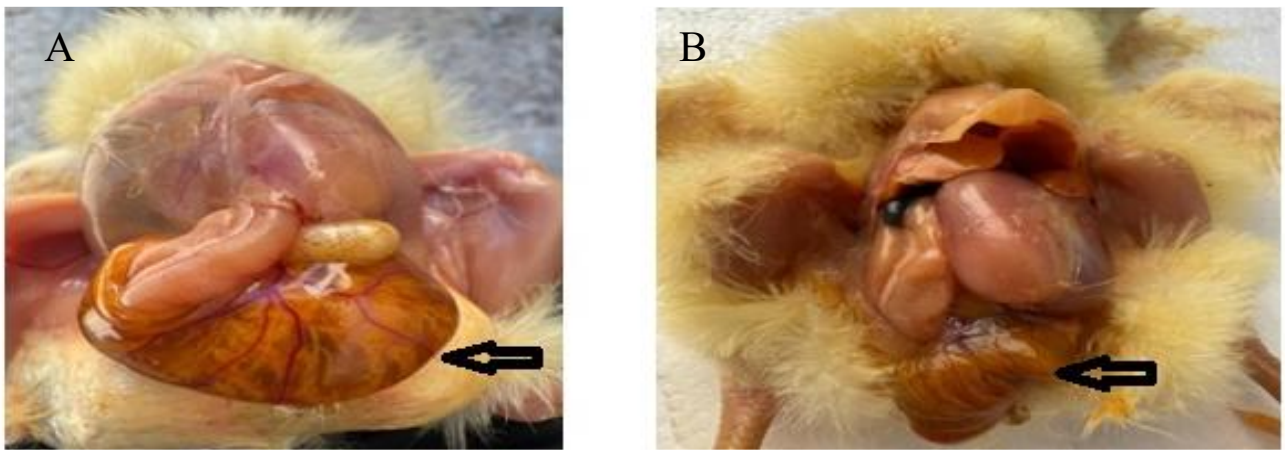

Fig. 1: A: The navel is inflamed, and the yolk sacs are distended with abnormal contents, and the blood vessels are hyperemic in a broiler in 3-day-old chick. B: The yolk sac is distended and filled with abnormal, yellowish-green, sticky contents in a 4-day-old broiler chick.

One hundred seventeen distinct bacterial strains were identified in 60 dead birds that were positive for omphalitis, suggesting the presence of mixed bacterial infections. Among the recognized bacterial categories, there were significant differences in the distribution of bacterial isolates (Chi-square goodness-of-fit test, χ^2 = 152.67, df = 6, P<0.0001). Post-hoc analysis using standardized residuals showed that *S. aureus* (47 isolates; 78.3%, SR = 7.41) and *E. coli* (46 isolates; 76.7%, SR = 7.16), which were identified at a significantly greater frequencies than expected (P<0.05). The isolation rates of the microorganisms *S. aureus* and *E. coli* did not show statistically significant difference (P>0.05).

The frequency of isolation of *Klebsiella* spp. (12 isolates; 20.0%, SR = -1.15) was significantly higher than that of *Pseudomonas* spp., *Shigella* spp., *Proteus* spp., and *S. epidermidis* (P<0.05), but significantly lower than that of *S. aureus* and *E. coli*. *Pseudomonas* spp., *Shigella* spp., *Proteus* spp. and *S. epidermidis* did not differ significantly from one another. Data are presented in Table 2b.

Table 2b: Prevalence of 117 bacteria isolates recovered from omphalitis during first 6 days of life.

Microorganism	Microorganisms isolated from 60 infected chicks with omphalitis	
	No. of infected chicks	No. of isolates (%) *
S. aureus	47	47 (78.3) [a]
E. coli	46	46 (76.7) [a]
Klebsiella spp.	12	12 (20.0) [b]
Pseudomonas spp.	4	4 (6.7) [c]
Shigella spp.	3	3 (5.0) [c]
Proteus spp.	3	3 (5.0) [c]
S. epidermidis	2	2 (3.3) [c]
Total	60 infected chicks	117 bacterial isolates

*Values sharing the same superscript letter are not significantly different ($P > 0.05$), whereas values with different letters differ significantly ($P < 0.05$), based on Chi-square goodness-of-fit test with post-hoc interpretation using standardized residuals.

