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ORIGINAL ARTICLE

Prevalence and Molecular Profile of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in Broiler Chickens, Morocco

Mohamed Amzil [1, 2], Kaotar Nayme [2], Abdelwahid Assaidi [3], Mohammed Timinouni [4], Asmaa Dihmane [5], Abouddihaj Barguigua [5], Aboubakr Khazaz [2, 6], Omar Charafeddine [7], Ahmed A. Bousfiha [1, 8], Assiya El Kettani [1, 9, 10]

[1] *Clinical Immunology, Inflammation and Allergy Laboratory (LICIA), Faculty of Medicine and Pharmacy, Hassan II University, Casablanca, Morocco*
[2] *Molecular Bacteriology Laboratory, Pasteur Institute of Morocco - Casablanca, Morocco*
[3] *Agri-Food and Health Laboratory, Faculty of Science and Technics, Hassan First University, Settat, Morocco*
[4] *Laboratory of Biotechnology and Bioinformatics, School of Advanced Studies in Biotechnology and Health (EHEB), Casablanca, Morocco*
[5] *Team of Biotechnology and Sustainable Development of Natural Resources, Department of Biology-Geology, Polydisciplinary Faculty, Sultan Moulay Slimane University, Beni Mellal, Morocco*
[6] *Microbiology and Antimicrobial Agents Research Team, LB2VE, Department of Biology, Faculty of Sciences, Chouaib Doukkali University, El Jadida, Morocco*
[7] *Laboratory of Physical Chemistry and Biotechnology of Biomolecules and Materials, Mohammedia Faculty of Science and Technology, Hassan II University, Casablanca, Morocco*
[8] *Clinical Immunology Unit, Department of Infectious Diseases, Abderrahim El Harouchi Children Hospital, University Hospital Center Ibn Rochd, Casablanca, Morocco*
[9] *Laboratory of Bacteriology, Virology and Hospital Hygiene, Ibn Rochd University Hospital, Casablanca, Morocco*
[10] *Laboratory of Bacteriology and Virology, Faculty of Medicine and Pharmacy, Hassan II University, Casablanca, Morocco*

SUMMARY

Background: **The prevalence, the characterization of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli (E. coli)* strains isolated from broiler chickens in the Casablanca-Settat region of Morocco, and their susceptibility to antibiotics were investigated.**

Methods: **A total of 216 intestinal samples were collected (100 samples in 2017 and 116 samples in 2019). *E. coli* isolates were screened for reduced susceptibility to Ceftazidime (CAZ) and subjected to antibiotic susceptibility testing. ESBL production was confirmed phenotypically and genotypically using PCR to detect bla_{TEM}, $bla_{CTX\text{-}M1}$, and bla_{SHV} genes.**

Results: **Results revealed a high prevalence (83.5%) of *E. coli*, with a significant decrease in prevalence observed in 2019 compared to 2017 (69%). Reduced susceptibility to Ceftazidime assay showed that 37% (n = 66) of the 178 *E. coli* isolates were resistant to Ceftazidime (CAZ-R), which indicates the potential likelihood of having ESBL. ESBL phenotypic testing showed a prevalence of 10.32%, with a slight increase in 2019 (11.5%), compared to 2017 (9%, $p > 0.05$). Antibiotic susceptibility testing demonstrated high resistance levels, notably in 2019, observed for Amoxicillin (94%), Ceftazidime (81%), and Aztreonam (69%). Multidrug resistance (MDR) significantly increased from 2017 to 2019, with 93% of isolates tested in 2019 resistant to at least three antibiotic families. Genotypic analysis revealed the presence of the $bla_{CTX\text{-}M15}$ (n = 1) and bla_{TEM1} (n = 2) genes in 2017, with an increase of bla_{TEM1} in 2019 (n = 9).**

Conclusions: **These findings highlight the emergence and the increases of ESBL-producing *E. coli* strains in Moroccan broiler chickens, signaling a growing concern for antimicrobial resistance in poultry farming. Enhanced surveillance and control measures are imperative to mitigate the spread of resistant pathogens in both animal and human populations.**

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Correspondence:
Mohamed Amzil
Clinical Immunology
Inflammation and Allergy Laboratory (LICIA)
Faculty of Medicine and Pharmacy
Hassan II University
Casablanca
Morocco
Email: amzilmohamed20012@gmail.com

KEYWORDS

Escherichia coli, broiler chicken, ESBL, antibiotics resistance, genes resistance

INTRODUCTION

Since the 1960s, the poultry sector has experienced substantial growth worldwide. The United States leads as the largest producer of poultry meat, accounting for 17% of global production, followed by China, Brazil, and the Russian Federation [1]. Over the period from 1961 through 2020, global poultry meat production surged from 9 to 133 million tones, with broiler meat alone contributing approximately 40% to global meat production in 2020 [1]. Similarly, Morocco has seen significant growth in poultry production, increasing from 55,000 to 640,000 tones between 1981 and 2021 [2]. However, the COVID-19 pandemic caused an economic and financial disruption that led to a notable decline in this vital sector in 2020 [3].

Poultry meat, due to its rich nutrient content, provides an ideal environment for microbial proliferation, leading to numerous cases of food poisoning [4]. *E. coli* strains are among the most important agents responsible for foodborne illnesses and are recognized as emerging pathogens in public health [5]. Indeed, the global burden of foodborne diseases exceeds 300 million illnesses, and nearly 200,000 deaths are caused by diarrhoeal *E. coli* worldwide every year [6]. Thus, major epidemics following the consumption of food contaminated with Enterohemorrhagic *E. coli* (EHEC) have occurred around the world. For example, the most serious were in the United States (Washington) in 1993 (linked to the consumption of hamburgers, with 501 patients, 3 deaths) [7] and in Scotland in 1996 (linked to the consumption of beef, with 120 cases of illness and 16 deaths) [8]. Similarly, in Central Europe; mainly in Germany; an outbreak of EHEC infections, also known as the cucumber crisis, was declared in 2011 (linked to the consumption of cucumbers, with 900 patients, 54 deaths) [9]. Food poisoning is also a major public health concern in Morocco; in 2019, it represented as the third most frequent cause of foodborne illness, responsible for 12.5% of all reported cases. [10]. In addition, around 100 episodes of foodborne outbreaks are reported each year, involving some 1,500 cases in all the country's provinces and regions. A striking example of this occurred on June 18, 2017, in Rabat, when 30 students at a training institute were severely affected by *E. coli* O157 [11].

