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Epidemiological study and identification of *Escherichia coli* strains associated with clinical events in Avian farming

Estudio epidemiológico e identificación de cepas de *Escherichia coli* asociadas a episodios clínicos en avicultura

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ABSTRACT

Avian pathogenic *Escherichia coli* (APEC) represents a major challenge for the poultry industry, causing significant economic losses. This problem is exacerbated by the misuse use of antibiotics in Veterinary Medicine, leading to the emergence of resistant strains and thus creating a significant risk to Public Health. This study, carried out on 38 poultry farms in Algeria, involved the collection of 200 samples for the isolation of *E. coli* strains. The resistance of these strains to frequently used antibiotics was assessed using the agar diffusion method. Multiple Correspondence Analysis (MCA) was used to determine potential risk factors. The obtained results revealed that *E. coli* was present in 30% of samples. Alarming levels of resistance were observed against Tetracycline (81.6%), Ampicillin (78.3%), Ciprofloxacin (68.3%) and Nalidixic acid (60%). Stressful environmental conditions in poultry houses, such as temperature variations, high humidity, poor ventilation and stocking density were identified as key factors in the development of avian colibacillosis. In conclusion, the current study highlights the urgent need to strictly monitor and regulate the use of antibiotics in Veterinary Medicine and improve animal welfare in order to minimize the risk it pose to Public Health originated in the farms. In addition, it is essential that farmers maintain optimal environmental conditions in chicken rearing.

Key words: Algeria; antibiotic resistance; avian colibacillosis; avian pathogenic; *Escherichia coli*; risk factors

RESUMEN

La *Escherichia coli* patógena aviar (EPA) representa un importante reto para la industria avícola, causante de cuantiosas pérdidas económicas. Este problema se ve agravado por el uso inadecuado y excesivo de antibióticos en medicina veterinaria, que conduce a la aparición de cepas resistentes y crea así un riesgo importante para la salud pública. El presente estudio, realizado en 38 granjas avícolas de Argelia, consistió en la recogida de 200 muestras para el aislamiento de cepas de *E. coli*. La resistencia de estas cepas a antibióticos de uso frecuente se evaluó mediante el método de difusión en agar. Para determinar los posibles factores de riesgo se utilizó el Análisis de Correspondencias Múltiples (ACM). Los resultados obtenidos revelaron que *E. coli* estaba presente en el 30 % de las muestras. Se observaron niveles alarmantes de resistencia frente a la tetraciclina (81,6 %), la ampicilina (78,3 %), la ciprofloxacina (68,3 %) y el ácido nalidíxico (60 %). Las condiciones ambientales estresantes en los gallineros, como variaciones de temperatura, mayor humedad, ventilación deficiente y densidad de población, se identificaron como factores clave en el desarrollo de la colibacilosis aviar. En conclusión, este estudio pone de relieve la urgente necesidad de vigilar y regular estrictamente el uso de antibióticos en medicina veterinaria y mejorar el bienestar animal para minimizar el riesgo para la salud pública. Además, es esencial que los granjeros mantengan unas condiciones ambientales óptimas en la cría de pollos.

Palabras clave: Argelia; resistencia a los antibióticos; colibacilosis aviar; *Escherichia coli*; patógena aviar; factores de riesgo

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INTRODUCTION

Avian pathogenic *Escherichia coli* (APEC) is an extra-intestinal pathogen responsible for local and systemic infections in poultry [1]. The most frequent manifestations of APEC infection in chickens (*Gallus gallus domesticus*) include pericarditis, omphalitis, aerosacculitis, egg-related peritonitis, salpingitis, cellulitis, as well as osteomyelitis, and arthritis. These are commonly known as avian colibacillosis [2], which is recognized as a disease with considerable economic consequences in Algeria and Worldwide [3]. This leads to substantial losses, resulting in high mortality and reduced performance [2].

APEC infections can be primary or secondary, if they occur because of immunosuppressive disease or environmental stress. The bacteria are introduced via the oral and respiratory tracts [4]. Chickens become infected through contaminated feed and water and can be transmitted to other birds via the fecal-oral route or by aerosol. In addition, APEC can be transmitted vertically by infected breeding stock via contaminated eggs [3].

The pathogenicity of APEC lies in its ability to deploy various virulence and pathogenesis factors such as adhesins, invasins, toxins, host serum resistance and iron acquisition systems [5]. These factors allow escape from the host immune system, colonization and systemic dissemination of APEC, facilitating the establishment of infection in poultry [5].