Three distinct groups were used to analyze 60 chickens from six different hatcheries (A-F). *S. aureus* isolation rates in the first group varied from 65.0% in hatchery B to 100% in hatchery E. The Chi-square analysis, however, revealed no statistically significant correlation between bacterial isolation rate and hatchery (χ^2 = 5.58, df = 5, P=0.35). *E. coli* isolation rates ranged from 57.1% in hatchery F to 100% in hatchery D in the second group, but there was no discernible variation between hatcheries (χ^2 = 4.02, df = 5, P=0.55). With no statistically significant difference found (χ^2 = 2.32, df = 5, P=0.80), the frequency of isolation of *Klebsiella* spp. in the third group was usually lower across all hatcheries, ranging from 0% in hatchery D to 28.6% in hatchery F. The lack of significant connections in every comparison was verified by Fisher's exact test (P>0.05). Data are illustrated in Table 2c.

In vitro antimicrobial resistant patterns in bacterial isolates recovered from omphalitis

Antibiotic resistance patterns in *E. coli* isolates: The tested *E. coli* isolates recovered from omphalitis exhibited a high resistance rate toward several antimicrobial agents such as aminopenicillin (ampicillin 100%, amoxicillin 97.8%); cephalosporins (cefoxitin 97.8%, Ceftiofur 65%); fluoroquinolone (ciprofloxacin 63%, norfloxacin 71.7%, levofloxacin 71.7%, enrofloxacin 80.4%) sulfonamides (sulfamethoxazole/trimethoprim 71.7%); amphenicols (florfenicol 71.7%); aminoglycoside (kanamycin 60.9%) and aminocyclitol (spectinomycin 63%). Moderate resistance rate was demonstrated toward aminoglycoside (gentamicin 39.1%, neomycin 36.9%); tetracyclines (doxycycline, 39.1%); cephalosporins (ceftriaxone 36.9%, cephalexin 28.3%) and phosphonic antibiotic (fosfomycin 26.1%). However, low resistance rate was revealed against polymyxins (polymyxin B 15.2%, polymyxins E (colistin) 10.9%). Data of the tested 46 *E. coli* isolates collected from omphalitis against 20 antimicrobial agents are demonstrated in Table 3. In addition, 97.8% (45 out 46) of *E. coli* isolates were multidrug resistant (MDR) and demonstrated resistance against more than two classes of antibiotics. Approximately 52% of isolates were MDR against 7-10 classes of antimicrobial agents. These Forty-five of *E. coli* isolates (one isolate did not show resistant to these tested antibiotics) showed 44 antibiotic-resistant patterns (data are not shown).

Table 2c: Frequency of isolation of predominant bacterial species from cases of yolk sac infections in six hatcheries.

Isolated bacterial	Hatchery	No. examined	N (%)
S. aureus	A	8	6 (75)
	B	20	13 (65)
	C	10	9 (90)
	D	7	6 (85.7)
	E	8	8 (100)
	F	7	5 (71.4)
E. coli	A	8	6 (75)
	B	20	16 (80)
	C	10	7 (70)
	D	7	7 (100)
	E	8	6 (75)
	F	7	4 (57.1)
Klebsiella spp.	A	8	2 (25)
	B	20	4 (20)
	C	10	2 (20)
	D	7	0 (0)
	E	8	2 (25)
	F	7	2 (28.6)

Antibiotic resistance pattern in *Klebsiella* spp. Isolates: The tested *Klebsiella* spp. isolates recovered from omphalitis demonstrated a high resistance rate against several antimicrobial agents such as aminopenicillin (ampicillin 100%, amoxicillin 100%); cephalosporins (cefoxitin 91.7%, ceftiofur 66.7%); fluoroquinolone (ciprofloxacin 66.7%, norfloxacin 83.3%, enrofloxacin 83.3); sulfonamides (sulfamethoxazole/trimethoprim 75%); amphenicols (florfenicol 83.3%); aminoglycoside (gentamicin 66.7%, kanamycin 58.3%) and aminocyclitol (spectinomycin 66.7%) and tetracyclines (doxycycline 58.3%). Intermediate resistance rate was demonstrated toward fluoroquinolone (levofloxacin 50%); cephalosporins (cephalexin 50%); aminoglycoside (neomycin 41.7%) and phosphonic antibiotic (fosfomycin (33.3%). However, low resistance rate was detected against cephalosporins (Ceftriaxone 25.0%) and polymyxins (polymyxin B 25%, polymyxins E (colistin) 25%). Data of the tested 12 *Klebsiella* spp. isolates collected from omphalitis against 20 antimicrobial agents are presented in Table 3. In addition, 91.7% (11 out 12) of *Klebsiella* spp. isolates were MDR and demonstrated resistance against 6 to 9 classes of antibiotics. Approximately, 73% of MDR isolates were resistant toward 7-9 classes of antimicrobial agents. All *Klebsiella* spp. isolates (12 isolates) showed 11 antibiotic-resistant patterns (data are not shown).

