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The Relationship of Antimicrobial Sensitivity and Biofilm Formation of *Salmonella spp*. Isolated from Broiler Chicken Farms in Biskra, Algeria

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ABSTRACT

Salmonella is a pathogen implicated in foodborne illnesses. Antibiotic resistance in *Salmonella* has emerged and is increasingly prevalent, posing a significant global problem. The current study aimed to study the antimicrobial resistance and biofilm formation capacity of *Salmonella* spp. isolated from broiler chicken farms in Biskra, Algeria. Two hundred and seventy samples were collected from various sources within broiler chicken farms (fecal matter, cloacal swabs, surface swabs, reservoir water, and water from final dispenser lines). Conventional methods were used to isolate and identify *Salmonella* species and confirmed using API 20E test strips, and the disk diffusion method was used to perform a sensitivity test, in addition to the crystal violet microtiter plate method to assay the biofilm formation capacity of the isolates. 45 strains isolated with a prevalence of 16.66%. The result of the sensitivity test showed resistance against ampicillin, Ciprofloxacin and tetracycline at 97.78%, 95,56% and 91.11%, respectively. Meanwhile, the third-generation cephalosporins and cefadroxil showed moderate sensitivity. The results also showed a difference in the ability of the isolates to produce biofilms; most of them were weak, medium and non-productive biofilms at a rate of 55.56%, 37.78% and 6.67%, respectively. A negative correlation of a statistically significant relationship between antibiotic resistance and the capacity for biofilm formation in strains of *Salmonella* was observed. This study revealed a high prevalence of antibiotic resistance of *Salmonella* strains isolated from broiler chicken farms, indicating the necessity for effective measures to ensure food safety in the poultry industry in Algeria.

Keywords: Algeria, Antibiotic resistance, Biofilm formation, Broiler chicken farms, *Salmonella* spp.

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INTRODUCTION

Salmonella is a Gram-negative, rod-shaped bacterium that can survive in anaerobic conditions and is pathogenic to humans and animals. It causes two major diseases: non-typhoidal gastroenteritis (Salmonellosis) and *Salmonella* human typhoid fever often called intoxication **(Wibisono *et al.*, 2023)**. *Salmonella* is commonly associated with consuming contaminated poultry, eggs, meat, milk, and seafood **(Pui *et al.*, 2011).** In 2018, the World Health Organization (WHO) reported 94 million cases of non-typhoidal *Salmonella* gastroenteritis globally, resulting in 500,000 deaths annually **(WHO, 2018).** In Algeria, more than 5,000 human cases are reported each year, although the true number is likely higher **(Boughaba *et al.*, 2019).**

The most prevalent zoonotic *Salmonella* serotypes are *Salmonella enterica* serotype Enteritidis and *Salmonella enterica* serotype Typhimurium, responsible for salmonellosis in humans and animals (**Mohammed and Dubie, 2022).** Salmonella Gallinarum (*S. Gallinarum*) and Salmonella Pullorum

(*S. Pullorum*) are host-specific pathogens in poultry, responsible for fowl typhoid and pullorum disease, respectively. These biovars cause septicemic infections that lead to high mortality rates (**Filho *et al*., 2016**).

However, the growing threat of antimicrobial resistance (AMR) has led to an alarming increase in public health. The overuse of antibiotics in animal farming is especially concerning, as it can lead to the development of multi-drug-resistant bacteria, particularly in *Salmonella*, to commonly used antimicrobials in veterinary sectors. This issue is more pronounced in developing countries, where antibiotic misuse and lack of regulation worsen the problem **(Castro Vargas *et al*., 2020).**

The global spread of *Salmonella*-producing β-lactamases represents a major public health concern, as these bacteria have developed resistance to multiple antimicrobial agents. **(Fakorede *et al*., 2023).** Similarly, the World Health Organization (WHO) has reported an increase in carbapenem-resistant *Enterobacteriaceae*, a critical priority pathogen group worldwide **(Sleiman *et al*., 2021).**

The dissemination and persistence capacity of *Salmonella* spp. is associated with biofilm formation in the environment outside the host **(Iniguez-Moreno *et al*., 2018),** which serves to reduce the efficacy of sanitation procedures in poultry **(Borges et *al*., 2018)** and helps *Salmonella* to survive in harsh conditions, such as those found on poultry farms **(Wang *et al*., 2013).** However, little is known about the role of biofilms in enhancing the virulence and antibiotic resistance of *Salmonella* **(Musa *et al*., 2024).**

