Characterisation of ESBL-producing *E. coli* isolated from healthy broilers in Tunisia

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Abstract

The aim of this study was to investigate the frequency of antimicrobial resistance and the rate of extended-spectrum β-lactamase (ESBL) in *Escherichia coli* isolated from caecum content collected in slaughterhouses located in three regions of Tunisia. *E. coli* isolates from chicken caecal samples, obtained using media supplemented with cefotaxime, were screened for antimicrobial susceptibility by the disk diffusion method. ESBL production was assessed by the double-disk synergy test, and the presence of β-lactamase encoding resistance genes was evaluated by PCR. Out of 111 faecal samples, 108 samples were positive for *E. coli* isolated from media supplemented with cefotaxime with 70% of ESBL-producing isolates. Alarming proportions of resistance against most of the 21 tested antibiotics were observed with 90% of multidrug resistant strains. Most strains exhibited resistance to amoxicillin, cefepime, tetracycline, cephalothin, piperacillin, cefotaxime, aztreonam, streptomycin, ceftazidime, cefuroxime, chloramphenicol, and florfenicol. The same resistance patterns were found in comparison between the three chicken slaughterhouses. A high prevalence of β-lactamase genes was observed, with $bla_{\text{CTX-M-G1}}$-ESBL in 79.6% of strains, bla_{TEM} in 45.4%, and bla_{SHV} in 33.3%. The $bla_{\text{CTX-M-G1}}$ and bla_{TEM} genes were significantly more frequent in strains from slaughterhouses A and C. Additionally, carbapenem resistance genes, $bla_{\text{OXA-48}}$ and bla_{IMP}, were demonstrated in 13% and 6.5% of strains, respectively. The study showed high frequency of ESBL-producing *E. coli* and high antibiotic resistance in broilers. Poultry farms could represent a significant reservoir of ESBL-producing bacteria, suggesting the dissemination of these pathogens to humans and environment. These findings indicate the need for achievement of control and surveillance system.

Antimicrobial resistance, Enterobacterales, multidrug-resistant bacteria

Escherichia coli (*E. coli*) is a Gram-negative rod-shaped bacterium that commonly exists as a commensal organism in the intestinal tract, where it is generally non-pathogenic. It can become pathogenic by acquiring virulence genes located on plasmids or other mobile genetic elements via horizontal gene transfer, thus enabling certain strains of *E. coli* to cause intestinal or extraintestinal disease (Khong et al. 2023).

Extended-spectrum β-lactamase (ESBL)-producing bacteria are capable of hydrolysing the β-lactam ring found in penicillins and oxyimino-cephalosporins (e.g., cefotaxime, cefepime, and ceftazidime), as well as monobactams like aztreonam. As a result, these bacteria become resistant to all penicillins, most cephalosporins, and monobactams, while typically remaining susceptible to carbapenems and cephamycins. This enzymatic degradation, mediated by hydrolytic amidases produced by ESBLs, compromises the efficacy of many β-lactam antibiotics and significantly narrows therapeutic options, particularly when these organisms also acquire resistance to carbapenems (Mojtahedi 2017).

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Since the beginning of the 1990s, the increase in the prevalence of ESBLs among clinical *E. coli* isolates in human medicine has been a cause of great concern. Different reports have alerted in the last few years about the dissemination of ESBL/AmpC-producing *E. coli* in healthy food-producing animals in different countries (Perestrelo et al. 2023).

The emergence of resistance to carbapenems (such as imipenem, meropenem, doripenem, and ertapenem) represents one of the most critical challenges in the fight against antibiotic resistance, as therapeutic alternatives are extremely limited. Carbapenem resistance was primarily associated with a specific group of carbapenemase enzymes capable of efficiently hydrolysing carbapenems. Over time, the clinical significance and diversity of carbapenemases increased markedly, with a notable rise in prevalence by the mid-2000s, especially among *Enterobacteriaceae*. More recently, this resistance has also been identified in animals, particularly in companion animals, raising concerns about cross-species transmission and the implications for One Health (Elshamy and Aboshanab 2020).