Antibiotic resistance patterns in *S. aureus* isolates: The tested *S. aureus* isolates recovered from omphalitis demonstrated a high resistance rate against several antimicrobial agents such as aminopenicillin (ampicillin 100%, penicillin 97.9%, amoxicillin 85%); aminocyclitol (spectinomycin 91.5%); cephalosporins (Cefoxitin 93.6%, ceftriaxone 59.6%, ceftiofur 57.4%); macrolides (erythromycin 89.4%); fluoroquinolone (ciprofloxacin 83.9%, norfloxacin 85%, levofloxacin 83.9%, enrofloxacin 89.4%); amphenicols (Florfenicol 78.7%); sulfonamides (Sulfamethoxazole/trimethoprim 74.5%); aminoglycoside (Kanamycin 70.2%); phosphonic antibiotic (fosfomycin 68.1%) and tetracyclines (doxycycline 55.3%). However, moderate resistance rate was demonstrated against cephalosporins (cephalexin 47.8%) and aminoglycoside (gentamicin 42.5%, neomycin 48.9%). Data of the tested 47 *S. aureus* isolates collected from omphalitis toward 20 antimicrobial agents are demonstrated in Table 3. In addition, 100% of *S. aureus* isolates were MDR and demonstrated resistance against more than two classes of antibiotics. Approximately, 79% of *S. aureus* isolates detected in this study were MDR against more than 6 classes of antimicrobial agents, and about 23% of *S. aureus* isolates were resistant to 10 classes of antimicrobial agents. All *S. aureus* isolates exhibited 45 antibiotic-resistant patterns (data are not shown).

Multi-drug resistance index of *E. coli*, *Klebsiella* spp. and *S. aureus*: Results of this study showed that the MARI of *E. coli* isolates ranged from 0.00 to 0.85 with average MARI 0.59±0.15. In addition, results showed that 97.8% of *E. coli* isolates (45 out 46) had MARI ≥0.25. The major number of isolates resistant to 11, 12 and 15 antimicrobial agents with a MARI ranged from 0.55, 0.60 and 0.75, respectively (45.7%, n = 21), which had 49.5% of the whole aggregate antibiotic resistance score. Results are shown in Table 4a.

The MARI of *Klebsiella* spp. isolates ranged from 0.15-0.85 with average MARI 0.62±0.20. Also, results exhibited that 91.7% of *Klebsiella* spp. isolates (11 out 12) had MARI ≥0.45. The major number of isolates resistant to 14 and 16 antimicrobial agents with a MARI ranged from 0.70 to 0.80 (41.7%, n = 5), which had 51.0% of the whole aggregate antibiotic resistance score. Results are demonstrated in Table 4b.

The MARI of *S. aureus* isolates ranged from 0.45-1.00 with average MARI 0.75±0.13. Furthermore, results showed that 100% of *S. aureus* isolates (47 out 47) had MARI ≥0.45. The most common isolates resistant to 14 to 18 antimicrobial agents with a MARI ranged from 0.70 to 0.90 (72.3%, n = 34), which had 77.9% of the whole aggregate antibiotic resistance score. Results are demonstrated in Table 4c.

Table 3: The antibiotic susceptibility profile of the *E. coli*, *Klebsiella* spp. and *S. aureus* isolates recovered from omphalitis.