Antibiotics are widely used in the poultry industry to combat avian colibacillosis. In many countries, the administration of antimicrobial agents is not limited to therapeutic purposes [6]. Antimicrobials are also used to improve productivity and feed conversion rates [4]. However, the continued administration of these molecules leads to the emergence of resistant strains [6]. These strains can be transmitted to humans through the food chain, posing a serious risk to Human Health [6].

The aim of this study was to isolate and identify *E. coli* strains associated with clinical events in poultry farms, to establish their antibiotic resistance profile and to identify potential risk factors contributing to the development of infection.

MATERIAL AND METHODS

Ethical statement

The animal experiment was approved by the Ethics Committee of the Mouloud Mammeri University in Tizi-Ouzou. The committee gave an approval number: UMMTO/2022/Ani021. Written informed consent was obtained from all participants prior to publication of this study.

Samples collection

Two hundred samples were taken from organs (mixed liver and heart, lungs, intestines, and joints) of sick chickens showing typical *E. coli* lesions, from 38 poultry farms located in the wilaya of Tizi-Ouzou in Algeria during the year 2022. Even if these animals are not intended for consumption, resistant bacterial strains can potentially contaminate the environment, other animals or people who come into contact with them, which could ultimately present risks to public health.

Isolation and identification of isolates

Samples were crushed and homogenized in BHIB and incubated at 37 degrees for 24 h, then plated on Hektoen medium. Identification of *E. coli* was based on morphological, microscopic and biochemical differential tests, including oxidase, indole, urea and citrate permease, then confirmed using the API 20E kit (Bio Mérieux, France).

Antibiotic susceptibility testing

Antimicrobial susceptibility was determined using Muller-Hinton agar (Oxoid) disc diffusion method and interpreted according to CLSI M100 2020, using interpretive categories zone diameters breakpoints for each tested antibiotic. This approach allowed us to assess the susceptibility of bacterial strains to the antibiotics used in our study and classify them into 3 categories: susceptible, intermediate, and resistant. It also allowed us to evaluate the current status of antibiotic resistance in *E. coli* in the Algerian poultry industry, rather than the effectiveness of clinical treatment. However, the epidemiological investigation provides additional insights into the factors favoring the occurrence of cases of avian colibacillosis, but is independent of the study of antibiotic resistance [7]. The tested antibiotics (Oxoid, UK) were: Ampicillin AMP (10 µg), Amoxicillin/Clavulanic acid AMC (30 µg), Piperacillin PIP (100 µg), Cefazolin KZ (30 µg), Cefoxitin FOX (30 µg), Cefotaxime CTX (30 µg), Ceftazidime CAZ (30 µg), Cefepime FEP (30 µg), Aztreonam ATM (30 µg),Imipenem IMP (10 µg), Meropenem MEM (10 µg), Gentamicin CN (10 µg/disc), Tetracycline TE (30 µg), Sulfamethoxazole SXT (1.25/23.75 µg), Nalidixic acid NA (30 µg), (20 µg), Ciprofloxacin CIP (5 µg), Amikacin AK (30 µg), Nitrofurantoin N (300 µg) and Chloramphenicol CHL (30 µg). *Escherichia coli* ATCC 29522 strain was used as quality control. The panel of antibiotics was selected taking into account their common use in the poultry industry and their critical importance in human medicine.

Phenotypic detection of extended-spectrum beta-lactamases (ESBL)

The presence of extended-spectrum beta-lactamases (ESBL) was detected by the double-disk synergy method and confirmed by the E-test CT/CLT.

Survey form

An investigation was carried out on these 38 poultry farms to collect data on clinical episodes of avian colibacillosis. Information was collected using a survey form, including variables such as age of chickens, rearing season, number of birds, mortality and morbidity rates, clinical symptoms, type of building, type of aeration, water source, Feed type, humidity level and ventilation type. The data were analyzed to identify trends and associated factors.

Statistical analysis

A Multiple Correspondence Analysis (MCA) was performed to identify potential risk factors associated with colibacillosis, using SPSS software (version 25.0).

RESULTS AND DISCUSSIONS

Isolation and identification

The prevalence of *E. coli* was estimated at 30%, while the remaining isolates involving other germs, such as *Klebsiella* and *Enterobacter*. 46.6% Isolates were derived from different anatomical sites, with the majority originating from the intestine (46.6%), followed by the lungs (31.6%), the heart and liver (18.3%), and the joints (3.3%)..These observations are discordant with the results of other studies carried

out in Algeria, such as Halfaoui *et al.* [8], who identified 156 strains out of 180 samples (86.66%), and Benklaouz *et al.* [9], who recorded 145 isolates out of 290 samples (50%). These discrepancies in *E. coli* detection rates could be attributed to a multitude of factors, including the methodology used, the study period, environmental variations and husbandry practices.