These statistics, combined with the growing demand for poultry, underscore the importance of examining foodborne pathogens like *E. coli* in Morocco.

These pathogens can cause a range of clinical manifestations, from mild diarrhea to severe conditions like hemorrhagic colitis and hemolytic uremic syndrome (HUS), which can be life-threatening and necessitate antibiotic treatment [5].

The discovery of antibiotics stands as one of the most significant breakthroughs of the century, saving millions of lives and revolutionizing livestock production [12]. The use of antibiotics in poultry farming is crucial, contributing to the evolution of industrial breeding from both economic and health perspectives [13]. The global veterinary drug market has experienced remarkable growth in turnover, paralleled by a 65% increase in global antibiotic consumption between 2000 and 2015 [14].

β-lactams are among the most extensively used antibiotics in veterinary and human medicine. Since 1950, antibiotics have been the primary class of veterinary drugs employed to combat bacterial infections in food-producing animals [15]. However, declining antibiotic costs have led to their widespread use, not only for treating infections but also for promoting growth [16]. Overuse and inappropriate use of these molecules in livestock, including poultry, and humans results in the selection of resistant bacteria in the gut microbiota [17]. These bacteria, which are excreted into the environment via animal and human feces, constitute a reservoir of resistance genes that is disseminated into aquatic and terrestrial ecosystems via manure and slurry used to fertilize fields and crops [18,19]. Domestic and wild animals play a key role in this cycle of resistance gene transfer. Domestic animals, often in direct contact with humans, can carry resistant strains and transmit them to humans [20]. In addition, wild animals can carry resistant bacteria over long distances, contributing to their spread in different natural environments [19]. Resistant bacteria can infect humans indirectly through undercooked meat, contaminated raw vegetables, or direct contact with infected animals. Human-to-human transmission, particularly in hospitals, plays a role in the spread of resistance genes [21]. This complex cycle of contamination between the environment, the human community, hospitals, and animal populations contributes to the ongoing spread of resistance, which is a major public health challenge. Each year, drug-resistant bacteria cause an estimated 35,000 deaths in the United States and 33,000 deaths in Europe [22]. Several studies have demonstrated shared resistance genes between *E. coli* isolates from chickens and humans, highlighting the interconnectedness of resistance emergence [23]. A strong statistical correlation has been observed between antibiotic resistance in litter and agri-cultural soils in India [24]. These critical issues often go unaddressed by breeders,

and antibiotic misuse remains prevalent in agriculture to this day [25,26].

This study aimed to determine the prevalence and the characterization of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* strains isolated from broiler chickens in the Casablanca-Settat region of Morocco and to describe their susceptibility to antibiotics.

MATERIALS AND METHODS

Study area

This study was conducted across various locations in the Casablanca-Settat region of Morocco, situated on the Atlantic coast in the central-western part of the country. The region encompasses seven cities with a combined population of 6,861,739. The average altitude of the area is approximately 253 meters above sea level, with an average temperature of 17.4°C and annual precipitation of 342.7 millimeters.

Sample collection and transportation

A total of 216 fresh intestinal samples of ready-to-eat broilers (slaughtered poultry) were randomly collected in the same geographical region (Casablanca-Settat region) on a nonrepetitive basis from traditional butchers in the most vulnerable retail outlets and weekly markets during two different periods: 100 samples in 2017 and 116 samples in 2019. To account for potential seasonal variations, samples were collected during the summer period in each year. To minimize microbial contamination, the external surfaces of the samples were disinfected using 70% alcohol before collection. Using sterile scissors and forceps, intestinal pieces were collected separately into sterile bags. After collection, between 10:30 a.m. and 3:30 p.m., the samples were coded and entered into the food sample traceability register, along with additional information (vendor's address, daily temperature, hygiene conditions observed, etc.). They are transported in isothermal boxes at 2 to 8°C with cold accumulators. The delivery time did not exceed two hours after sampling. The analysis was carried out within 24 hours of arrival at the laboratory [27].

Isolation of *E. coli* strains

E. coli strains from intestinal samples were isolated as previously described [28]. Briefly, 25 g portions of each sample were aseptically excised with a scalpel, placed into separate stomacher bags containing 225 mL of sterile peptone water (Difco, Detroit, USA), and homogenized at 230 rpm for 2 minutes. From the homogenate, 100 µL aliquots were plated onto MacConkey agar (Difco, Detroit, USA) and incubated for 24 hours at 44°C [28]. Three to five lactose-fermenting colonies from different regions of multiple plates were subcultured onto eosin-methylene blue agar (Difco, Detroit, USA). Only one *E. coli* isolate was selected from each food sample based on colonies showing a dark blue color with a characteristic metallic sheen, identified as *E. coli* using API 20E commercial strips (bioMérieux, Paris, France).

Screening of *E. coli* with reduced susceptibility to ceftazidime

To identify ESBL strains, a screening for *E. coli* strains with decreased susceptibility to CAZ was conducted. Isolated strains were cultured on MacConkey agar supplemented with 1 mg/L of Ceftazidime (Bio-rad) and incubated at 37°C for 24 hours [29,30].

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the disk diffusion method according to European Committee for Antimicrobial Susceptibility Testing (EUCAST 2019) recommendations. This testing was conducted on all isolated strains with reduced susceptibility to Ceftazidime to determine their antibiotic resistance profiles against a panel of antibiotics. The following antibiotics were tested: Amoxicillin-Clavulanic acid (AMC) (20/10 µg/mL), Amoxicillin (AM) (25 µg/mL), Ceftazidime (CZD) (30 µg/mL), Cefotaxime (CTX) (30 µg/mL), Cefoxitin (FOX) (30 µg/mL), Cefepime (FEP) (30 µg/mL), Imipenem (IPM) (10 µg/mL), Aztreonam (ATM) (30 µg/mL), Amikacin (AK) (30 µg/mL), Tobramycin (TOB) (10 µg/mL), Gentamycin (GEN) (15 µg/mL), Ciprofloxacin (CIP) (5 µg/mL), Sulfamethoxazole-trimethoprim (SXT) (1.25 - 23.75 µg/mL), Tetracycline (TE) (30 µg/mL). In our study, multidrug resistant (MDR) bacteria are defined as bacteria exhibiting resistance to at least three distinct antibiotic families [31].