Group	Antibiotic	*S. aureus* (n=47)			*E. coli* (n=46)			*Klebsiella* (n=12)		
		R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)
Fluoroquinolone	Ciprofloxacin	39 (83.9)	2 (4.3)	6 (12.7)	29 (63)	15 (32.6)	2 (4.3)	8 (66.7)	3 (25)	1 (8.3)
	Norfloxacin	40 (85)	3 (6.4)	4 (8.5)	33 (71.7)	11 (23.9)	2 (4.3)	10 (83.3)	1 (8.3)	1 (8.3)
	Enrofloxacin	42 (89.4)	0 (0)	5 (10.6)	37 (80.4)	6 (13)	3 (6.5)	10 (83.3)	1 (8.3)	1 (8.3)
	Levofloxacin	39 (83.9)	2 (4.3)	6 (12.7)	33 (71.7)	13 (28.3)	0 (0)	6 (50)	4 (33.3)	2 (16.7)
Aminoglycoside	Gentamicin	20 (42.5)	21 (44.7)	6 (12.7)	18 (39.1)	26 (56.5)	2 (4.3)	8 (66.7)	2 (16.7)	2 (16.7)
	Neomycin	23 (48.9)	16 (34)	8 (17)	17 (36.9)	29 (63)	0 (0)	5 (41.7)	4 (33.3)	3 (25)
	Kanamycin	33 (70.2)	9 (19.1)	5 (10.6)	28 (60.9)	15 (32.6)	3 (6.5)	7 (58.3)	4 (33.3)	1 (8.3)
Cephalosporins	Cephalexin	22 (47.8)	18 (38.3)	7 (14.9)	13 (28.3)	29 (63)	4 (8.7)	6 (50)	6 (50)	0 (0)
	Ceftriaxone	28 (59.6)	9 (19.1)	10 (21.3)	17 (36.9)	25 (54.3)	4 (8.7)	3 (25)	9 (75)	0 (0)
	Ceftiofur	27 (57.4)	15 (31.9)	5 (10.6)	30 (65)	15 (32.6)	1 (2.2)	8 (66.7)	4 (33.3)	0 (0)
	Cefoxitin	44 (93.6)	3 (6.4)	0 (0)	45 (97.8)	1 (2.2)	0 (0)	11 (91.7)	1 (8.3)	0 (0)
Aminopenicillin	Amoxicillin	40 (85)	6 (12.7)	1 (2.1)	45 (97.8)	0 (0)	1 (2.2)	12 (100)	0 (0)	0 (0)
	Ampicillin	47 (100)	0 (0)	0 (0)	46 (100)	0 (0)	0 (0)	12 (100)	0 (0)	0 (0)
	Penicillin	46 (97.9)	1 (2.1)	0 (0)	-	-	-	-	-	-
Polymyxins	Polymyxins E (Colistin)	-	-	-	5 (10.9)	41 (89.1)	0 (0)	3 (25)	9 (75)	0 (0)
	Polymyxin B	-	-	-	7 (15.2)	39 (84.8)	0 (0)	3 (25)	9 (75)	0 (0)
Tetracyclines	Doxycycline	26 (55.3)	13 (27.7)	8 (17)	18 (39.1)	20 (43.5)	8 (17.4)	7 (58.3)	5 (41.7)	0 (0)
Sulfonamides	Sulfamethoxazole/trimethoprim	35 (74.5)	10 (21.3)	2 (4.3)	33 (71.7)	13 (28.3)	0 (0)	9 (75)	3 (25)	0 (0)
Amphenicols	Florfenicol	37 (78.7)	7 (14.9)	3 (6.4)	33 (71.7)	13 (28.3)	0 (0)	10(83.3)	2 (16.7)	0 (0)
Phosphonic Antibiotic	Fosfomycin	32 (68.1)	8 (17)	7 (14.9)	12 (26.1)	34 (73.9)	0 (0)	4 (33.3)	8 (66.7)	0 (0)
Aminocyclitol	Spectinomycin	43 (91.5)	3 (6.4)	1 (2.1)	29 (63)	12 (26.1)	5 (10.9)	8 (66.7)	2 (16.7)	2(16.7)
Macrolides	Erythromycin	42 (89.4)	3 (6.4)	2 (4.3)	-	-	-	-	-	-

Table 4a: Antimicrobial resistant patterns of MARI of 46 *E. coli* isolates collected from omphalitis in North of Palestine using 22 different types of antibiotics.

MARI of *E. coli* isolates					
Antibiotic resistant pattern	*MARI	No. of isolates (%)	Range of MARI	Aggregate antibiotic resistance score	**Mean of MARI for all isolates ±SD
0	0.00	1 (2.2)		0	
5	0.25	1 (2.2)		5	
7	0.35	2 (4.3)		14	
8	0.40	3 (6.5)		24	
9	0.45	4 (8.7)		36	
10	0.50	3 (6.5)	0.00-0.85	30	0.59±0.15
11	0.55	9 (19.7)		99	
12	0.60	6 (13.0)		72	
13	0.65	4 (8.7)		52	
14	0.70	4 (8.7)		56	
15	0.75	6 (13.0)		90	
16	0.80	2 (4.3)		32	
17	0.85	1 (2.2)		17	
Total		46 (≈100)		527	

*MARI: Multiple Antibiotic Resistance Index, **SD: Standard Deviation.