The current understanding of *Salmonella* spp. contamination in Algerian broiler farms is limited. While several studies conducted in different regions of the country have identified the presence of various pathogenic *Enterobacteriaceae* in foods of animal origin **(Leila Dib *et al*., 2019)**. The antibiotic resistance of *Salmonella* remains underexplored. The relationship between AMR and biofilm formation is a new concern in the locality of Biskra because the failure of biosecurity in poultry farms is explained by the novelty of the poultry industry and the misuse of antibiotics. Thus, this study aimed to examine the resistance of *Salmonella* spp. strains isolated from poultry in Biskra, Algeria, to antimicrobial agents and their potential to form biofilms.

MATERIALS AND METHODS

Ethical Approval

This study was conducted according to the guidelines of the Institutional Committee for the Protection of the National Administration for Higher Education and Scientific Research in Algeria under the law (98-11, Act of August 22, 1998).

Study Area

The Biskra region, located 400 km southeast of Algiers (between longitudes 04.92°E and 06.77°E, latitudes 34.28°N and 35.30°N), covers an area of 10,245.7 km²**(Fig.1).** It serves as a transitional zone between the northern Atlas Mountains and the Sahara. Biskra has an arid to hyper-arid climate, with December to February being the coldest months (average temperature 12.3°C–13.5°C) and June to August the hottest (average temperature 31.2°C–34.8°C). Rainfall is minimal, with October being the wettest month (22.87 mm) and July the driest (1.66 mm) **(Reghais *et al*., 2023).**

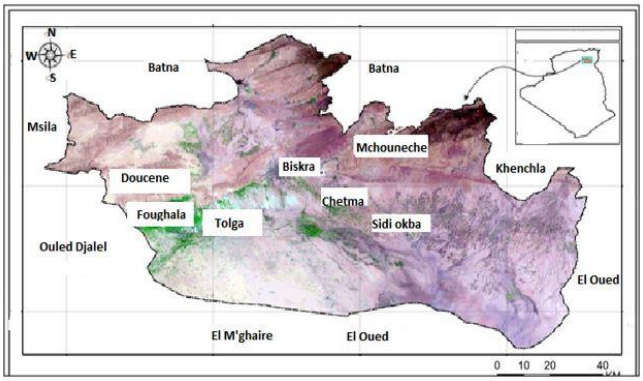

Fig.1: Map of Biskra region **(Reghais *et al*., 2023).**

Sampling

This study focused on broiler farms in Biskra Wilaya between March 2021 and May 2023, 27 farms were visited, each with 2 to 4 poultry buildings and 2,500 to 15,000 birds. Farms were located across various regions of the Wilaya, with earthen floors and wood shavings as bedding. Sampling took place 10 to 15 days before slaughter, with five types of samples collected: fecal, cloacal swabs, surface swabs, and water from reservoirs and final dispenser lines, totaling 270 samples.

Samples were handled under strict biosafety protocols to prevent contamination. They were transported in ice chests and stored at 3 ± 1°C for no longer than 1 hour before enrichment. Droppings (100g) were collected from multiple locations in the litter to ensure representatively, and 25g were analyzed. Five cloacal swabs were randomly taken from different areas of the poultry house. Two 500ml water samples were collected one from the reservoir and one from the final dispenser line and 25ml of each were analyzed. Surface samples were taken from wall cracks and floor junctions using sterile swabs for each area **(Carrique-Mas and Davies, 2008).**

Isolation and Identification of *Salmonella*

Salmonella isolation followed **ISO 6579-1:2017** guidelines. Samples were pre-enriched in 1:10 Buffered Peptone Water (Merck, Germany) (2.5%) and incubated at 37°C for 24 hours. After incubation, 1 ml

of the pre-enrichment broth was transferred to 9 ml of Rappaport Vassiliadis broth (Liofilchem, Italy) and incubated at 41.5°C for 24 hours. Following incubation, 0.01 ml of the culture was streaked onto Xylose Lysine Desoxycholate (XLD) and Hektoen Enteric (HE) agar plates, (HiMedia, India) which were incubated at 37°C for 24 hours. Suspected *Salmonella* colonies (slightly transparent red with a black center on XLD, and transparent with back center on HE agar) were selected. Five colonies with typical *Salmonella* characteristics were streaked onto pre-dried nutrient agar plates. The isolates were then transferred to Triple Sugar Iron (TSI) (Condolab, Spain) slants and tested for urease activity and Tryptophan Desaminase (TDA) (Merck, Germany). Finally, *Salmonella* phenotypes were confirmed using API 20E test strips (BioMérieux, France) **(Yang *et al.*, 2015).**