Antimicrobial susceptibility testing

According to the results of the disk diffusion test shown in FIG. 1 and TABLE I, the highest rates of prevalent resistance were recorded against TE and AMP, reaching 81.6% and 78.3%, respectively. Percentage of resistance detected were also notable for PIP at 76.6%, CIP at 68.3%, STX at 65%, and NA at 60%. CHL and KZ exhibited resistance at rates of 33.3% and 28.33%, respectively. In contrast, the antibiotics AMC, CTX, ATM, FEP, CN, and N showed relatively low rates of resistance. All strains were susceptible to FOX, CAZ, IMP, MEM and AK. Only one strain tested positive for ESBL production.

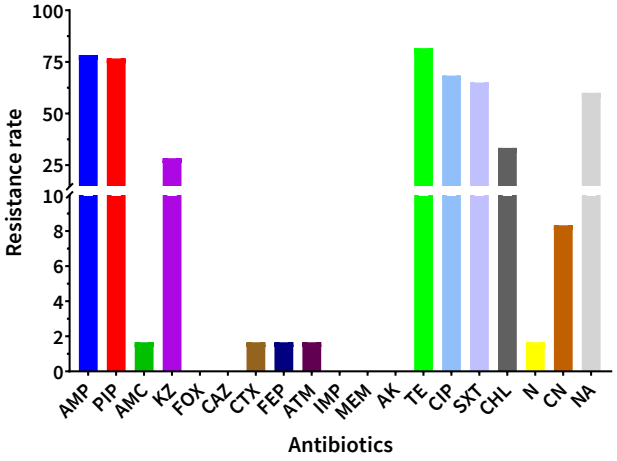

FIGURE 1. Frequency of Antibiotic Resistance

TABLE I
Results of antimicrobial susceptibility testing

Strains	AMP	PIP	AMC	KZ	FOX	CAZ	CTX	ATM	FEP	IMP	MEM	NA	AK	TE	CIP	CN	SXT	N	CHL
	S ≥ 17	S ≥ 21	S ≥18	S ≥ 23	S ≥ 18	S ≥ 21	S ≥ 26	S ≥ 21	S ≥ 25	S ≥ 23	S ≥ 22	S ≥19	S ≥ 17	S ≥ 15	S ≥ 26	S ≥ 15	S ≥ 16	S ≥ 17	S ≥ 18
	R ≤ 13	R ≤ 17	R≤13	R ≤ 19	R ≤ 14	R ≤ 17	R ≤ 22	R ≤ 17	R ≤ 18	R ≤ 19	R ≤ 18	R ≤ 13	R ≤ 14	R ≤ 11	R ≤ 21	R ≤ 12	R ≤ 10	R ≤ 14	R ≤ 12
E coli ATCC 25922	24	25	24	24	25	29	31	32	35	34	33	25	26	22	32	30	25	24	26
E1	6	17	17	21	22	29	30	30	34	30	33	6	25	15	12	25	6	20	6
E2	6	17	19	21	21	30	30	31	33	30	33	6	25	6	13	25	6	18	21
E3	6	12	18	17	22	30	29	30	33	30	33	6	21	6	6	22	27	23	6
E4	6	15	18	20	22	30	29	30	34	30	32	6	21	6	15	15	6	24	26
E5	6	17	21	20	22	31	29	25	34	32	33	6	23	6	17	20	6	22	6
E6	6	14	18	18	22	31	30	30	34	32	33	15	23	6	25	25	6	29	6
E7	6	17	22	21	22	30	29	33	34	30	32	6	21	6	14	19	6	27	6
E8	18	29	21	22	22	27	30	30	35	30	33	21	22	23	35	20	27	23	25
E9	6	16	19	20	22	30	30	30	35	30	32	6	21	6	13	20	6	17	24
E10	17	25	20	22	22	30	30	28	35	30	32	6	20	21	21	20	21	19	24
E11	6	12	17	17	22	26	23	29	33	30	33	6	19	6	6	24	29	21	24
E12	6	15	19	20	21	25	29	30	31	30	29	12	21	6	20	16	6	25	24
E13	6	16	18	20	24	29	29	26	30	30	29	16	19	6	21	16	30	24	24
E14	21	28	22	24	22	30	29	30	33	30	30	15	21	14	25	21	6	29	6
E15	6	22	20	21	22	31	28	35	34	31	33	6	21	6	21	20	6	27	6
E16	6	18	18	20	21	27	29	30	33	30	30	6	21	10	31	20	6	27	25
E17	0	15	16	15	22	30	28	32	34	30	32	6	22	6	12	18	6	21	6
E18	6	17	18	20	18	30	26	30	35	30	32	6	21	6	16	18	6	25	6
E19	6	15	18	20	22	29	23	30	34	30	30	6	22	6	10	19	6	15	6
E20	6	12	16	18	22	28	25	30	34	30	30	6	20	6	6	20	6	25	6
E21	6	12	17	19	22	27	23	30	33	30	30	18	22	6	27	18	6	21	6
E22	6	14	16	17	22	28	25	30	34	30	30	6	23	6	11	20	30	19	22
E23	6	18	18	18	22	30	25	30	33	30	30	6	22	27	14	20	6	25	15
E24	6	18	16	20	23	30	25	30	33	32	30	6	21	6	6	20	6	19	6
E25	6	14	22	20	22	28	30	29	31	30	30	6	21	6	10	21	20	22	20