Table 4b: Antimicrobial resistant patterns of MARI of 12 *Klebsiella* spp. isolates collected from omphalitis in North of Palestine using 22 different types of antibiotics.

Klebsiella spp.					
Antibiotic resistant pattern	**MARI	No. of isolates (%)	*Range of MARI	Aggregate antibiotic resistance score	**MARI of all isolates ±SD
3	0.15	1 (8.3)		3	
9	0.45	1 (8.3)		9	
10	0.50	2 (16.7)		20	
11	0.55	1 (8.3)		11	
13	0.65	1 (8.3)		13	
14	0.70	2 (16.7)	0.15-0.85	28	0.62±0.20
16	0.80	3 (25)		48	
17	0.85	1 (8.3)		17	
Total		12 (≈100)		149	

*MARI: Multiple Antibiotic Resistance Index, **SD: Standard Deviation.

Table 4c: Antimicrobial resistant patterns of MARI of 47 *S. aureus* isolates recovered from omphalitis in North of Palestine using 20 different types of antibiotics.

S. aureus					
Antibiotic resistant pattern	*MARI	No. of isolates (%)	Range of MARI	Aggregate antibiotic resistance score	**MARI of all isolates ±SD
9	0.45	1 (2.1)		9	
10	0.5	3 (6.4)		30	
11	0.55	2 (4.3)		22	
12	0.6	3 (6.4)		36	
13	0.65	3 (6.4)		39	
14	0.7	5 (10.6)	0.45-1	70	0.75±0.13
15	0.75	6 (12.8)		90	
16	0.8	7 (14.9)		112	
17	0.85	11 (23.4)		187	
18	0.9	5 (10.6)		90	
20	1	1 (2.1)		20	
Total		47 (100)		705	

*MARI: Multiple Antibiotic Resistance Index, **SD: Standard Deviation.

S. aureus isolates had significantly higher MARI values than both *Klebsiella* spp. and *E. coli* isolates. The mean MARI for *S. aureus* 0.75, followed by *Klebsiella* spp. (0.62) and *E. coli* (0.59). The three bacterial groups had significantly different MAR indices (Kruskal–Wallis H = 36.78, df = 2, P<0.0001). *S. aureus* isolates exhibited the highest MAR index (mean rank = 51.96), followed by *Klebsiella* spp. (mean rank = 33.33) and *E. coli* isolates (mean rank = 26.93), according to the mean ranks. The MAR index of *S. aureus* was substantially higher than both *E. coli* and *Klebsiella* (P<0.001), whereas there was no significant difference between *E. coli* and *Klebsiella* (P=0.696), according to post-hoc pairwise comparisons using Dunn's test with Bonferroni correction. These findings imply that, in comparison to the other two bacterial species, the *S. aureus* isolates in our investigation show a significantly higher rate of multi-antibiotic resistance. Data are shown in Table 5.

Table 5: Analyses of MAR index using Kruskal–Wallis and Dunn's Post-Hoc software.

Bacterial isolates	No. of isolates	Mean Rank*
E. coli	46	26.93[a]
Klebsiella spp.	12	33.33[a]
S. aureus	47	51.96[b]

*Values sharing the same superscript letter are not significantly different (P>0.05), whereas values with different letters differ significantly (P<0.05), based on Chi-square goodness-of-fit test with post-hoc interpretation using standardized residuals.

DISCUSSION

Omphalitis is more likely to occur in unsanitary conditions, where opportunistic bacterial infections thrive. Infected flocks exhibit reduced hatch rates, increased mortality, and a higher rate of culling due to alterations in immunoglobulin protein production, which are associated with bacterial infections and ultimately lead to immunodeficiency (Mohibbullah *et al.*, 2022). Yolk sac infections primarily result from bacterial contamination of the eggshell when the cuticle remains damp after laying. Contamination can stem from several factors, including poor nest hygiene, eggs resting on the ground, incubation of dirty or defective eggs, and the concurrent collection of both clean and contaminated eggs (Melese *et al*., 2017; Yousef *et al*., 2023). Prolonged egg storage, incorrect incubation temperatures, and humidity were recognized as risk factors leading to an increased incidence of omphalitis (Jalob *et al*., 2015). The total prevalence of omphalitis in this study was 52.6%, aligning with the findings reported by Kaboudi *et al*. (2021), who found a prevalence rate of 55.3% in Tunisia. Nonetheless, the outcome of the present study exceeds the findings reported earlier (Amare *et al*., 2013), which indicated a prevalence rate of 33.1% in Ethiopia and rates of 32.0 and 37.2% in Egypt (Abdel-Tawab *et al*., 2016; Shaheen *et al*., 2024). Results of the present study showed lower prevalence rate of omphalitis than other results published previously from Ethiopia (Melese *et al*., 2017) and Egypt (Mowafy and El Oksh, 2017), who reported a prevalence rate of 96.1 and 68.3%, respectively. In our study, a higher prevalence rate was noticed in 5 days old chicks (68.2%), which is consistent with results of other studies, 64.3% in Ethiopia (Amare *et al*., 2013) and 76.0% in Tunisia (Kaboudi *et al*., 2021). Early omphalitis is caused by a variety of reasons, including unhygienic conditions at the hatchery, poor transportation circumstances, and difficulties in transportation from the hatchery to the farm.