Poultry are recognized as a significant reservoir for the dissemination of antimicrobial-resistant bacteria, particularly ESBL-producing strains, into both the human population and the environment. Pathogenic *E. coli* in poultry poses a direct threat to animal health and productivity, as well as to public health, due to the increasing prevalence of infections that are difficult to treat with conventional antibiotics (Hussain et al. 2017). Several studies have identified a high frequency of ESBL-producing *E. coli* in poultry from Egypt, Ghana, Algeria and Tunisia (Mnif et al. 2012; Abdallah et al. 2015; Mezhoud et al. 2015; Akenten et al. 2023).

Antimicrobial resistance (AMR) is a current public health problem in Tunisia. In the last 15 years, the country experienced a strong increase of antibiotic resistant bacteria, from humans as well as from animals or foods of animal origin, in strict relation to overuse or misuse of antimicrobials. A huge impact on the Tunisian poultry sector is related to higher resistance rates than those observed in Tunisia's bovine and ovine industries (Di Francesco et al. 2021).

The aim of this research was to detect the presence of *E. coli* in caeca of healthy broilers that are intended for slaughter, in order to evaluate their antimicrobial resistance profiles and to determine the prevalence of ESBL-producing strains.

Materials and Methods

Sample collection

From December 2021 to March 2022, individual caeca samples from 111 broiler chickens were taken from 3 different slaughterhouses in Tunisia (Nabeul, Sousse, Sfax). The samples were collected aseptically, placed into a sterile screw capped flacon tube and transferred in an ice box to the Microbiology Laboratory of National School of Veterinary Medicine of Sidi Thabet to be tested for bacterial characterization.

Isolation and identification of *E. coli*

One gram of faecal contents from each caeca sample was suspended into a 1 ml buffered peptone water (BPW) and were incubated overnight at 37 °C for enrichment. The suspensions were subsequently streaked onto MacConkey agar (Thermo Scientific™, Warrington, UK) supplemented with cefotaxime (CTX 1 µg/ml) to selectively isolate strains exhibiting resistance to broad-spectrum cephalosporins, such as cefotaxime and ceftazidime and incubated overnight at 37 °C. One bacterial colony was picked up from MacConkey agar with CTX and plated on brain heart infusion agar (Merck, Darmstadt, Germany). Bacterial isolates were identified by API 20E galleries (BioMérieux, Lyon, France) which are more convenient than the conventional biochemical tests method (Sharifi-Yazdi and Azimi 1998).

Antimicrobial susceptibility testing

The disk diffusion method was used to assess the antibiotic susceptibility of all *E. coli* isolates on Mueller-Hinton agar plates (Thermo Scientific™), following the guidelines and breakpoints of the Antibiogram Committee of the French Society for Microbiology using 21 antimicrobial discs comprising (per disk): amoxicillin (20 µg), cefepime (30 µg), piperacillin (30 µg), ticarcillin (85 µg), cephalothin (30 µg), amoxicillin-clavulanic acid (30 µg), cefuroxime (30 µg), ceftazidime (10 µg), cefoxitin (30 µg), cefotaxime (30 µg), aztreonam (30 µg), ertapenem (10 µg), florfenicol (30 µg), chloramphenicol (30 µg), tetracycline (30 µg), nalidixic acid (30 µg),

ciprofloxacin (5 µg), gentamicin (10 µg), and streptomycin (25 µg). Moreover, the susceptibility to colistin was tested by colispot test (Jouy et al. 2017) and colistin resistance was confirmed by determination of the minimum inhibitory concentrations by the broth microdilution test.

Confirmation of ESBL-producing strains

ESBL production among potential ESBL-producing isolates was confirmed phenotypically using synergy test. Comparison of the zone of inhibition was made for the ceftazidime, cefotaxime, and ceftazidime discs placed 25–30 mm (centre to centre) away from a disc of amoxicillin and clavulanic acid (Jacoby and Han 1996).

Bacterial DNA extraction

Genomic DNA was extracted using the boiling method from overnight bacterial culture nutrient agar. Bacterial cells were suspended in 1 ml of sterile deionized water and centrifuged at 13,000 *g* for 5 min. The resulting pellet was re-suspended in 100 µl of deionized water and incubated at 95 °C for 10 min. The suspensions were then diluted with 500–600 µl of distilled water and stored at −20 °C for use as DNA templates in polymerase chain reaction (PCR) assays.