TABLE I
Results of antimicrobial susceptibility testing (cont...)

E26	6	15	18	20	20	27	23	28	33	30	30	6	19	6	9	19	6	16	22
E27	6	15	16	18	20	28	24	29	34	30	30	6	22	6	6	6	6	22	6
E28	6	16	18	20	20	28	23	30	34	30	30	6	20	6	10	20	6	17	24
E29	6	15	11	18	16	28	22	30	34	30	30	6	22	6	6	6	6	22	6
E30	6	10	20	6	25	22	6	15	18	30	30	6	21	6	25	18	6	25	25
E31	6	17	13	13	18	25	20	30	34	31	30	6	22	6	10	19	6	20	6
E32	6	16	20	20	22	31	25	31	35	30	30	6	22	6	27	6	20	22	24
E33	6	17	21	15	22	33	34	30	33	35	34	6	19	6	22	18	20	21	20
E34	6	17	21	14	22	30	29	29	32	33	32	6	20	6	6	18	6	21	22
E35	19	25	24	23	22	29	30	29	32	33	32	6	20	6	6	18	6	15	21
E36	20	27	24	23	24	31	32	32	33	31	31	6	20	6	26	20	20	22	24
E37	6	13	22	13	24	29	31	29	30	31	30	6	22	6	6	18	6	23	24
E38	6	14	20	18	22	32	33	30	30	32	30	6	20	6	6	12	6	22	23
E39	6	15	23	12	22	30	29	30	30	33	32	6	20	6	6	19	6	21	21
E40	6	17	23	21	21	28	31	30	30	33	32	6	21	6	6	25	6	14	23
E41	6	12	20	16	24	31	33	30	30	30	32	6	20	6	6	6	6	18	23
E42	6	16	22	22	24	30	32	30	30	32	30	19	20	6	15	23	6	19	21
E43	6	18	22	21	23	30	32	30	32	30	30	19	20	6	6	25	26	21	24
E44	6	16	22	20	24	30	32	30	31	30	31	20	20	6	33	25	6	20	23
E45	21	27	24	29	24	30	30	32	31	30	30	19	18	6	25	23	6	21	21
E46	21	30	24	28	25	33	34	30	34	31	30	19	22	6	25	27	27	21	20
E47	6	15	24	20	25	28	29	30	30	30	31	20	20	6	6	24	6	22	6
E48	20	23	20	18	25	30	30	30	32	30	31	20	20	25	30	25	27	22	20
E49	6	15	23	20	25	26	30	30	32	30	30	20	20	6	15	25	6	21	25
E50	6	15	22	20	23	29	30	30	32	33	30	19	20	6	27	25	24	18	24
E51	25	31	25	25	25	31	30	31	35	32	31	21	23	25	27	18	25	18	27
E52	6	16	21	22	25	30	31	31	34	33	32	20	23	6	12	28	6	19	6
E53	6	13	20	20	24	30	31	30	34	30	32	19	20	6	6	24	6	20	20
E54	6	16	21	20	24	30	31	31	35	32	30	19	20	6	15	24	25	19	21
E55	20	29	24	22	25	29	32	31	33	32	31	19	22	25	28	24	26	20	26
E56	20	28	24	23	25	31	30	32	35	32	31	19	21	25	30	34	26	19	25
E57	6	15	19	20	22	31	30	30	34	32	31	21	19	6	6	24	6	19	6
E58	6	16	24	20	25	30	30	30	32	30	31	21	22	6	6	26	6	21	6
E59	19	30	22	23	25	29	30	31	35	30	31	21	20	23	30	24	25	22	25
E60	19	27	21	23	25	30	32	31	34	30	31	20	20	22	30	24	23	21	21