Phenotypic detection of extended-spectrum β-lactamase production

ESBL production was screened using the double disk synergy test and agar diffusion test, involving a central Amoxicillin/clavulanic acid disk (20/10 µg) and a third-generation Cephalosporin disk (Cefotaxime 30 µg and Ceftazidime 30 µg), placed 30 mm apart (center to center). Standard strains *E. coli ATCC 25922* and *Klebsiella pneumoniae ATCC 700603* were used as negative and positive controls for ESBL production, respectively [32].

Genotypic study

The genotypic study involved total DNA extraction and typing of genes encoding β-lactamases (bla_{TEM}, $bla_{CTX\text{-}M1}$, bla_{SHV}).

DNA extraction

The total DNA was extracted by heat shock method, for each *E. coli* strain, a single colony was dissociated in 100 µL of nuclease-free water, this suspension was heated at 100°C for ten minutes, then immediately cooled in iced water. After ten minutes of centrifugation at 10,000 revolutions per minute, the supernatant is transferred into a tube and then stored at -20°C until use

Table 1. Primers used for PCR amplification.

Gene	Primer	Primer sequence	Amplicon (bp)	Reference
bla$_{CTX-M1}$	CTX-M1 (+)	GGTTAAAAAATCACTGCGTC	863	[35]
	CTX-M1 (-)	TTGGTGACGATTTTAGCCGC		
bla$_{TEM}$	a216 (+)	ATAAAATTCTTGAAGACGAAA	1,079	
	a217 (-)	GACAGTTACCAATGCTTAATCA		
bla$_{SHV}$	os-5 (+)	CGCCGGGTTATTCTTATTTGTCGC	795	
	os-6 (-)	TCTTTCCGATGCCGCCGCCAGTCA		

(+) - primer sense, (-) - primer antisense.

Table 2. PCR amplification conditions for *bla.*

Amplification steps	Temperature conditions/time		Reference
	bla$_{SHV}$, *CTX-M-group 1*	*bla*$_{TEM}$	
Initial denaturation	94 °C/5 minutes	94 °C/5 minutes	[35]
Cyclic denaturation	94 °C/1 minute	94 °C/1 minute	
Hybridisation	60 °C/1 minute	50 °C/1 minute	
Cyclic elongation	72 °C/1 minute	72 °C/1 minute	
Final elongation	72 °C/7 minute	72 °C/7 minute	
Number of cycles	30	30	

Table 3. Profile of antibiotic resistance to different antibiotics.

Antibiotic family	Antibiotic	Percentage of resistance (%) number of strains (n *)		p-value
		2017 % (n = 50)	2019 % (n = 16)	
β-lactam antibiotics	Amoxicillin-Clavulanic acid	88 (44)	81 (13)	0.493
	Amoxicillin	NR	94 (15)	NA
	Ceftazidime	66 (33)	81 (13)	0.248
	Cefotaxime	2 (1)	56 (9)	p < 0.001
	Cefoxitin	84 (42)	31 (5)	p < 0.001
	Cefepime	0 (0)	12.5 (2)	p < 0.001
	Imipenem	0 (0)	0 (0)	NA
	Aztreonam	0 (0)	69 (11)	p < 0.001
Aminoglycosides	Amikacin	0 (0)	67 (11)	p < 0.001
	Tobramycin	14 (7)	44 (7)	0.011
	Gentamycin	2 (1)	0 (0)	0.569
Quinolones	Ciprofloxacin	52 (26)	69 (11)	0.240
Sulfamides	Sulfamethoxazole-trimethoprim	42 (21)	50 (8)	0.575
Cyclines	Tetracycline	96 (48)	100 (16)	0.417

NR - Not realized, NA - Not analyzed, NS - Not significant, n - number.

Table 4. Antibiotic resistance profile of suspected ESBL-producing *E. coli* (2017).

Profile of suspect ESBL-producing *E. coli* strains (2017)					
Phenotypic study				Genotypic study	
Code	Antibiotic resistance profile	NB Family ATB	Association between antibiotic families	*bla*-type	Remark
E29	AMC FOX CAZ TE	2	B C	*CTXM15*	Not MDR
E65	AMC FOX CAZ TOB SXT TE	4	B A S C	*TEM1*	
E64	AMC FOX CAZ CIP SXT TE	4	B Q S C	*TEM1*	

B - β-lactam antibiotics, A - Aminoglycosides, Q - Quinolones, S - Sulfamides, C - Cyclines.
P - Positive, N - Negative, NB - number, MDR - Multidrug-resistant.

Table 5. Antibiotic resistance profile of suspected ESBL-producing *E. coli* (2019).

Profile of suspect ESBL-producing *E. coli* strains (2019)				
Phenotypic study				Genotypic study
Code	Antibiotic resistance profile	NB Family ATB	Association between antibiotic families	*bla*-type
CS14	AMC AM CTX FOX FEP ATM TOB CIP TE	4	B A Q C	*TEM1 CTX15*
CS45	AMC AM FEP CIP TE	3	B Q C	*TEM1*
CS75	AMC AM FEP ATM SXT TE	3	B S C	*TEM1*
CS84	AMC AM CTX FOX FEP ATM TOB CIP SXT TE	5	B A Q S C	*TEM1*
CS86	AMC AM CTX FEP ATM TOB CIP SXT TE	5	B A Q S C	*TEM1*
CS90	AMC AM FEP ATM CIP SXT TE	4	B Q S C	*TEM1*
CS95	AMC AM FOX FEP ATM AK TOB SXT TE	4	B A S C	*TEM1*
CS98	AMC AM CTX FEP AK TE	3	B A C	*TEM1*
CS101	AMC AM CTX FEP ATM AK TOB CIP TE	4	B A Q C	*CTX15*
CS110	AMC AM CTX FEP ATM SXT TE	3	B S C	*TEM1*

B - β-lactam antibiotics, A - Aminoglycosides, Q - Quinolones, S - Sulfamides, C - Cyclines.
P - Positive, N - Negative, NB - number.