Detection of β-lactamase genes

The presence of the β-lactamases encoding genes; $bla_{\text{CTX-M- Grouip 1 (G1),}}$ bla_{TEM}, and bla_{SHV}, as well as genes encoding carbapenem resistance; bla_{IMP}, bla_{VIM}, bla_{NDM}, bla_{KPC} and $bla_{\text{OXA-48}}$ was investigated using PCR amplification; PCR was performed using a thermal cycler (2720 Applied Biosystems thermal cycler by Life Technologies). The PCR reaction condition was as follows: initial denaturation at 95 °C for 5 min; 35 cycles of denaturation at 95 °C for 1 min, annealing for 1 min at specific temperature (Table 1), extension at 72 °C for 1 min; and a final extension (72 °C, 10 min).

Data analysis

The data of these strains were analysed by SPSS version 26 software (IBM Corporation, Somers, NY), Chi-square test were carried out to determine to investigate the differences significant in antibiotic resistance, ESBL productions and risk factors. The significance level was set at $P < 0.05$.

Table1. Primers and PCR conditions employed to amplify the β-lactamase genes among *E. coli* isolates.

Target gene	Primer sequence	Size (bp)	Annealing temperature	Reference
$bla_{\text{CTX-M-G1}}$	F :5'-ATG GTT AAA AAA TCACTGCG-3'			
	R :5'-TTA CAA ACC GTC GGTGAC-3'	876	49 °C	(Batchelor et al. 2005)
bla_{SHV}	F : 5'-CACTCAAGGATGTATTGTG-3'			
	R : 5'-TTAGCGTTGCCAGTGCTCG-3'	885	54 °C	(Jouini et al. 2007)
bla_{TEM}	F : 5'-ATTCTTGAAGACGAAAGGGC-3'			
	R : 5'-ACGCTCAGTGGAACGAAAAC-3'	1150	50 °C	
$bla_{\text{OXA-48}}$	F : 5'-GCGTGGTTAAGGATGAACAC-3'			
	R : 5'-CATCAAGTTCAACCCAACCG-3'	438	56 °C	(Hatrongjit et al. 2018)
$bla_{\text{NDM-1}}$	F : 5'-GGTTTGGCGATCTGGTTTTC-3'			
	R : 5'-CGGAATGGCTCATCACGATC-3'	621	56 °C	
bla_{IMP}	F : 5'-GGAATAGAGTGGCTTAAYTCTC-3'			
	R : 5'-GGTTTAAYAAAACAACCACC-3'	203	56 °C	
bla_{VIM}	F: 5'-GATGGTGTTTGGTCGCATA-3'			
	R : 5'-CGAATGCGCAGCACCAG-3'	390	52 °C	
bla_{KPC}	F : 5'-CGTCTAGTTCTGCTGTCTTG-3'			
	R : 5'-CTTGTCATCCTTGTTAGGCG-3'	798	56 °C	

Results

In this study, 108 (97.3%) *E. coli* isolates were obtained on MacConkey agar supplemented with cefotaxime (1 µg/ml) from 111 faecal samples of chicken from three suppliers (A, B, C); 51 samples from A, 30 from B and 30 from supplier C (Table 2). The isolates were further analysed to determine and characterize their antibiotic resistance profile.

Table 2. The prevalence of *E. coli* within 3 different suppliers.

Supplier	No. of samples examined	No. of positive samples	Rate (%)
A	51	49	96
B	30	30	100
C	30	29	97

Table 3. Antimicrobial susceptibility patterns of 108 *E. coli* isolates.

Antibiotics	Susceptible (n) %	Intermediate (n) %	Resistant (n) %	Non-susceptible (n) %
Amoxicillin	0	0	108	108
	0%	0%	100%	100%
Piperacillin	4	3	101	104
	4%	3%	93%	95%
Amoxicillin/clavulanic acid	47	3	58	61
	42%	3%	55%	56.5%
Ticarcillin/clavulanic acid	33	42	33	66
	31%	39%	31%	69%
Cephalothin	0	4	104	108
	0%	4%	96%	100%
Cefuroxime	10	17	81	98
	10%	15%	75%	90.7%
Cefoxitine	82	12	14	26
	76%	11%	13%	24.1%
Cefotaxime	9	9	90	99
	8.3%	8.3%	83.3%	91.6%
Cefepime	0	0	108	108
	0%	0%	100%	100%
Ceftazidime	8	18	82	100
	7.40%	16.6%	75.92%	92.5%
Aztreonam	16	8	84	92
	14%	7%	78%	85%
Ertapenem	70	9	29	38
	65%	8%	27%	35%
Gentamicin	66	7	35	42
	59%	6%	34%	38.9%
Streptomycin	15	10	83	93
	14%	9%	77%	86%
Tetracycline	3	0	105	105
	3%	0%	97%	97%
Chloramphenicol	33	0	75	75
	30%	0%	70%	70%
Florfenicol	34	3	71	74
	31.4%	2.7%	64%	68.5%
Sulphamethoxazole/trimethoprim	57	0	51	51
	53%	0%	47%	47%
Nalidixic acid	49	1	58	59
	46%	1%	53%	54%
Ciprofloxacin	58	2	48	50
	55%	2%	43%	46%
Colistin	100	-	8	8
	93%		7%	7%