A multiresistant strain is a bacterium that exhibits resistance to at least three different classes of antimicrobials [6]. In this study, 80% of isolates were found to be multi-resistant to at least three different classes of antibiotics. Specific resistance rates were 15.33% for five classes of antibiotics, 26.66% for six classes and 18.33% for seven classes. A high prevalence of multiresistance (56.66%) was observed for six specific antibiotics (TE, AMP, PIP, CIP, STX, NA). A total of 32 antibiotypes were obtained from APEC isolates (Table II), antibiotic profiles are the result of the agar diffusion test, reflecting a diversity of antibiotic sensitivity/resistance among isolated strains and providing information on the state of antibiotic resistance in the poultry sector. The most prevalent resistance profile being: AML, PIP, NA, TE, CIP, SXT, CHL.

In the present study, it was examined the resistance of *E. coli* strains to 19 antibiotics. Tetracycline showed the highest rate of resistance (81.6%), which is similar to the results reported by Aggad *et al.* [10] and Belmahdi *et al.* [11], where resistance rates were 87 and 90%, respectively. Resistance to Ampicillin (78.3%) was comparable to the findings of Halfaoui *et al.* [8] and Mansouri *et al.* [12] in Algeria, and Dou *et al.* [13] in China, where the

TABLE II
Antibiotic resistance profiles of isolated strains

Strains	Antibiotypes
E1	AMP, PIP, NA, CIP, SXT, CHL
E2, E12	AMP, PIP, NA, TE, CIP, SXT
E3	AMP, PIP, KZ, NA, TE, CIP, CHL
E4, E16, E28	AMP, PIP, NA, TE, CIP, SXT
E5, E7, E24, E20, E17, E18, E19	AMP, PIP, NA, TE, CIP, SXT, CHL
E6	AMP, PIP, KZ, TE, SXT, CHL
E9, E47, E52, E57, E58	AMP, PIP, TE, CIP, SXT, CHL
E10	AN, CIP
E11, E22	AMP, PIP, KZ, NA, TE, CIP
E14	SXT, CHL
E15	AMP, NA, TE, CIP, SXT, CHL
E21	AMP, PIP, KZ, TE, SXT, CHL
E23	AMP, PIP, KZ, NA, CIP, SXT
E25	AMP, PIP, NA, TE, CIP
E26	AMP, PIP, NA, TE, CIP, SXT
E27	AMP, PIP, KZ, NA, TE, CIP, CN, SXT, CHL
E29	AMP, PIP, AMC, KZ, NA, TE, CIP, CN, SXT, CHL
E30	AMP, PIP, KZ, CTX, ATM, FEP, NA, TE, SXT
E31	AMP, PIP, AMC, KZ, NA, TE, CIP, SXT, CHL
E32	AMP, PIP, NA, TE, CN
E33	AMP, PIP, KZ, NA, TE
E34, E37, E39	AMP, PIP, KZ, NA, TE, CIP, SXT
E35	NA, TE, CIP, SXT
E36	NA, TE
E38, E41	AMP, PIP, KZ, NA, TE, CIP, CN, SXT
E40	AMP, PIP, KZ, NA, TE, CIP, SXT, F
E42, E49, E53, E13	AMP, PIP, TE, CIP, SXT
E43, E54	AMP, PIP, TE, CIP
E44	AMP, PIP, TE, SXT
E45	TE, SXT
E46	TE
E50	AMP, PIP, TE

resistance rate was 80.3%. The high levels of resistance to Tetracycline and Ampicillin are partly due to their prolonged use as growth promoters and therapeutic treatments in the poultry sector [14].

In addition, the incorporation of heavy metals into poultry feed as additives has enabled bacteria to acquire resistance to these metals [15], which can be accompanied by antibiotic resistance due to the phenomenon of co-selection. Indeed, metal and antibiotic resistance genes can be located on the same genetic structure, such as plasmids or transposons [15]. This phenomenon has been observed in a variety of situations, including co-resistance to copper, silver, mercury and tetracycline; and co-resistance to copper, silver, β-lactam and fluoroquinolone [16]. In addition, it has been reported that some microorganisms use coregulation, a mechanism based on regulatory proteins, to coordinate resistance to heavy metals and antibiotics, enabling them to simultaneously develop defense mechanisms against both types of substance [16].