One of the main factors for yolk sac storage, which is a suitable culture medium for bacteria growth, is the delay in feeding after hatching. This means that early feeding could speed up yolk passage into the intestine resulting in intestinal motility. The emergence and spread of microorganisms, the adsorption of toxins, and the lack of nutrients and maternal-derived antibodies will all be worse by a retained yolk sac. However, under stressful conditions, the unabsorbed yolk sac may be a source of certain pathogens such as *Campylobacter* spp. and *Salmonella Enteritidis*, which will be discharged in droppings and transferred to the carcass of subsequent broilers (Kaboudi *et al*., 2021; Elibol *et al.*, 2023).

At necropsy exam, lesions described in this study were in agreement with other previously published studies (Amare *et al*., 2013; Jalob *et al*., 2015; Kaboudi *et al*., 2021).

In the current investigation, results demonstrated that Gram-negative bacteria isolates were the predominant in cases of omphalitis. The frequency of isolation of Gram-negative bacteria was 58.1% (68/117). These findings were in agreement with other published research studies from Egypt (Shaheen *et al*., 2024), Bangladesh (Nasrin *et al*., 2012; Mohibbullah *et al*., 2022) and Ethiopia (Melese *et al*., 2017), which showed that the common bacteria isolated from omphalitis were Gram-negative bacteria. However, our results were in contrast to a report published from Tunisia (Kaboudi *et al*., 2021), which illustrated that the common bacteria recovered from omphalitis were Gram-positive bacteria. In the present study, out of 60 cultures from omphalitis cases, mixed infections were the most common (49 chicks; 81.7%). This finding differs from other published research from Tunisia (Kaboudi *et al*., 2021) and Egypt (Mowafy and El Oksh, 2017), which indicated that single infection occurred in 71.3 and 68.3%, respectively, of positive cultures. Findings from the present study aligned with earlier research conducted in Ethiopia (Amare *et al.*, 2013), indicating 59.37%, along with studies in Egypt (Abdel-Tawab *et al*., 2016; Shaheen *et al*., 2024), which reported 29 and 71.4%, respectively, of positive cultures showcasing mixed bacterial infections

In the current research, the major common recovered bacterial isolate was *S. aureus* (78.3%), followed by *E. coli* (76.7%), *Klebsiella* spp. (20%), *Pseudomonas* spp. (6.7%), *Shigella* spp. (5%), *Proteus* spp. (5%) and *S. epidermidis* (3.3%). These findings were consistent with previous study exhibited that *S. aureus* was the most frequent isolated from cases of omphalitis in Egypt (Khalil and El-Shamy, 2012). *S. aureus* has also been reported as the second most isolated bacteria in cases of omphalitis in broilers in Egypt and Ethiopia (Amare *et al*., 2013; Abdel-Tawab *et al*., 2016).

The findings disagreed with multiple research studies from Pakistan. (Iqbal *et al*., 2006), Ethiopia (Amare *et al*., 2013; Melese *et al*., 2017), Tunisia (Kaboudi *et al*., 2021), Egypt (Abdel-Tawab *et al*., 2016; EL-Sawah *et al*., 2016; Mowafy and El Oksh, 2017; Saad *et al*., 2017; Shaheen *et al*., 2024), Bangladesh (Mohibbullah *et al*., 2022; Hasan *et al*., 2024**)**, which reported that *E. coli* isolates were the most frequent recovered bacteria from omphalitis.