Table 4. Distribution of the prevalence of antibiotic resistance according to suppliers.

Antibiotics	Supplier A		Supplier B		Supplier C		Total		*P* value
Amoxicillin	49	100%	30	100%	29	100%	108	100%	–*
Piperacillin	45	92%	30	100%	29	100%	104	98%	0.082
Amoxicillin/ clavulanic acid	22	45%	20	67%	24	83%	66	65%	0.003
Ticarcillin/ clavulanic acid	24	49%	26	69%	28	96%	78	71%	0.000
Cephalothine	49	100%	30	100%	29	100%	108	100%	–
Cefuroxime	49	96%	30	100%	18	62%	100	87%	–
Cefoxitin	12	24%	8	26%	12	41%	32	30%	0.089
Cefotaxime	43	88%	30	100%	26	89%	99	92%	0.145
Cefepime	49	100%	30	100%	29	100%	108	100%	–
Ceftazidime	46	94%	27	93%	27	93%	103	93%	0.809
Aztreonam	34	69%	30	100%	28	96%	92	88%	0.000
Ertapenem	8	16%	13	43%	17	59%	38	35%	0.003
Gentamycin	15	31%	13	43%	12	41%	42	45%	0.828
Streptomycin	42	86%	29	79%	26	90%	100	93%	0.057
Tetracycline	46	94%	30	100%	29	100%	110	97%	0.156
Chloramphenicol	33	67%	26	86%	19	65%	78	55%	0.114
Florfenicol	31	63%	23	77%	19	65%	73	51%	0.275
Sulphamethoxazole/ trimethoprim	16	33%	18	60%	16	55%	53	62%	0.18
Nalidixic acid	11	22%	27	90%	21	72%	59	54%	0.000
Ciprofloxacin	5	10%	20	67%	22	76%	47	51%	0.000
Colistin	1	2%	4	13%	2	7%	7	7%	0.140

*– No statistics are computed because the values are constant

Antibiotic resistance profile

High levels of resistance were observed against several antibiotics, including amoxicillin, cefepime, tetracycline, cephalothin, piperacillin, cefotaxime, aztreonam, streptomycin, ceftazidime, cefuroxime, chloramphenicol, and florfenicol, with resistance rates ranging from 64% to 100%. Moderate resistance rates (27% to 55%) were noted for amoxicillin/clavulanic acid, nalidixic acid, sulphamethoxazole/trimethoprim, ciprofloxacin, gentamicin, ticarcillin/clavulanic acid, and ertapenem (Tables 3 and 4). Low resistance levels were detected for cefoxitin (13%) and colistin (7%). Notably, 98% (106/108) of the isolates were classified as multidrug-resistant (MDR). Extended-spectrum β-lactamase production was confirmed in 70% (76/108) of the isolates.

Antibiotic resistance according to suppliers

Among 108 *E. coli* strains, those from supplier A showed significantly lower resistance to ticarcillin, amoxicillin/clavulanic acid, nalidixic acid, aztreonam, and ciprofloxacin compared to the others, particularly supplier C. High resistance to amoxicillin, cefotaxime, tetracycline, and other antibiotics was observed across all suppliers. ESBL-producing strains were more frequent in supplier B (90%) than in A (63%) and C (62%) ($P = 0.021$). Multidrug resistance was nearly universal (98% overall), with suppliers B and C at 100% and A at 96% (Table 2).