[33].

Polymerization chain reaction

In order to detect the genes encoding the β-lactamases of the type: bla_{TEM}, $bla_{CTX\text{-}M1}$, and bla_{SHV}, we used the PCR technique as described by Guessennd et al. (2008) [34,35]. The primers used are listed in Table 1. The reaction mixture contained PCR buffers, specific primers, and Taq DNA polymerase [34]. The PCRs conditions are summarized in Table 2, carried out in the thermocycler (Perkin2400-Elmer Cetus, Notwalk, Conn). PCR products were analyzed by electrophoresis in 1% agarose (Invitrogen) [34]. The reference strains used as positive controls are *E. coli* U2A21790 (*CTX-M1*), *Salmonella spp*. U2A1446 (*TEM*), and *Salmonella spp*. U2A1446 (*SHV*) [36]. Negative control was done without DNA [34].

Sequencing

All amplified products were sequenced to validate their identities. Both strands of the purified amplicons were sequenced with a Genetic Analyzer 3130x1 sequencer (Applied Biosystems), using the BigDye Terminator Cycle Sequencing kit and the same primers used for PCR amplification. Comparisons with known sequences are performed with the BLAST program of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov) [35,36].

Statistical analysis

Data were analyzed with SSPS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). The χ^2 test was used to determine the statistical significance of the data. A p-value of < 0.05 was considered significant [37]. In this study, the p-value chosen is two-tailed, as

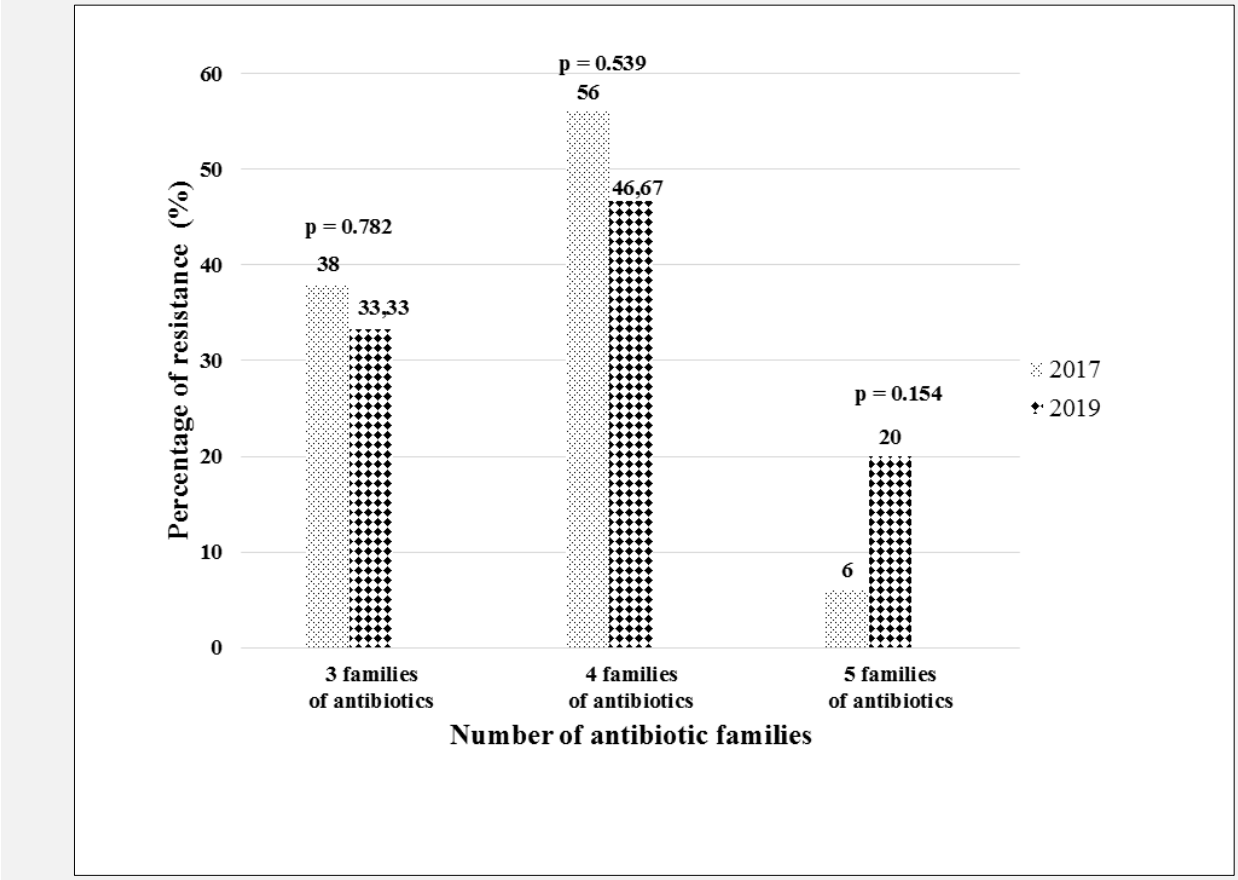

Figure 1. Evolution of multidrug resistance in *E. coli* strains in 2017 and 2019.

the statistical analysis was performed to determine the significance of the variation observed in resistance over a two-year period.

RESULTS

Prevalence of *E. coli* in intestinal samples of broilers

The microbiological analysis of the 216 samples collected in the Casablanca - Settat region showed the presence of 213 thermotolerant coliforms. The results of the biochemical analysis identified 178 strains as *E. coli*, a percentage of 83.5%. In addition, *E. coli* was found with a lower prevalence for the year 2019 (69%) compared to 2017, for which a prevalence of 100% is noted. The prevalence of *E. coli* experienced a statistically significant decrease ($p < 0.001$).