Antimicrobial susceptibility Test

The antibiotic susceptibility of *Salmonella* isolates was assessed using the agar disc diffusion method on Mueller-Hinton Agar (MH) (HiMedia, India) according to **EUCAST guidelines (2024).** The following 19 antimicrobial agents were tested:

AMP (ampicillin) 10µg, AMC (amoxicillin clavulanic-Acid) 20µg, PIP (piperacillin) 30µg, TET (tetracycline) 30µg, CIP (ciprofloxacin) 5µg, (NA) nalidixic acid 30µg, LVX (levofloxacin) 5µg, (AZM) azithromycin 15µg, CN (gentamycin) 30µg, AMK (amikacin) 15µg, (IMP) imipenem 10µg, (ETP) ertapenem 10µg, SXT (trimethoprim-sulfamethoxazole) 1,25/3,75 µg, FEP (cefepime) 30µg, CAZ (ceftazidime) 30µg, CTX (cefotaxime) 10µg, FOX (cefoxitin) 5µg, CFX (cefadroxil) 30µg, NIT (nitrofurantoin) 100µg, (Biorad, France).

A suspension of 3-4 colonies from each *Salmonella* isolate was prepared in 10 ml of 0.9% NaCl, adjusted to a 0.5 McFarland turbidity standard (1×10^7 CFU/ml). The suspension was swabbed onto MH agar plates, and antibiotic discs were applied at their respective dosages. Plates were incubated at 37°C for 24 hours. After incubation, the zones of inhibition were measured using a digital caliper and interpreted as sensitive (S), intermediate (I), or resistant (R) according to **CA-SFM Vet (2023)** and **EUCAST** breakpoints **(2024).** Strains resistant to three or more antimicrobial classes were classified as multi-resistant **(Table 1).**

Biofilm assay

Before inoculation, all strains were transferred from the original culture to Tryptone Soy Broth (TSB) (HiMedia, India) and incubated at 37°C for 24 hours. The suspension was vortexed, and turbidity was adjusted to match a McFarland 0.5 standard (1×10^7 CFU/ml) **(Leonhard *et al.*, 2019).** Then the wells of the microtiter plate are filled with 200 µl of TSB supplemented with 1% glucose is poured into the well. The negative control wells contain broth only: 200 µl of TSB supplemented with 1% glucose per well. **(Stepanovic *et al.*, 2007).**

The crystal violet microtiter plate assay was performed as described by **O'Toole *et al.*, (2011).** In order to minimize errors and provide reliable analysis of the data obtained, it is essential to perform testing of each strain at least in triplicate (three wells per strain). Six wells should be used for the negative control. After incubating for 48 hours at 37°C, non-adherent cells were removed by inverting the plate and rinsing twice with phosphate-buffered saline (PBS). A 1% crystal violet solution (Prolab, France) was then added and incubated for 10 minutes. Following three washes with PBS, the biofilm was treated with an 80% ethanol and 20% acetone solution (4:1) for 10 minutes. Control wells contained medium with no bacteria as a negative control. Absorbance was measured at 570 nm using a microplate reader.

To interpret the results, a cut-off value (ODc) was established to differentiate biofilm-producing strains from non-producing ones. This was calculated as the average OD of the negative control plus three standard deviations (ODc = average OD of negative control + 3 SD) **(Stepanovic *et al.*, 2007).**

Final optical density (OD) values for tested strains were determined by subtracting the strain's average OD from the ODc. Negative values were recorded as zero, while positive values indicated biofilm production. Strains were categorized accordingly **(Table 1) (Stepanovic *et al.*, 2007).**

Table1: The formula and the interpretation of the OD values.

The formula	The interprétation
OD ≤ ODC	(0) No biofilm formation
ODC < OD ≤ 2 x ODC	(+ or 1) Weak biofilm formation capacity
2 x ODC < OD ≤ 4 x ODC	(++ or 2) Moderate biofilm formation capacity
4 x ODC < OD	(+++ or 3) High biofilm formation capacity

Statistical analysis

Statistical analysis of the relationship between antibiotic resistance and biofilm formation in *Salmonella* strains was performed using Spearman's rank correlation. Results were visualized as a heatmap in IBM SPSS (Version 24). Data preparation was done in Excel 2016, and calculations were optimized with Python 3.13.1.