Detection of β-lactamase genes

The identification of β-lactamase genes in the isolates revealed that ESBL-$bla_{\text{CTX-M-G1}}$ was the most prevalent broad-spectrum β-lactamase gene, detected in 79.6% (86/108) of isolates, followed by bla_{TEM} (45.4%, 49/108) and bla_{SHV} (33.3%, 36/108). Carbapenem resistance genes, $bla_{\text{OXA-48}}$ and bla_{IMP}, were identified in 13% and 6.5% of isolates, respectively. The genes $bla_{\text{CTX-M-G1}}$-ESBL and bla_{TEM} were predominantly found in strains from suppliers A and C, with significant differences ($P = 0.016$ and $P = 0.002$, respectively). Moreover, bla_{SHV} was significantly more prevalent in strains from supplier A compared to other suppliers ($P = 0.001$). Additionally, the carbapenem resistance gene $bla_{\text{OXA-48}}$ was most frequently found in strains from suppliers B and C ($P = 0.008$) (Table 5).

Table 5. Distribution of the prevalence of β-lactamase genes according to the suppliers.

Antibiotic	Supplier A (n = 49)		Supplier B (n = 30)		Supplier C (n = 29)		Total (n = 108)		*P* value
$bla_{\text{CTX-M-G1}}$	40	81%	19	63%	27	93.1%	86	79.6%	0.016
bla_{TEM}	30	61%	6	20%	13	44.8%	49	45.4%	0.002
bla_{SHV}	25	51%	10	33.3%	1	3.4%	36	33.3%	0.001
bla_{IMP}	2	4.1%	2	6.6%	3	10.3%	7	6.5%	0.554
$bla_{\text{OXA-48}}$	1	2.05%	6	20%	7	24.1%	14	13%	0.008

Discussion

Poultry products are widely consumed worldwide, being popular food choices due to their fairly reasonable price. In this investigation, we assessed the antibiotic resistance in *E. coli* isolates obtained from the caeca of healthy chicken broilers collected in poultry slaughterhouses across three key geographical regions: Great Tunis in the north, Mahdia in the central-east, and Sfax in the southeast of Tunisia.

In Tunisia, as in many other countries, a wide range of antimicrobials are used in poultry for both prophylactic and therapeutic purposes, including penicillins, tetracyclines, macrolides, colistin, sulphonamides, aminoglycosides, and quinolones. This widespread use raises concerns about antimicrobial resistance and its potential transmission to humans (Economou et al. 2015).

Our findings highlight a concerning discovery that broilers intended for human consumption may harbour MDR *E. coli* strains exhibiting resistance to most commonly used antibiotics. The potential spread of these resistant strains to humans and the environment raises alarm. This poses a significant challenge for Tunisia, a developing country with a shortage of adequately qualified personnel and economic challenges that impact overall health situations.

The overall prevalence of cefotaxime-resistant *E. coli* carriage in broiler caecum was 97%, broken down as follows: 96% for supplier A, 100% for supplier B and 97% for supplier C. This slight variation is non-significant ($P = 0.558$). Since *E. coli* is a predominant constituent of the intestinal microbiota of warm-blooded animals, including broilers, its isolation from poultry samples is expected to be frequent, often approaching 100%. For example, studies have reported isolation rates close to 100% in Tunisia, Iran (Pourhossein et al. 2020), and Algeria (Messaili et al. 2019) and slightly lower in Belgium (92.3%) (Persoons et al. 2011). However, it is important to emphasize that while *E. coli* colonization is common, the detection of cefotaxime-resistant *E. coli* reflects an additional public health concern the commensal bacteria in poultry.

Furthermore, *E. coli* was isolated from 159 out of 170 (93.5%) chicken caecal samples collected in Nepal, following inoculation on MacConkey agar (Koju et al. 2022). In the present study, the resistance rate to the combination of amoxicillin and clavulanic acid was 55%, which is in agreement with the results in Tunisia (43.3%) (Abbassi et al. 2021), and in Italy (68.7%) (Ghodousi et al. 2015). An older Tunisian study reported no resistance to amoxicillin and clavulanic acid (Soufi et al. 2009) while in the Czech Republic study, the resistance was 32% (Pavlickova et al. 2017). On the contrary in China, the resistance rate was extremely high at over 90% (Tang et al. 2022).