Screening of *E. coli* with reduced susceptibility to ceftazidime

The screening results for *E. coli* with reduced susceptibility to CAZ revealed that in 2017 50% (n = 50) of isolates exhibited notable resistance to CAZ, while in 2019, this percentage decreased to 20.51% (n = 16). This shows an overall prevalence of 37% for extended-resistant Ceftazidime (ERC3G), suggesting the presence of potential MDR or ESBL-producing strains. A significant decline in Ceftazidime susceptibility was observed in 2019 compared to 2017 ($p < 0.001$).

Double synergy assay

The findings from the ESBLs phenotype research revealed a synergy between AMC and Cephalosporins (indicated by the champagne cork image), which may suggest the presence of enzymes associated with this phenotype. In 2017, 9% (9/100) of isolates exhibited this synergy, compared to 11.5% (13/113) in 2019, resulting in an overall prevalence of 10.32%. There was a

statistically insignificant increase observed in 2019 compared to 2017 (p = 0.593).

Antibiotics susceptibility

The evolution of resistance over the two collection years, particularly with reduced susceptibility to CAZ, is detailed in Table 3. Intermediate strains were categorized with resistant forms as they could signify the early stages of resistance. Notably, the highest levels of resistance among the selected *E. coli* strains were observed in 2019 (Table 3). Analyzing the frequency of resistant strains to each antibiotic revealed varying levels of resistance within the β-Lactam family. In 2019, the highest frequencies were seen for Amoxicillin (94%), Ceftazidime (81%), Aztreonam (69%), Cefotaxime (56%), and Cefepime (12.5%). Conversely, in 2017, the highest resistance frequencies for Amoxicillin with Clavulanic acid and Cefoxitin were noted at 88% and 84%, respectively. Notably, no resistance to Carbapenems (Imipenem) was detected. Furthermore, in 2017, there was no resistance to fourth-generation cephalosporins (Cefepime). Tetracycline resistance ranged from 96% (2017) to 100% (2019), while resistance to Aminoglycosides varied with the highest frequency observed in 2019 (44% for Tobramycin, 67% for Amikacin), except for Gentamicin, which remained effective against these strains (98 - 100% sensitivity). For Quinolones, resistance to Ciprofloxacin was 69% in 2019 and 52% in 2017. The highest frequency of resistance to Sulfonamides (Sulfamethoxazole-trimethoprim) was recorded in 2019 (50%), compared to 42% in 2017. The resistance rates of *E. coli* ERC3G to Cefotaxime, Cefepime, Aztreonam, Tobramycin, and Amikacin increased significantly in 2019 compared to 2017. There was, however, a statistically insignificant increase in resistance to Ceftazidime, Ciprofloxacin, Tetracycline, and Sulfamethoxazole-trimethoprim. On the other hand, resistance to Amoxicillin with Clavulanic Acid and Gentamicin showed a non-significant decrease over the same period. In contrast, a statistically significant decrease in resistance to Cefoxitin was observed in 2019 compared with 2017.

Evolution of multidrug resistance in *E. coli* strains in 2017 and 2019

The results of our antibiogram analysis showed that the overall proportion of multiresistant *E. coli* strains tested (50 in 2017 and 16 in 2019) increased over time, from 64% in 2017 to 93% in 2019 (p = 0.022). More specifically, the percentage of strains resistant to exactly three antibiotic families decreased slightly, from 38% in 2017 to 33.33% in 2019 (p = 0.782), and similarly, strains resistant to four families decreased from 56% to 46.67% (p = 0.539). In contrast, resistance involving five antibiotic families showed a statistically insignificant increase from 6% in 2017 to 20% in 2019 (p = 0.154) (Figure 1). These results highlight a worrying escalation of multidrug resistance in *E. coli* strains over the studied period.

Genotypic study

E. coli strains suspected of being broad spectrum Beta-lactamase producers (n = 9 in 2017 and n = 13 in 2019) were screened using Simplex PCR to detect the presence of $bla_{CTX\text{-}M1}$, bla_{TEM}, and bla_{SHV} genes. DNA bands were visualized on agarose gel through electrophoresis under UV light. The genotypic analysis revealed that among the 9 *E. coli* strains tested in 2017, 11% (n = 1/9) carried the $bla_{CTX\text{-}M15}$ gene, while 22% (n = 2/9) harbored the bla_{TEM1} gene, with no strains possessing the bla_{SHV} gene (Table 4). Conversely, in 2019, genes encoding $bla_{CTX\text{-}M15}$ type β-lactamase and bla_{TEM1} were detected at frequencies of 15% (n = 2/13) and 69% (n = 9/13), respectively (Table 5). Comparing the types and frequencies of detected genes between the two collection years revealed a notable increase in the frequency of the bla_{TEM} gene encoding β-lactamases in 2019 (Table 5).

DISCUSSION

Our study identified a significant resistance rate to CAZ of 50% in 2017, which decreased to 20.51% in 2019, resulting in an overall prevalence of extended-resistant Ceftazidime (ERC3G) of 37% in the analyzed broiler intestine samples. This observed level of resistance to third generation Cephalosporins (3CG) is relatively high compared to rates reported in India (28.2%) by Kaushik et al. in 2018 [38] and in China by Yassin et al. (9.8%) [39], but lower than that reported by Belmahdi et al. in Algeria [41], where over 90% of isolates were resistant to CAZ. Our CAZ-resistant strains displayed varying frequencies of resistance within the β-lactam family, with the highest rates observed in 2019 for Amoxicillin (94%), Ceftazidime (81%), Aztreonam (69%), and Cefotaxime (56%). Conversely, the highest levels of resistance to Amoxicillin with Clavulanic Acid and Cefoxitin were observed in 2017, with respective resistance frequencies of 88% and 84%. Resistance to Tetracycline increased from 96% in 2017 to 100% in 2019. Among the aminoglycosides, our isolates exhibited the highest frequency of resistance in 2019, except for Gentamicin (44% for Tobramycin, 67% for Amikacin), which remained active against these strains (98 - 100% sensitivity). Regarding Quinolones, 69% of strains were resistant to Ciprofloxacin in 2019, compared to 52% in 2017. Sulfamethoxazole-trimethoprim resistance reached its highest frequency in 2019 (50%), while a resistance rate of 42% was recorded for the same antibiotic in 2017. It should be noted that in 2017, the high proportion of strains with reduced susceptibility to Ceftazidime (during screening), accompanied by lower prevalence of resistance to β-lactam antibiotics (during antibiotic susceptibility testing), suggests that the tested strains harbor other β-lactam resistance genes, such as the *AmpC* gene, which encodes an AmpC-type β-lactamase. These enzymes generally have greater activity against Cefoxitin, but overproduction of the