Ciprofloxacin showed a resistance rate of 68.33%, which corroborates the results obtained by Meguenni *et al.* [17]. Regarding Nalidixic acid, in the present study revealed a resistance rate of 60%, which remains lower than that reported by some studies carried out in Algeria, which reported rates ranging from 90 to 95% [18, 19]. Veterinarians often use Nalidixic acid and Ciprofloxacin to prevent early chick mortality and contain the spread of avian diseases, due to their affordability on the Algerian market [20]. However, the widespread use of quinolones and fluoroquinolones in poultry farming has led to a growing problem of resistance. Part of this resistance could be attributed to the persistence of residues of these antibiotics in poultry drinking water [21]. Chickens consuming water contaminated with these residues progressively develop resistance, especially in the event of prolonged exposure to antibiotics, thus promoting the transfer of resistance genes between different bacteria in the gastrointestinal tract [22]. It is essential to note that quinolones and fluoroquinolones are classified as "critically important antimicrobials" by the World Health Organization (WHO), due to their importance in Human Medicine [23]. Resistance of avian bacteria to these antibiotics may represent a risk to Human Health due to their potential transmission through the food chain via cross-contamination [24].

The rate of resistance to Trimethoprim sulfonamides was 65%, concordant with the findings of Benameur *et al.* [25] and Aberkane *et al.* [19]. Even higher resistance rates (95.5%) were observed in the study by Ibrahim *et al.* [26] in Jordan. The difference could be attributed to a variety of factors, including variations in bacterial strains, antibiotic use practices and local conditions specific to each Region. These molecules are commonly used in Veterinary Medicine to prevent and treat various avian diseases, which could explain the high levels of resistance observed [27].

The rate of resistance to Chloramphenicol was 33.33%, similar to the results of Halfaoui *et al.* [8]. This resistance could be due to the persistence of pre-existing resistances or to the misuse of this substance, as it is prohibited in the breeding context, as well as to the phenomenon of co-selection [28].

Resistance to Cefazolin (28.33%) could be explained by co-selection resulting from the frequent or inappropriate use of other antibiotics in the same class, such as Ampicillin [29]. The low resistance to Gentamicin (8.3%) agrees with the study carried out by Levy *et al.* [3] in Bangladesh (8.3%) and by Kiiti *et al.* [30] in Tanzania. This could be the result of inappropriate use, given that this antibiotic is banned in Veterinary Medicine in Algeria. By comparing our results to previous studies, we may observe a trend towards an increase in antimicrobial resistance, but this would require in-depth data analysis and an understanding of contextual factors specific to each study [17].

The molecules AMC, CTX, ATM, FEP and N showed the lowest resistance rates, with only 1.6% resistance. It should be noted that these antibiotics are not used in Veterinary Medicine [8]. While all *E. coli* strains were sensitive to FOX, CAZ, IMP, MEM and AK, as these substances are not used in avian pathology [31, 32].

Finally, only one strain was identified as positive for ESBL production. Such strains have also been reported by Benklaouz *et al.* [9] and Halfaoui *et al.* [8]. Recent studies have shown a widespread

spread of ESBL-producing *E. coli* in animals intended for human consumption in Algeria, despite the rare use of third generation Cephalosporins in poultry farming [11]. This resistance could result from the selection of ESBL-producing *E. coli* strains due to excessive use of other antibiotics, notably quinolones [9]. Furthermore, the use of Ampicillin could favour the appearance of mutations leading to the emergence of ESBL-producing mutants derived from the bla TEM-1 or bla SHV-1 genes [28].

Frequent use of antimicrobial agents results in selective pressure leading to resistance to anti-APEC antimicrobials [33]. In addition, the constant use of low-dose antibiotics in poultry feed, mainly for growth promotion, promotes the production and spread of antibiotic resistance genes, thus contributing to the emergence of antibiotic resistance [21].

The increasing use of disinfectants also plays a role in the rise of bacterial resistance [34]. It has been reported that antibiotic resistance is not solely dependent on the use of antibiotics but may also result from excessive use of disinfectants and biocides [33], as these products contribute to the cross-selection of resistance mechanisms [27].

Risk factors

Multiple correspondence Analysis (MCA) revealed two principal components: dimension 1 and dimension 2 (FIG. 2-A, B).

The most discriminating variables for axis 1 are: type of building, season, clinical symptoms, water source and soil type, while for axis 2, they are: age and stocking density. The most relevant variables are age, density, building type and symptoms (FIG. 2-C). Age is significantly correlated with building type, density is correlated with symptoms, and season is correlated with soil type and building type.