In the last decade, the emergence of carbapenem resistance in *Enterobacteriaceae* has increased significantly in clinical settings, becoming a major global health concern that impacts both human and veterinary medicine (Das et al. 2023). In the present study, for carbapenems represented by ertapenem, the resistance rate was 35%, which is a very high rate given that this antibiotic is not used in food-producing animals. Carbapenems are essential antibiotics for managing MDR bacterial infections; however, the emergence of carbapenem resistance severely limits available treatment options and poses a significant clinical challenge (Smith et al. 2024). Colistin, serving as the final option for treating MDR Gram-negative bacteria, exhibited resistance in 7% of our isolates. It is important to remember that colistin is a critically important antibiotic that is used as a last resort drug in human medicine. Consequently, this antibiotic could not be recommended as the preferred drug for managing MDR *E. coli* in our region despite the low resistance rate. Colistin resistance is particularly concerning due to plasmid-mediated transmission and its designation as a last-resort antibiotic in human medicine (Jansen et al. 2022). A survey among 662 European veterinary practitioners revealed that the majority (51.9%) did not use it or had ceased its use, 33.4% had decreased its use, 10.4% had stabilized use, and only 2.7% had increased it (Jerab et al. 2022). There was a significant prevalence of mobilized colistin resistance, with 64 (69.5%) of the ESBL-producing isolates carrying the *mcr* gene, as reported by Reem Ali in Egypt in 2024 (Ali et al. 2024).

In our study, we observed a resistance rate of 7%, to this antibiotic. In South Asian countries, resistance rate to colistin was 30% (Dawadi et al. 2021). The concerning increase in colistin resistance, facilitated by various mechanisms especially plasmid-mediated transfer, underscores the importance of rigorous antimicrobial stewardship and effective wastewater management (Mondal et al. 2024).

In the present study, the tetracycline resistance rate among isolates was 97%, which is comparable to the rate reported in Tunisia (93.9%) by Abbassi et al. (2021). High resistance rates were also observed in Algeria (90%) (Messaili et al. 2019) and in several European countries (89.6%) (Ceccarelli et al. 2020). In contrast, lower rates were reported in another Tunisian study (76%) (Saidani et al. 2019). Notably, a study in Germany by Grobbel et al. (2022), which compared resistance rates between intensively and organically reared broilers, concluded to respective rates of 40.2% and 9.7%.

The resistance rate recorded in our study for chloramphenicol which is banned for use in animals in Tunisia, was 70% versus 64% for florfenicol, these resistances being correlated, resulting possibly from cross-resistance between these two phenicols. In Tunisia, Abbassi et al. (2021) found no resistance to chloramphenicol, in contrast to the moderate rate of 27% reported by Soufi et al. (2009) and the rates of 56% and 44% reported by Saidani et al. (2019) for chloramphenicol and florfenicol, respectively. High resistance to chloramphenicol was reported in China (88.7%) (Zhu et al. 2014). In Peru, the resistance rate to chloramphenicol and florfenicol in non-organic broilers was 62.3% and 52.3%, respectively, while significantly lower values were reported for chloramphenicol (8.6%) and florfenicol (6%) in organic broilers (Murray et al. 2021). It should be noted that cross-resistance between florfenicol and chloramphenicol may explain the high rate of chloramphenicol resistance observed. Although the use

of chloramphenicol on Tunisian farms is officially restricted, its use cannot be entirely ruled out and remains to be confirmed.

The current study reported high resistance rate to quinolones, particularly nalidixic acid (53%), even though it is not used for therapeutic purposes in animals in Tunisia. Similar finding was reported in Tunisia (Abbassi et al. 2021) and in Colombia (Donado-Godoy et al. 2015). However, this rate is lower than in previous studies in Tunisia (74%) (Saidani et al. 2019) and in Saudi Arabia (70.3%) (Altalhi et al. 2010). The resistance rate to ciprofloxacin was 43%, which is in agreement with Colombian and Indian reports of 50.3% and 66%, respectively (Donado-Godoy et al. 2015; Rawat et al. 2022). Resistance to ciprofloxacin showed considerable variation among the three suppliers, with rates of 76%, 67%, and 10% for suppliers C, B, and A, respectively, the difference was highly significant ($P = 0.000$).

The resistance to gentamicin was 34%, higher than the rates reported previously in Tunisia, with 2% of resistant strains in poultry meat (Soufi et al. 2009), whereas another study reported a rate of 22% in faeces of healthy chicken (Saidani et al. 2019) and in Italy, 14.1% of the *E. coli* strains from retail chicken meat were gentamicin resistant (Ghodousi et al. 2015).