RESULTS

The results showed that out of 270 samples from different sources, 45 were positive for the isolation of *Salmonella* spp. at a rate of 16.66% **(Table 2).** The biochemical analysis with the API 20E system identified 45 isolates, revealing three main profiles: 75.6% had the 6704752 profiles, 17.8% had the 6704712 profiles, and 6.7% had the 6705742 profiles. Using the API Web V4.1 software (BioMerieux), the strains were identified as *Salmonella* spp. with 99.9% identification accuracy.

Table2: Prevalence of *Salmonella* spp. in different samples

Samples	No of samples	N°of *Salmonella*+Ve	Prevalence of *Salmonella* (%)	95% Confidence Interval (CI)
Fecal matter	27	12	26,66	[27,59% – 62,69%]
Cloacal swabs	135	12	26,66	[5,16% – 14,89%]
Surfaces swabs	54	8	17,66	[7,70% – 26,60%]
Reservoir water	27	5	11,11	[8,18% – 36,70%]
Final dispenser lines water	27	8	17,66	[15,85% – 48,48%]
Total	270	45	16,66	[12,70% – 21,57%]

Antimicrobial Resistance

High levels of resistance were observed in *Salmonella* strains against several antibiotics **(Table 3).** The most resistance was seen for ampicillin, tetracycline and ciprofloxacin at rates of 97.78%, 95.56%, and 91.11%, respectively. Other notable resistances included azithromycin, piperacillin and nalidixic acid at rates of (82.22%), (86.67%), and (77.78%), respectively. Moderate resistance was observed for amikacin, levofloxacin and gentamicin at rates of (64.44%), (60%), and (55.56%), respectively. In contrast, imipenem (33.33%) and ertapenem (24.44%) showed lower resistance. The least resistance was seen for cefoxitin (15.56%), trimethoprim-sulfamethoxazole (17.78%), and ceftazidime (8.89%), with nitrofurantoin, cefadroxil, and cefotaxime showing minimal resistance (2.22%-4.44%). These results appear better in the histogram where multiresistance is seen for beta-lactams, quinolones, aminoglycosides, tetracyclines and macrolides while sensitivity is more visible to cephalosporins **(Fig.2).**

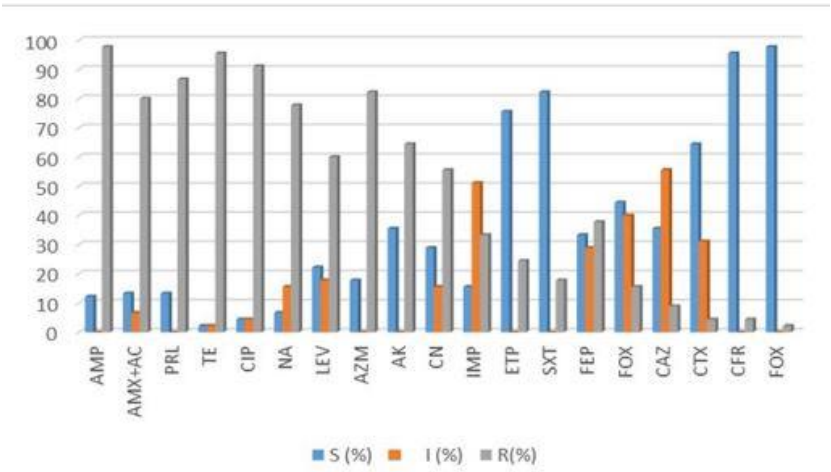

Fig.2: The Resistance and Sensitivity of Salmonella spp strains to Different Antibiotics. **S:** sensible. **I:** intermediary. **R:** resistance. Ampicillin (AM), Amoxicillin-Clavulanic Acid AMX+AC), Cefoxitin (FOX), Cefadroxil (CFR), Piperacillin (PRL), Gentamicin (CN), Imipenem (IMP), Ertapenem (ETP), Nalidixicacid (NA), Azithromycin (AZM), Amikacin (AK), Nitrofurantoin (FOX), Tetracycline (TE), Ciprofloxacin (CIP), TrimethoprimSulfamethoxazole (SXT), Levofloxacin (LEV), Cefepime (FEP), Ceftazidime (CAZ), Cefotaxim (CTX).