According to the survey (TABLE III), the majority of colibacillosis-infected chickens (n=55) are separated into two groups, the first in the start-up phase (58.33%) and the second in the growth phase (30%). They are generally housed in solid-structure buildings (90.0%), and rearing takes place either in winter (50%) or spring (26.66%). Population density remains high in 76.66% of cases, with more than 2,000 individuals per building, and drinking water comes mainly from the tap in 75% of cases. The most frequent symptoms are severe diarrhoea (50%) and respiratory disorders (31.66%).

A minority group of infected chickens (n=5) are in the finishing phase and are housed in greenhouse-type buildings during the summer season, with numbers exceeding 6,000 individuals per building. These birds tended to drink well water and showed joint symptoms.

It is also interesting to note that there is a small group of infected chickens (6.66%) that share some common factors with the two previously mentioned groups.

The results of the MCA indicate that poultry age, numbers, symptoms, type of construction, season, soil type and water source are key factors to consider when assessing the risks associated with avian colibacillosis in poultry flocks [2, 35].

Due to the development of their immune systems, young chickens are more vulnerable to bacterial infections, particularly secondary infections [36].

Colibacillosis in poultry can manifest itself in different ways throughout their growth [37]. Common symptoms include diarrhoea, caused by invasion of the intestinal mucosa by *E. coli*, leading to inflammation and disruption of intestinal function [38]. In addition, APEC infections can cause respiratory problems [38]. Finally, although less common, joint stiffness can occur when the bacterium spreads through the bloodstream, reaching the joints and causing painful inflammation that results in stiffness and difficulty of movement in chickens [39].

The systematic use of gas incubators to heat rearing buildings in winter can pose problems of temperature regulation, which can have significant consequences for chicken health. Chickens are

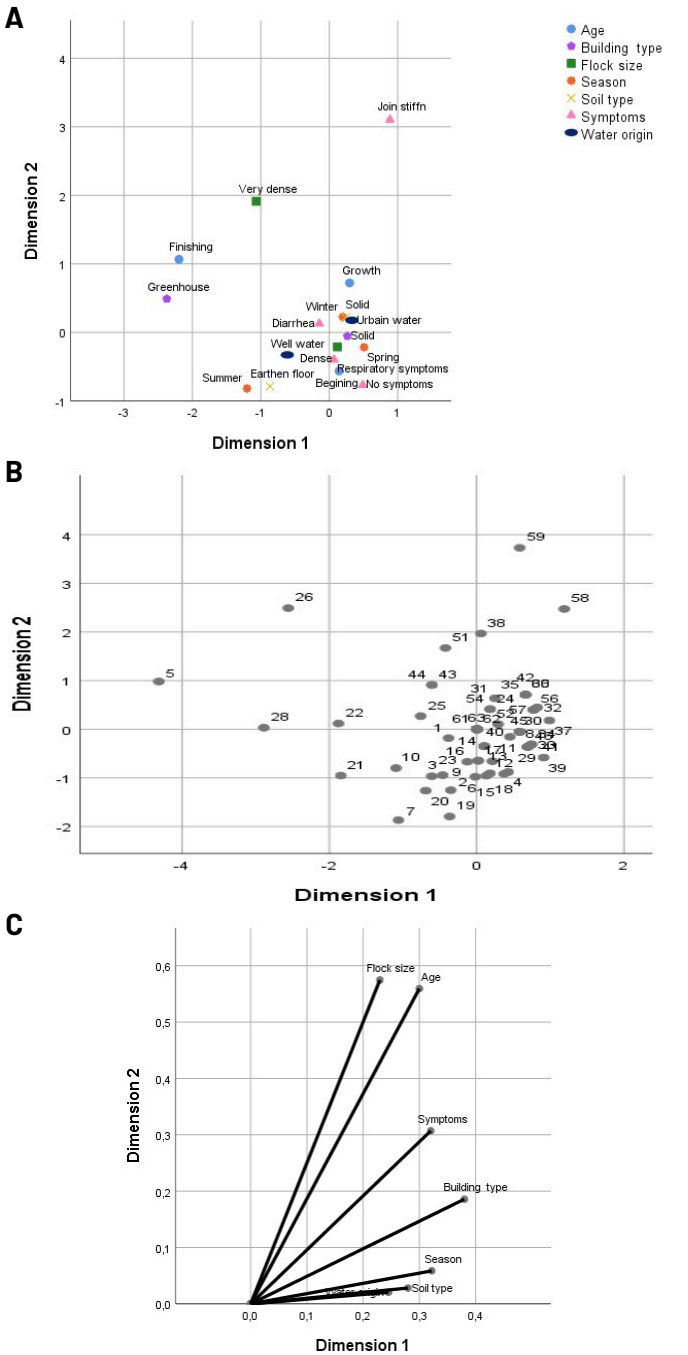

FIGURE 2. Multiple Correspondence Analysis. (A) Joint Plot of Category Points. (B) Object Points Labeled by Observation Numbers. (C) Discrimination Measures