AmpC β-lactamase enzyme can also hydrolyse 3rd generation Cephalosporins (e.g. Ceftazidime) in case of overexpression of the gene [41,42]. This may be supported by the high rate of Cefoxitin (C2G) resistance in antibiotic susceptibility testing (84% of Cefoxitin resistance in 2017). In addition, Cefoxitin was used as a marker for the production of β-lactamases AmpC [42]. Therefore, it can be inferred that during 2017, we screened strains for resistance to third-generation Cephalosporins (Ceftazidime) mediated by the *bla* gene as well as other genes (*AmpC*). However, in 2019, the rate of resistance to Cefoxitin (C2G) during antibiotic susceptibility testing showed a remarkable decrease (31% of resistance in 2019), accompanied by an increase in resistance to C3G (Ceftazidime and Cefotaxime). This means that during 2019, we screened strains resistant to third-generation Cephalosporins (Ceftazidime), which is mediated by the *bla* gene.

The increase in resistance to critically important antibiotics, such as β-lactams, observed between 2017 and 2019 could be attributed to several factors. One possible explanation could be the excessive and inappropriate use of antibiotics in veterinary and agricultural practices, often without regulation, throughout the Casablanca-Settat region. Antibiotics are often administered not only for therapeutic purposes but also as growth promoters in poultry production, a practice that has been linked to the increase in antibiotic-resistant bacterial strains [16]. In Morocco, despite efforts to regulate the sale of antibiotics, enforcement of these regulations often remains limited, allowing farmers and veterinarians easier access to antimicrobials. Changing farming practices may also contribute to this concern, as the intensification of broiler farming could lead to increased use of antibiotics to prevent disease. This change in farming practices could explain the escalation in multidrug resistance we observed, in particular, in 2019, where 93% of resistant strains were resistant to at least three antibiotic families. A study by Abo Amer et al. in 2018 in Saudi Arabia [31] reported an even higher rate (99%). Furthermore, variations in antibiotic use in agricultural practices may influence resistance patterns. A study conducted in China by Tang et al. in 2017 [43] revealed similar trends, showing that frequent use of antibiotics in poultry farming is correlated with a higher prevalence of multidrug-resistant strains. Multidrug-resistant bacteria in food-producing animals pose a significant risk to human health, as these strains can be transmitted to humans through consumption of contaminated food [44]. Given the international trade in poultry and poultry products, this risk extends beyond Morocco. Our research on ESBL phenotypes showed a percentage of 9% (9/100) in 2017, increasing to 11.5% (13/113) in 2019, resulting in a total prevalence of 10.32%. A study by Dhaouadi et al. published in 2020 [45] reported a higher rate (30%) of ESBL. *E. coli* strains suspected of being broad spectrum Beta-lactamase producers (9 in 2017 and 13 in 2019) underwent genotypic analysis to detect bla_{SHV}, $bla_{CTX\text{-}M1}$, and bla_{TEM} genes encoding ESBLs. Among these, 14 were β-lactamase-producing *E. coli.* The bla_{TEM} gene (type 1) gene was the most prevalent among our tested *E. coli* strains, other similar results have been reported in Egypt by Osman et al. [46] and in Jordan by Ibrahim [33]. The detection of $bla_{CTX\text{-}M15}$ gene in 3 strains aligns with reports from Nigeria [47] and Algeria [40]. Notably, none of our strains carried the bla_{SHV} gene, unlike other studies conducted by Abo Amer et al. [31] and Younis et al. that successfully detected this gene [48]. The predominance of the bla_{TEM1} gene, in particular, could be due to the excessive and sometimes inappropriate use of β-lactam antibiotics in community and hospital settings, which exerts selective pressure; thus favoring the spread of this resistance gene, given that the bla_{TEM1} gene is often carried by mobile genetic elements, allowing it to move easily between different bacteria. The high prevalence of β-lactamase-producing *E. coli* strains, particularly those carrying the bla_{TEM1} gene, is alarming as they limit the effectiveness of β-lactam antibiotics, which are one of the most commonly used classes of antibiotics to treat bacterial infections. Consequently, the increase in the bla_{TEM1} gene could lead to an increase in morbidity associated with resistant infections.

Although this study provides an overview of extended-spectrum β-lactamase-producing *E. coli* strains, it has several limitations that need to be considered when interpreting the results, including that the absence of precise information on the types and quantities of antibiotics used in broiler farms complicates efforts to link observed trends in antibiotic resistance with specific usage practices. Additionally, we did not screen for other β-lactam resistance genes (e.g. *AmpC*) or conduct conjugation tests to assess resistance gene transfer.

CONCLUSION

This finding showed that *E. coli* isolates of avian origin could constitute a reservoir of resistance genes coding for ESBLs and that the massive and unreasonable use of antibiotics at the broiler production level would contribute considerably to the appearance and increase of the prevalence of MDR bacteria. To limit this growing threat, we recommend the implementation of a comprehensive surveillance program that routinely monitors antimicrobial use and resistance patterns at both regional and national levels. This surveillance should include regular sampling of poultry products on farms to track the spread of resistant bacteria. In addition, we support stricter regulations on the sale and use of antibiotics in poultry production, including restrictions on the use of critically important antibiotics such as Cephalosporins and Fluoroquinolones. Consideration of alternative measures, such as improved biosecurity and vaccination, should be prioritized to mitigate the reliance on antibiotics in poultry production. Furthermore, targeted public health initiatives are essential to enhance awareness among farmers and veterinarians regarding the

risks associated with antibiotic misuse. These efforts should be integrated into broader antimicrobial stewardship programs to promote responsible antibiotic use and limit the emergence and dissemination of resistant bacterial strains.