Table 3: The Resistance and Sensitivity of *Salmonella* spp strains to Different Antibiotics

Type of Antibiotics	*Salmonella* spp		
	S (%)	I (%)	R(%)
Ampicillin "AMP"	12,22	0	97,78
Amoxicillin-Clavulanic Acid "AMX+AC"	13,33	6,67	80
Piperacillin "PRL"	13,33	0	86,67
Tetracycline "TE"	2,22	2,22	95,56
Ciprofloxacin "CIP"	4,44	4,44	91,11
Nalidixic acid "NA"	6,67	15,56	77,78
Levofloxacin "LEV"	22,22	17,78	60
Azithromycin "AZM"	17,78	0	82,22
Amikacin "AK"	35,56	0	64,44
Gentamicin "CN"	28,89	15,56	55,56
Imipenem "IMP"	15,56	51,11	33,33
Ertapenem "ETP"	75,56	0	24,44
Trimethoprim-Sulfamethoxazole "SXT"	82,22	0	17,78
Cefepime"FEP"	33,33	28,89	37,78
Cefoxitin "FOX"	44,44	40	15,56
Ceftazidim "CAZ"	35,56	55,56	8,89
Cefotaxim "CTX"	64,44	31,11	4,44
Cefadroxil "CFR"	95,56	0	4,44
Nitrofurantoin	97,78	0	2,22
S: sensitive	I: intermediate		R: resistant

This multiresistance is seen for 13 antibiotics, is significant especially for 09 and for 13 antibiotics, moderate for 10, 12, 5-6 and weak for the others **(Table 4).**

Table 4: Strains of *Salmonella* spp showing multi-drug resistance to different number of antibiotics

Antibiotic types	Antibiotic Number	No of strains	Percentage
AK/CN	2	1/45	0.2%
PRL/AZM/AK/TE/FEP;AM/IMP/ETP/AZM/AK/TE	5-6	4/45	6.6%
AM/PRL/CN/IMP/NA/AZM/AK/TE/CIP/AMX+AC AM/PRL/NA/AZM/AK/TE/CIP/AMX+AC AM/PRL/AZM/AK/TE/CIP/AMX+AC	7-8-11	2/45	4.4%
AM/PRL/NA/AZM/AK/TE/CIP/LEV/AMX+AC	9	15/45	33.3%
AM/FOX/PRL/AZM/AK/TE/CIP/SXT/LEV/AMX+AC	10	5/45	11.11%
AM/FOX/CFR/PRL/CN/NA/AZM/AK/TE/CIP/LEV/AMX+AC	12	4/45	8.88%
AM/PRL/CN/ETP/NA/AZM/AK/TE/CIP/LEV/FEP/AMX+AC/CAZ	13	9/45	20%

Biofilm formation capacity

Based on the final optical density (OD) value of the strain, they were classified into the following categories according to Table 2: (OD≤0.67) No biofilm formation (0.67 < OD ≤ 1.34). weak biofilm formation (1.34 < OD ≤ 2.68) Moderate biofilm formation (2.68 < OD HBF) High biofilm formation. As previously expected, the ability of the strains to form biofilms was variable. Most strains (55.56%) formed weak biofilms, while (37.78%) and (6.67%) did not form biofilms and formed moderate biofilms, respectively **(Table 5).**

Table 5: The ability of the strains of *Salmonella* to form biofilms.

Strains	OD value	Results
S4,S5,S12,S13,S14,S16,S17,S18,S19,S20,S26,S28, S32, S33, S36, S37, S39	0,05-0,64	NBF(37.78%)
S1, S2, S3, S6, S7, S8, S10, S11, S15, S21, S22, S23, S24, S25, S27, S29, S30, S31, S34, S35, S38, S40, S41, 44, S45	0,68-1,27	WBF(55.56%)
S9, S42, 43	2,02-2,15	MBF(6.67%)

NBF: No biofilm formation. WBF: Weak biofilm formation. MBF: Moderate biofilm formation of the strains studied, 17 are classified as Non-Biofilm Formers (NBF) with a low capacity to form biofilms, 25 are Weak Biofilm Formers (WBF) forming moderate biofilms, and 3 are Moderate Biofilm Formers (MBF).

The *Salmonella* strains here appear to form small quantities of biofilms (25) in large majority, a fairly large proportion (17) do not form biofilms, only a few strains (3) form moderate biofilms **(Fig.3).**

Fig.3: The ability of the strains of *Salmonella* to form biofilms (%).