Nevertheless, in other previous studies from Iraq (Jalob *et al*. 2015) and Bangladesh (Shahjada *et al*., 2017), *Streptococcus* spp. and S*almonella* spp. were the most common recovered bacteria from omphalitis cases, respectively. In addition, although *Salmonella* spp. was detected in many reports from many countries as from Bangladesh (Nasrin *et al*., 2012; Shahjada *et al*., 2017; Mohibbullah *et al*., 2022; Hasan *et al*., 2024), Egypt (Abdel-Tawab *et al*., 2016; Amer *et al*., 2017; Saad *et al*., 2017; Shaheen *et al*., 2024), Ethiopia (Melese *et al*., 2017), Tunisia (Kaboudi *et al*., 2021), *Salmonella* spp. was not detected in this study.

To prevent the use of unsuccessful antibiotics against pathogenic bacteria isolated from omphalitis cases, it is crucial to determine the antibiotic susceptibility of bacterial isolates. The majority of the *E. coli* isolates that were assessed in this study exhibited high levels of antibiotic resistance rate to a number of antimicrobial drugs, including ampicillin, amoxicillin, cefoxitin, ceftiofur, enrofloxacin, levofloxacin, norfloxacin, sulfamethoxazole/trimethoprim, florfenicol, ciprofloxacin, kanamycin, and spectinomycin. Results from other published studies either disagreed with our findings or only partially agreed with them (Iqbal *et al*., 2006; Nasrin *et al*., 2012; Abdel-Tawab *et al*., 2016; Melese *et al*., 2017; Saad *et al*., 2017; Kaboudi *et al*., 2021; Hasan *et al*., 2024). In the present study, moderate prevalence of drug resistance rate was demonstrated toward gentamicin, neomycin, doxycycline, ceftriaxone, cephalexin, and fosfomycin. The rate of resistance to doxycycline was consistent with a recently published study (Kaboudi *et al*., 2021). Additional reports (Amare *et al*., 2013; Abdel-Tawab *et al*., 2016; Melese *et al*., 2017; Saad *et al*., 2017; Mohibbullah *et al*., 2022; Hasan *et al*., 2024; Shaheen *et al*., 2024) indicating *E. coli* isolated from omphalitis cases were very sensitive to gentamicin, but this did not validate our findings.

While agar diffusion techniques are not advised by EUCAST (European Committee on Antimicrobial Susceptibility Testing) and CLSI (Bentaher *et al*., 2025), broth microdilution is currently regarded as the gold standard method. But the agar diffusion methods are still used to assess colistin (El Seedy *et al*., 2019; Benklaouz *et al*., 2020; Dhaouadi *et al*., 2020; Kaboudi *et al*., 2021; Limbachiya *et al*., 2022; Tofani *et al*., 2022; Aberkane *et al*., 2023; Saeed *et al*., 2023; Adwan *et al*., 2024; Bahadur *et al*., 2024; Abuseir *et al*., 2026). The present investigation showed a low prevalence of resistance rate to polymyxin B and polymyxins E (colistin). These findings were consistent with a recent study (Kaboudi *et al*., 2021) that showed low resistance (8.6%) of *E. coli* isolates recovered from omphalitis toward colistin.

A high level of resistance rate to a number of antimicrobial agents, including ampicillin, amoxicillin, cefoxitin, ceftiofur, enrofloxacin, norfloxacin, ciprofloxacin, sulfamethoxazole/trimethoprim, florfenicol, gentamicin, kanamycin, spectinomycin, and doxycycline, was revealed by subjecting *Klebsiella* spp. isolates to antibiotic susceptibility test. These findings were somewhat compatible with earlier research (Iqbal *et al*., 2006; Kaboudi *et al*., 2021; Javed *et al*., 2024). Nonetheless, minor levels of resistance rate of *Klebsiella* spp. to ceftriaxone, polymyxin B, and polymyxin E (colistin) were found. The most effective drugs against *Klebsiella* spp. were polymyxins E and sulfamethoxazole/trimethoprim (Kaboudi *et al*., 2021).

The frequency of isolation of antibiotic resistance to 20 antimicrobial medications was determined by analyzing the *S. aureus* isolates that were evaluated in this investigation. All isolates showed a resistant rate between 42.5% for gentamicin and 100% for ampicillin. These results either partially support or contradict other published studies (Amare *et al*., 2013; Abdel-Tawab *et al*., 2016; Melese *et al*., 2017; Saad *et al*., 2017; Kaboudi *et al*., 2021).