Declaration of Interest:
No potential conflict of interest was reported by the authors.

References:

1. FAO. Gateway to poultry production and products. Production. FAO. 2024. https://www.fao.org/poultry-production-products/production/en/
2. Fagrach A, Fellahi S, Challioui MK, et al. Backyard poultry flocks in Morocco: Demographic characteristics, husbandry practices, and disease and biosecurity management. Animals (Basel) 2023;13(2):202. (PMID: 36670742)
3. Zaime Z, Ouahi L. Impact of Covid-19 on the Poultry Sector in Morocco: An Empirical Analysis. IBIMA Bus Rev 2023;2023: 963486. https://ibimapublishing.com/articles/IBIMABR/2023/963486/
4. Hamiroune M, Saidani K, Naceur R, Belarbi HS, Foughalia A, Berber A. Microbiological quality of Merguez in some retailing meat shops in the region of MSila (Algeria). Afr J Microbiol Res 2017;11(6):211-7. https://academicjournals.org/journal/AJMR/article-abstract/9ECD55C62743
5. Aijuka M, Buys EM. Persistence of foodborne diarrheagenic Escherichia coli in the agricultural and food production environment: Implications for food safety and public health. Food Microbiol 2019;82:363-70. (PMID: 31027795)
6. Lee W, Kim M-H, Sung S, et al. Genome-based characterization of hybrid Shiga toxin-producing and enterotoxigenic Escherichia coli (STEC/ETEC) strains isolated in South Korea, 2016-2020. Microorganisms 2023;11(5):1285. (PMID: 37317259)
7. Bell BP, Goldoft M, Griffin PM, et al. A multistate outbreak of escherichia coli o157: h7-associated bloody diarrhea and hemolytic uremic syndrome from hamburgers: the washington experience. JAMA 1994;272(17):1349-53. (PMID: 7933395)
8. Dundas S, Todd WT, Stewart AI, Murdoch PS, Chaudhuri AK, Hutchinson SJ. The central Scotland Escherichia coli O157: H7 outbreak: risk factors for the hemolytic uremic syndrome and death among hospitalized patients. Clin Infect Dis 2001;33(7): 923-31. (PMID: 11528561)
9. Karch H, Denamur E, Dobrindt U, et al. The enemy within us: lessons from the 2011 European Escherichia coli O104: H4 outbreak. EMBO Mol Med 2012;4(9):841-8. (PMID: 22927122)
10. Bouchriti Y, Kabbachi B, Achbani A, et al. Analysis on epidemiological characteristics of food poisoning events in Agadir prefecture, Morocco, from 2015 to 2017. E3S Web of Conferences 2021;319:01028. https://www.e3s-conferences.org/articles/e3sconf/pdf/2021/95/e3sconf_vigisan_01028.pdf
11. Moumni Abdou H, Dahbi I, Akrim M, et al. Outbreak investigation of a multipathogen foodborne disease in a training institute in Rabat, Morocco: case-control study. JMIR Public Health Surveill 2019;5(3):e14227. (PMID: 31573941)
12. Muteeb G, Rehman MT, Shahwan M, Aatif M. Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. Pharmaceuticals (Basel) 2023;16(11): 1615. (PMID: 38004480)
13. Mehdi Y, Létourneau-Montminy M-P, Gaucher M-L, et al. Use of antibiotics in broiler production: Global impacts and alternatives. Anim Nutr 2018;4(2):170-8. (PMID: 30140756)
14. Klein EY, Van Boeckel TP, Martinez EM, et al. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proceed Nat Acad Sci 2018;115(15):E3463-70. https://www.pnas.org/doi/full/10.1073/pnas.1717295115
15. Sanders P, Chauvin C, Toutain P-L. Use of antibiotics in farming and stakes in public health. INRA Prod Anim 2011;24(2):199-204. https://hal.science/hal-01191161
16. Yadav AS, Kolluri G, Gopi M, Kumaragurubaran K, Malik YS, Kuldeep D. Exploring alternatives to antibiotics as health promoting agents in poultry - a review. J Exp Biol Agric Sci 2016;4(3S): 368-83. https://www.researchgate.net/profile/Ajit-Yadav-2/publication/303528242_Exploring_alternatives_to_antibiotics_as_health_promoting_agents_in_poultry-_a_review/links/57485c0108ae18b6dce91ab4/Exploring-alternatives-to-antibiotics-as-health-promoting-agents-in-poultry-a-review.pdf
17. Cheng G, Ning J, Ahmed S, et al. Selection and dissemination of antimicrobial resistance in Agri-food production. Antimicrob Resist Infect Control 2019;8:158. (PMID: 31649815)
18. Checcucci A, Trevisi P, Luise D, et al. Exploring the animal waste resistome: the spread of antimicrobial resistance genes through the use of livestock manure. Front Microbiol 2020;11: 1416. (PMID: 32793126)
19. Barguigua A, Rguibi Idrissi H, Nayme K, Timinouni M. Virulence and antibiotic resistance patterns in E. coli, Morocco. Ecohealth 2019;16(3):570-5. (PMID: 30895488)
20. Zalewska M, Błażejewska A, Czapko A, Popowska M. Antibiotics and antibiotic resistance genes in animal manure–consequences of its application in agriculture. Front Microbiol 2021; 12:610656. (PMID: 33854486)
21. Founou LL, Founou RC, Essack SY. Antibiotic resistance in the food chain: a developing country-perspective. Front Microbiol 2016;7:1881. (PMID: 27933044)
22. Li T, Wang Z, Guo J, et al. Bacterial resistance to antibacterial agents: Mechanisms, control strategies, and implications for global health. Sci Total Environ 2023;860:160461. (PMID: 36435256)
23. Borges CA, Tarlton NJ, Riley LW. Escherichia coli from commercial broiler and backyard chickens share sequence types, antimicrobial resistance profiles, and resistance genes with human extraintestinal pathogenic Escherichia coli. Foodborne Pathog Dis 2019;16(12):813-22. (PMID: 31411497)
24. Bhushan C, Khurana A, Rajeshwari S, Nagaraju M. Antibiotic Resistance in Poultry Environment: Spread of Resistance from Poultry Farm to Agricultural Field. CSE 2017. https://cdn.cseindia.org/userfiles/report-antibiotic-resistance-poultry-environment.pdf
25. Hare D. Microbial resistance to antimicrobial drugs. Can Vet J 1998;39(6):329-31. (PMID: 9635166)
26. Burgos JM, Ellington BA, Varela MF. Presence of multidrug-resistant enteric bacteria in dairy farm topsoil. J Dairy Sci 2005; 88(4):1391-8. (PMID: 15778307)