Correlation between antibiotic resistance and biofilm formation

We investigated the relationship between biofilm formation and antibiotic resistance in *Salmonella* strains from broiler chicken farms. A Spearman correlation hit map was used to visualize these associations with statistically significant set at $p < 0.05$. The Spearman correlation Heat map shows the relationships between biofilm formation and resistance to different antibiotics. The correlation values range from -1 to +1. Biofilm formation appears to be mostly negatively correlated with antibiotic resistance in *Salmonella* strains. **(Fig.4)**

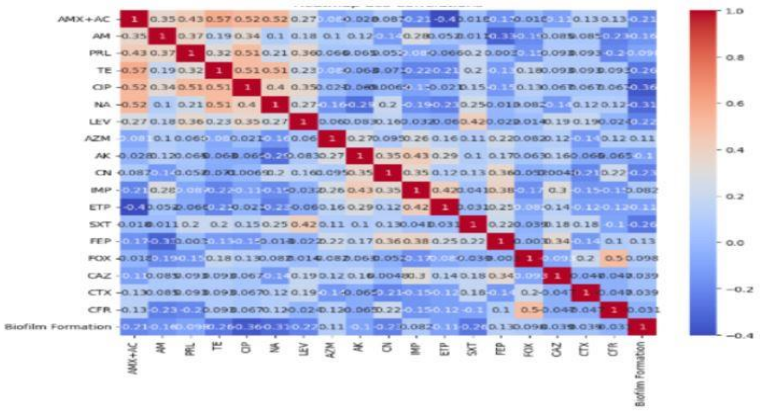

Fig.4: Heat map of Spearman correlations between biofilm formation and antibiotic resistance of *Salmonella* spp.

DISCUSSION

Salmonella can asymptomatically colonize poultry, contaminating products and the human food chain, making it a major cause of human salmonellosis **(Cosby *et al.,* 2015).** Rising antibiotic resistance has exacerbated this issue, making *Salmonella* a significant public health threat. *Salmonella Typhimurium* and *Salmonella Enteritidis*, both zoonotic pathogens, are prevalent in many countries **(Shaji *et al.,* 2023; Moghadam *et al.,* 2023).** Transmission to poultry occurs both horizontally and vertically, with infected animals spreading the pathogen throughout the poultry supply chain.

In this study, 45 *Salmonella* strains were isolated from 270 samples collected from broiler farms in Biskra, Algeria, yielding a prevalence of 16.66%. This result is consistent with **Jibril *et al.,* (2023)** in Nigeria, who reported a prevalence of 15.9%. It is lower than the 34.37% prevalence observed in Skikda, Algeria, by **Djeffal *et al.,* (2018),** likely due to differences in sampling locations, with slaughterhouses generally having higher contamination rates than farms. Our findings also surpass the 2.39% prevalence reported in Eastern Algeria by **Ayachi *et al.,* (2010).** In neighboring countries, similar prevalence rates were recorded, such as 19.9% in Tunisia **(Oueslati *et al.,* 2021)** and 9.8% in Morocco **(Zahli *et al.,* 2022)**; while Somalia reported a lower rate of 8.9% **(Cige *et al.,* 2023).**

The semi-arid climate of Biskra may contribute to higher infection rates in poultry. Heat and drought can weaken their immune systems, making them more susceptible to infection, while the dry conditions promote bacterial spread through dust. **Jibril *et al.,* (2023)** also highlighted the impact of poor feed, hygiene, and water management in such climates, further increasing contamination risks.

The samples collected from various sources, including feces, cloacal swabs, surface swabs, and water from reservoirs and drinking lines, resulted in a 16.6% isolation rate of *Salmonella*. Specifically, we recovered 26.6% of *Salmonella* from droppings and 28.71% from water, which aligns with findings by **Hassan *et al.,* (2019).** However, results from **Singh *et al.,* (2013)** and **Djeffal *et al.,* (2018)** were much lower, at 2.5% and 3.3%, respectively. **Julianingsih *et al.,* (2024)** reported varying rates of 2.7% and 16%, while **Mridha *et al.,* (2020)** recorded slightly higher rates of 40.09% and 17.9%.