TABLE III
Survey Sheet Results

Risk Factors	Prevalence
Stage	
• Start–up phase	58.33%
• Growth phase	30%
• Finishing phase	11.66%
Rearing season	
• Winter	50.0%
• Spring	26.66%
• Summer	23.33%
• Autumn	0%
Number of chickens by building	
• > 2000	76,66%
• > 6000	23,33%
Construction type	
• Bulding	90.0%
• Greenhouse	10.0%
Soil type	
• Hard flooring	78.33%
• Clay flooring	21.66%
Feed type	Pellets
Water source	
• Urban water supply	75%
• Well water	25%
Ventilation type	
• Dynamic ventilation dynamique	100.0%
• Others ventilation systems	0.0%
Pre–treatment with antibiotics	100%
Humidity level	High
Clinical symptoms	
• Severe diarrhea	50%
• Respiratory disorders	31.66
• Joint stiffness	3.33%
• Death with no apparent symptoms	15%

particularly sensitive to these temperature fluctuations, as they have no sweat glands and therefore rely heavily on thermal regulation by their feathers [37]. Heat stress can have a negative impact on chickens' performance, physiology and general health, and can make them more susceptible to infections, particularly colibacillosis [40].

In some cases, there is not enough space to contain the number of birds, which can lead to overcrowding. Overcrowding is known to induce stress in poultry, which can have a negative impact on their immune systems [41]. Stress disrupts various physiological functions and leads to reduced feed intake and growth, increasing their susceptibility to disease and reducing their ability to mount effective immune responses [35].

Poultry farms using solid buildings show a higher prevalence of the disease, which could be due to inadequate ventilation, favoring the accumulation of humidity and ammonia gases, which could stimulate the chickens' mucous membrane and cause pathological lesions of the tissues of the trachea and lungs [42]. These conditions offer a breeding ground for the bacteria responsible for colibacillosis [43].

Hard floors in livestock facilities have a rigid, porous surface, making them difficult to clean efficiently [44, 45]. This characteristic promotes the accumulation of dirt, organic debris and bacteria on floor surfaces [46]. In addition, these surfaces are conducive to the formation of biofilm, a microbial matrix that protects bacteria from cleaning and disinfection procedures [47].

Drinking water for poultry is not subject to any specific regulations in terms of microbiological, chemical and physical criteria [48]. This situation creates a potential opportunity for the transmission of pathogenic micro–organisms and contaminants and may also compromise the efficacy of drugs administered in the water [48]. In addition, the accumulation of organic matter in water supply systems, such as tanks, drinking troughs and battery pipes, could create a habitat conducive to the multiplication of micro–organisms in water [49].

Stressful environmental conditions in poultry houses, such as temperature variations, excessive humidity, poor ventilation and high stocking density, weaken the poultry immune system, making them more vulnerable to colibacillosis and other types of infections besides affecting animal welfare [35]. This disease is frequently treated with antibiotics, but inappropriate use creates antibiotic resistance, complicating treatment [50].

CONCLUSION

In conclusion, the emergence of multi–resistant strains of avian pathogenic *Escherichia coli* represents a growing threat to Animal and Public Health, as they compromise the efficacy of medical treatments and have the potential to spread between animals and humans. This study highlights the significant impact of stressful environmental conditions on the prevalence of colibacillosis in poultry. Indeed, to reduce the risks associated with this disease, it is crucial that poultry farmers and managers maintain optimal environmental conditions to minimize stress. At the same time, appropriate use of antibiotics is essential to maintain their continued effectiveness in treating bacterial infections. Concerted efforts involving both the livestock sector, health authorities, and researchers are necessary to curb the emergence of antibiotic resistance and preserve the efficacy of these critical drugs, for both animal and public health.

Availability of data and materials

The data sets during and/or analyzed during the current study are available from the corresponding author on reasonable request

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

S.S. performed experiments and wrote the manuscript. A.M H.S. co-directing work, S.S, Y.B, H.A.I. performed experiments, B.S. Statistical analysis, K.H. Directing work and onceived the experiments.

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