27. Ghailani I, Louajri A, Zawjal A, Khay EQ, Barrijal S. The microbiological quality of commercialized food products in Northwest of Morocco. Int J Innov Appl Stud 2020;30:535-42. https://ijias.issr-journals.org/abstract.php?article=IJIAS-20-163-07

28. Laarem M, Barguigua A, Nayme K, et al. Occurrence of plasmid-mediated quinolone resistance and virulence genes in avian Escherichia coli isolates from Algeria. J Infect Dev Ctries 2017; 11(2):143-51. (PMID: 28248675)

29. Wilson G, McCabe D. The use of antibiotic-containing agars for the isolation of extended-spectrum β-lactamase-producing organisms in intensive care units. Clin Microbiol Infect 2007;13(4): 451-3. (PMID: 17359334)

30. D'Agata E, Venkataraman L, DeGirolami P, Samore M. Molecular epidemiology of acquisition of ceftazidime-resistant gram-negative bacilli in a nonoutbreak setting. J Clin Microbiol 1997; 35(10):2602-5. (PMID: 9316915)

31. Abo-Amer AE, Shobrak MY, Altalhi AD. Isolation and antimicrobial resistance of Escherichia coli isolated from farm chickens in Taif, Saudi Arabia. J Glob Antimicrob Resist 2018;15:65-8. (PMID: 29842974)

32. Barguigua A, El Otmani F, Talmi M, Zerouali K, Timinouni M. Prevalence and types of extended spectrum β-lactamases among urinary Escherichia coli isolates in Moroccan community. Microb Pathog 2013;61:16-22. (PMID: 23643553)

33. Ibrahim RA, Cryer TL, Lafi SQ, Basha E-A, Good L, Tarazi YH. Identification of Escherichia coli from broiler chickens in Jordan, their antimicrobial resistance, gene characterization and the associated risk factors. BMC Vet Res 2019;15(1):1-16. (PMID: 31118039)

34. Guessennd N, Bremont S, Gbonon V, et al. [Qnr-type quinolone resistance in extended-spectrum beta-lactamase producing enterobacteria in Abidjan, Ivory Coast.] Pathol Biol (Paris) 2008;56(7-8):439-46. (PMID: 18926644)

35. Barguigua A, El Otmani F, Talmi M, et al. Characterization of extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolate s from the community in Morocco. J Med Microbiol 2011;60(Pt 9):1344-52. (PMID: 21546559)

36. Jamali L, Haouzane F, Bouchajou M. Prevalence of plasmid mediated quinolone resistance genes among enterobacteria isolates in Moroccan community. Int J Innov Sci Res 2014;11:387-99. https://ijisr.issr-journals.org/abstract.php?article=IJISR-14-236-02

37. Bouzari S, Farhang E, Hosseini SM, Alikhani MY. Prevalence and antimicrobial resistance of shiga toxin-producing Escherichia coli and enteropathogenic Escherichia coli isolated from patients with acute diarrhea. Iran J Microbiol 2018;10(3):151-7. (PMID: 30112152)

38. Kaushik P, Anjay A, Kumari S, Dayal S, Kumar S. Antimicrobial resistance and molecular characterisation of E. coli from poultry in Eastern India. Vet Ital 2018;54(3):197-204. (PMID: 30574996)

39. Yassin AK, Gong J, Kelly P, et al. Antimicrobial resistance in clinical Escherichia coli isolates from poultry and livestock, China. PLoS One 2017;12(9):e0185326. (PMID: 28934348)

40. Belmahdi M, Bakour S, Al Bayssari C, Touati A, Rolain J-M. Molecular characterisation of extended-spectrum β-lactamase-and plasmid AmpC-producing Escherichia coli strains isolated from broilers in Béjaïa, Algeria. J Glob Antimicrob Resist 2016;6:108-12. (PMID: 27530851)

41. Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev 2009;22(1): 161-82. (PMID: 19136439)

42. Derbyshire H, Kay G, Evans K, Vaughan C, Kavuri U, Winstanley T. A simple disc diffusion method for detecting AmpC and extended-spectrum β-lactamases in clinical isolates of Enterobacteriaceae. J Antimicrob Chemother 2009;63(3):497-501. (PMID: 19155228)

43. Tang KL, Caffrey NP, Nóbrega DB, et al. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. Lancet Planet Health 2017;1(8):e316-27. (PMID: 29387833)

44. Hocquet D, Muller A, Bertrand X. What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. J Hosp Infect 2016;93(4):395-402. (PMID: 26944903)

45. Dhaouadi S, Soufi L, Hamza A, et al. Co-occurrence of mcr-1 mediated colistin resistance and β-lactamase-encoding genes in multidrug-resistant Escherichia coli from broiler chickens with colibacillosis in Tunisia. J Glob Antimicrob Resist 2020;22:538-45. (PMID: 32251867)

46. Osman KM, Kappell AD, Elhadidy M, et al. Poultry hatcheries as potential reservoirs for antimicrobial-resistant Escherichia coli: A risk to public health and food safety. Sci Rep 2018;8(1):5859. (PMID: 29643424)

47. Chah KF, Ugwu IC, Okpala A, et al. Detection and molecular characterisation of extended-spectrum β-lactamase-producing enteric bacteria from pigs and chickens in Nsukka, Nigeria. J Glob Antimicrob Resist 2018;15:36-40. (PMID: 29908916)

48. Younis G, Awad A, Mohamed N. Phenotypic and genotypic characterization of antimicrobial susceptibility of avian pathogenic Escherichia coli isolated from broiler chickens. Vet World 2017; 10(10):1167-72. (PMID: 29184361)