Antimicrobial resistance in poultry poses significant public health risks, with *Salmonella* responsible for over 600,000 antibiotic-resistant infections annually **(Hagras *et al*., 2024).** Assessing the resistance of isolates to commonly used antibiotics is crucial for improving treatment and control strategies **(Arkali and Cetinkaya, 2020).** *Salmonella* from broiler chickens exhibits higher resistance rates compared to other domestic animals **(EFSA and ECDC 2020).**

This study tested 19 antimicrobial agents and found high resistance in *Salmonella* isolates from poultry farms in Biskra, Algeria, particularly against beta-lactams, tetracyclines, quinolones, and macrolides. The highest resistance was observed to ampicillin (97.78%), tetracycline (95.56%), ciprofloxacin (91.11%), piperacillin (86.67%), nalidixic acid (77.78%), levofloxacin (60%), and azithromycin (82.22%). These results are consistent with findings from **Gharaibeh *et al.,* (2024)** and **Habib *et al.,* (2024).** The widespread use of antimicrobials in poultry for therapeutic, prophylactic, and growth-promoting purposes likely contributes to this high resistance. Moderate resistance was observed for aminoglycosides (Amikacin 64.44%, Gentamicin 55.56%), carbapenems (Imipenem 33.33%, Ertapenem 24.44%), and sulfonamides (Sulfamethoxazole-Trimethoprim 17.78%), consistent with **Ogu *et al.,* (2021)** for aminoglycosides and **Wajid *et al.,* (2019)** for carbapenems. The irrational use of antimicrobials in poultry farming accelerates the development of resistant strains.

Low resistance was observed for cefepime (37.78%), while very low resistance occurred for cefoxitin (15.56%), ceftazidime (8.89%), cefotaxime (4.44%), and cefadroxil (4.44%). Given that cephalosporins are critical antibiotics in human medicine, their use in animals is banned for disease treatment, as for nitrofurans (2.22%), which are carcinogenic antibiotics. Our findings on beta-lactam resistance (Ampicillin, Amoxicillin-clavulanate, Piperacillin) align with **Hagras *et al.,* (2024)** who reported 100% resistance to these drugs and **Hassan *et al.,* (2019)** especially for Ampicillin. **Singh *et al.,* (2013)** also found long-standing resistance to beta-lactams, such as penicillin and oxacillin. The widespread use of beta-lactams has led to resistance through genetic mutations and the acquisition of resistance genes via mobile genetic elements like plasmids, transposons, and integrons **(Courvalain and Leclercq, 2012).**

Tetracycline resistance was observed at 95.56%, consistent with the findings of **Abdi *et al.,* (2017)** and **Al-Zenki *et al.,* (2007)** who reported resistance rates of 97.8% and 96.5%, respectively. Other studies, such as those by **Mridha *et al.,* (2020)** and **Assoumy *et al.,* (2021)** reported lower resistance rates of 80% and 82.4%. Tetracycline is widely used in veterinary medicine and as a growth promoter in poultry farming, contributing to the selection of resistant bacterial strains.

Resistance to quinolones (Ciprofloxacin, Levofloxacin, and Nalidixic acid) was high, with rates of 91.11%, 77.78%, and 60%, respectively. These findings are similar to those of **Oueslati *et al.*, (2022)** and **Magdy *et al.*, (2020)** who reported resistance to quinolones ranging from 58.5% to 82.78%. Quinoline resistance is often linked to the extensive use of drugs like nalidixic acid and flumequine in poultry treatment.

The resistance rate for Azithromycin was 82.22%, aligning with the 80% reported by **Mridha *et al.*, (2020)** but significantly higher than the 21.43% found by **Waghamare *et al.*, (2018)** and 18% by **Julianingsih *et al.*, (2024)** for *Salmonella Pullorum*. This resistance may result from the suboptimal use of azithromycin in veterinary practice due to its higher cost compared to other macrolides like tylosin and erythromycin. Aminoglycoside resistance was observed at 64.44% for Amikacin and 55.56% for Gentamicin. These rates were higher than those reported by **Nguyen *et al.*, (2021)** (0.55% and 6%) but lower than the 75% resistance for Gentamicin reported by **Magdy *et al.*, (2020).** These results are consistent with **Assoumy *et al.*, (2021)** who reported 47% and 52% resistance for Gentamicin and Streptomycin. Resistance to carbapenems (Imipenem 33.33%, Ertapenem 24.44%) was similar to the findings of **Wajid *et al.*, (2019)** who reported resistance rates of 70% for Imipenem and 24% for Ertapenem. In Algeria, carbapenems and aminoglycosides are often used illegally, and this trend is not adequately controlled.