Multidrug-resistant (MDR) isolates were detected in this study, approximately 97.8% (45 out 46) of *E. coli* isolates were multidrug resistant and demonstrated resistance against more than two classes of antibiotics. Approximately 52.0% of isolates were multi-drug resistant against 7 to 10 classes of antimicrobial agents. All MDR *E. coli* isolates showed 44 antibiotic-resistant patterns. In addition, 91.7% (11 out 12) of *Klebsiella* spp. isolates were multidrug resistant and demonstrated resistance against 6 to 9 classes of antibiotics. Approximately, 73.0% of multidrug-resistant isolates were resistant toward 7 to 9 classes of antimicrobial agents. All *Klebsiella* spp. isolates showed 11 antibiotic-resistant patterns. Also, 100% (47 out 47) of *S. aureus* isolates were multidrug resistant and demonstrated resistance against more than two classes of antibiotics. Approximately, 79% of *S. aureus* isolates detected in this study were multidrug resistant against more than 6 classes of antimicrobial agents, and about 23% of *S. aureus* isolates were resistant to 10 classes of antimicrobial agents. All *S. aureus* isolates exhibited 45 antibiotic-resistant patterns. Multidrug resistance was reported in many previous reports (Nasrin *et al*., 2012; Das *et al*., 2017; Melese *et al*., 2017; Shahjada *et al*., 2017; Kaboudi *et al*., 2021; Hasan *et al*., 2024). The findings indicated that appropriate antibiotics must be assessed and identified before the administration of antibiotic therapy in chickens.

These results showed that *S. aureus* isolates had significantly higher MARI values than both *Klebsiella* spp. and *E. coli* isolates. The mean MARI for *S. aureus* 0.75 with a range from 0.45-1, followed by *Klebsiella* spp. (0.62) with a range from 0.15-0.85 and *E. coli* (0.59) with a range from 0.00 to 0.85. Also, results exhibited that 91.7% of *Klebsiella* spp. isolates (11 out 12) had MARI ≥0.45. The major number of isolates resistant to 14 and 16 antimicrobial agents with a MARI ranged from 0.70 to 0.80 (41.7%, n = 5), which had 51.0% of the whole aggregate antibiotic resistance score. In addition, results showed that 97.8% of *E. coli* isolates (45 out 46) had MARI ≥0.25. The major number of isolates resistant to 11 and 12 antimicrobial agents with a MARI ranged from 0.55 to 0.60 (32.7%, n = 15), which had 32.4% of the whole aggregate antibiotic resistance score. Furthermore, results showed that 100% of *S. aureus* isolates (47 out 47) had MARI ≥0.45 and ranged from 0.45-1.00. The most common isolates resistant to 14 to 18 antimicrobial agents with a MARI ranged from 0.70 to 0.90 (72.3%, n = 34), and had 77.9% of the whole aggregate antibiotic resistance score. In previous research study, the MARI for *E. coli* and S*taphylococcus* spp. varied from 0.5 to 1.00, with an average of 0.77 (Hasan *et al*., 2024). The MAR index of 0.2 or higher denotes a high-risk source of contamination, whereas a MAR index of 0.4 or higher is linked to sources of contamination from feces (Adenaike *et al*., 2016). These findings of high MARI indicate that the isolates are high-risk, likely originating from an environment with heavy antibiotic use. The elevated MARI in these hatcheries indicates that improper use of antimicrobials may greatly impact the frequency of isolation of antibiotic-resistant isolates seen in hatcheries or in poultry farms (Saeed *et al*., 2023). High MARI indicating MDR and suggesting that the bacteria have experienced intensive antibiotic pressure. The resistance of the bacterial isolates to various antimicrobials discovered in this study may suggest that these antimicrobials are frequently used to treat bacterial infections in both humans and animals since these bacteria are environmental pollutants from clinical cases. This requires the careful enhancement of hatchery sanitation practices to develop alternative, efficient methods for preventing and controlling the spread of bacterial omphalitis (Melese *et al*., 2017; Khalefa *et al*., 2025).

Conclusions: The resistance of the bacterial isolates to various antimicrobials discovered in this study may suggest that these antimicrobials are frequently used to treat bacterial infections in both humans and animals since these bacteria are environmental pollutants from clinical cases. The frequency of isolation of multidrug-resistant pathogens in chicks with omphalitis is a problem since these pathogens may be transmitted to humans and animals, making bacterial illnesses more challenging to treat. To create guidelines for the care and prevention of microbial omphalitis in chicks in Palestine, more research is advised. Omphalitis was very common, as were the deaths associated with it. In order to manage and prevent such infections, more efforts should be taken to develop stringent hygiene requirements, proper antimicrobial stewardship, with a schedule of coordinated actions aimed to optimize the use of antimicrobials, strict farm-level biosecurity and proper disposal of dead birds. This would result in a general reduction in the use of antimicrobials in chicken farms.

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