Sulfonamide resistance (Sulfamethoxazole-Trimethoprim) was 17.78%, higher than the 12.5% reported by **Hassan *et al.*, (2019)** but lower than the 79.8% resistance rate found by **Gharaibeh *et al.*, (2024).** The relatively low use of sulfonamides in avian veterinary practices, due to their potential nephrotoxicity and need for prolonged administration, may contribute to these resistance rates. The lowest resistance rates were observed for cephalosporins (Cefoxitin 15%, Ceftazidime 8%, Cefotaxime 4%, and Cefadroxil 4%), consistent with the results of **Guechtouli *et al.*, (2024); Nguyen *et al.*, (2021)** and **Assoumy *et al.*, (2021)** who reported very low resistance to Ceftazidime (0%, 1.1%, 4%). These low resistance rates can be attributed to the restricted use of cephalosporins in poultry, as these antibiotics are primarily reserved for human medicine. The highest prevalence of multidrug resistance in *Salmonella* strains was observed against 15 antibiotics, with a resistance rate of 33%, followed by resistance to 9 antibiotics at 20%. The lowest resistance rates were observed for 4 and 5 antibiotics, with rates of 8% and 11%, respectively.

This study revealed high levels of multidrug resistance in *Salmonella* isolates from broiler farms, including resistance to AM, PRL, CN, ETP, NA, AZM, AK, TE, CIP, FEP, LEV, and AMX+AC, CAZ. The spread of these resistant strains through the environment, food-producing animals, and humans poses a significant public health risk. Some isolates exhibited resistance to at least six antibiotics, raising concerns due to the severe outcomes often associated with such infections **(Saha and Sarkar, 2021).** Multidrug-resistant infections limit treatment options and negatively impact both animal and human health **(Wang *et al.*, 2020).** This issue is particularly critical in developing countries with limited healthcare resources **(Saha and Sarkar, 2021).** Since October 2020, Algeria has been a member of the Global Surveillance System for Antibiotic Use and Multidrug Resistance (GLASS), a global initiative to standardize surveillance. However, surveillance systems for veterinary medicine have not yet been implemented **(Kamel *et al.*, 2024).**

Biofilm formation is a survival mechanism that allows pathogenic bacteria to resist harsh conditions, including antibiotics and disinfectants, while enhancing virulence. This trait is linked to the growing prevalence of antibiotic-resistant pathogens, particularly in environments with poor hygiene **(Sauer *et al.*, 2022).** *Salmonella* can form biofilms even in nutrient-limited conditions, and strains that form biofilms on surfaces like plastic are often strong producers **(Stepanovic *et al.*, 2004).** This study used microtiter plates to replicate materials commonly found in poultry production.

Biofilm formation varied among the *Salmonella* strains. Most strains (55.56%) were weak biofilm producers, 37.78% did not form biofilms, and 6.67% formed moderate biofilms. Similar studies have reported varying biofilm production levels, with **Agarwal *et al.*, (2011)** finding that 57.61% of *Salmonella* strains were moderate biofilm producers, while **Nair *et al.*, (2015)** suggested that weak biofilm producers, especially from poultry, may acquire genetic traits over time, increasing their pathogenic potential. **Musa *et al.*, (2024)** reported that 58.75% of strains formed strong biofilms, 36.25% formed moderate biofilms, and 6.25% were weak producers. Biofilms enhance gene transfer, including the transfer of virulence and antibiotic resistance genes, contributing to the rise of resistant pathogens **(Galloway *et al.*, 2012).** The biofilm matrix limits antimicrobial penetration, and bacteria within biofilms may exhibit resistance traits due to stress or nutrient deprivation **(Gilbert *et al.*, 2002).**

A negative significant relationship was found between antibiotic resistance and biofilm formation in this study, consistent with findings from **Cwiek *et al.*, (2020)** and **Voss-Rech *et al.*, (2023).** However, **Brito *et al.*, (2024)** reported a positive correlation between these two factors.

CONCLUSION

This study reveals that *Salmonella* spp. strains isolated from broiler chicken farms in Biskra, Algeria, exhibit high resistance to ampicillin, tetracycline and ciprofloxacin. Most strains demonstrated weak biofilm formation and a statistically significant negatively correlated relationship with antibiotic resistance in Salmonella strains. Future research should focus on exploring alternative treatment strategies, investigating the genetic determinants of resistance, and assessing their link to *Salmonella* isolates from poultry and their environment. To reduce antibiotic reliance and combat the spread of resistant bacterial strains, the use of vaccines or probiotics is strongly recommended. Additionally, the implementation of regular antimicrobial resistance monitoring programs, the adoption of strict biosecurity measures on farms, and improved hygiene practices throughout the production chain are essential to limit *Salmonella* contamination and the emergence of resistant strains.

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Conflict of Interest

The author declares that no conflict of interest is reported.

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