In our study, the resistance rate obtained for the sulphamethoxazole and trimethoprim combination was 48%, lower than the rate reported in Tunisia (80%) (Soufi et al. 2009) and in Italy (79.1%) (Ghodousi et al. 2015); however, this rate was higher than in Tanzania (10.8%) (Mgaya et al. 2021).

The study of antibiotic susceptibility revealed that multi-drug resistance was observed in 96% of strains isolated from factory-farmed chickens. These alarming results are in agreement with a Tunisian study carried out on chicken meat (Soufi et al. 2009). Another study reported that 98.9% of the strains were MDR (Hassen et al. 2021), with quinolones, tetracycline, and trimethoprim/sulphamethoxazole being the main antibiotics involved. In contrast, Abbassi et al. (2021) found only 48.8% MDR strains in Tunisia.

This result is higher than in previous studies in Korea (56.4%) (Seo et al. 2018), in Saudi Arabia (40.5%) (Altalhi et al. 2010), and in Brazil (79.3%) (Koga et al. 2015). On the other hand, low frequency of MDR was documented in the Czech Republic (13%) (Pavlickova et al. 2017). The high rate of MDR *E. coli* obtained in our investigations represents a very serious problem, threatening consumer health. This high rate could be due to the uncontrolled use of antibiotics by farmers, as a result of free access to antibiotics. In addition, indiscriminate treatment on poultry farms without recourse to antibiotic susceptibility testing could be one of the causes leading to the emergence of multi-resistant *E. coli* strains.

The ESBL-production was detected in 76 *E. coli* isolates (70%). This finding was lower than the ESBL-production rate observed in chicken from Ecuador (94.3%) (Vinueza-Burgos et al. 2019).

Many studies have confirmed the high frequency of ESBL-producing *E. coli* of avian origin and revealed that these bacteria, isolated from poultry, are significantly more resistant to antibiotics than isolates from other food-producing animals (Liu et al. 2022). Based on the earlier discoveries, the prevalence of ESBL-producing *E. coli* may vary depending on the source of samples and the geographical region. Our study confirmed the differences in ESBL rates according to the region and probably depending on the habits of antibiotic use.

The elevated occurrence of ESBL-producing strains poses a notable health hazard as these isolates have the potential to spread to food products like chicken meat and the environment, subsequently leading to transmission to humans.

The marked differences in antibiotic resistance profiles among *E. coli* strains from suppliers A, B, and C each located in distinct cities suggest potential regional variation in antimicrobial use patterns or farm management practices. Supplier A exhibited significantly lower resistance rates to ticarcillin, amoxicillin/clavulanic acid, nalidixic acid, aztreonam,

and ciprofloxacin compared to suppliers B and C, indicating potentially reduced selective pressure from these agents in its production system. In contrast, consistently high resistance levels (> 85%) to amoxicillin, cephalosporins (e.g., cefotaxime), and tetracycline across all suppliers point to the widespread misuse of critically important antibiotics in poultry farming. Notably, supplier B harboured the highest proportion of ESBL-producing isolates (90%, $P = 0.021$), likely driven by extended-spectrum cephalosporin use. Furthermore, the near-universal prevalence of multidrug resistance (96–100%) represents a serious public health concern. These findings, based on isolates from caecal faecal samples, underscore the urgent need for targeted, region-specific antimicrobial stewardship interventions to mitigate the spread of resistance through the food chain.

The β-lactamase resistance genes were demonstrated in the isolates; $bla_{\text{CTX-M}}$ gene was the most predominant gene and this finding is in agreement with an African study in Ghana (Akenten et al. 2023). The CTX-M-ESBL class is dominant and has become a major concern in health setting worldwide (D'Andrea et al. 2013). The detected genes bla_{TEM} and bla_{SHV} in this study have been reported among poultry in several countries (Messaili et al. 2019; Abdel-Rahman et al. 2023). The presence of these genes does not always correlate with their phenotypic expression, as not all variants of bla_{SHV} and bla_{TEM} confer an ESBL phenotype. The $bla_{\text{CTX-M-G1}}$ and bla_{TEM} genes were highly prevalent among the *E. coli* isolates. These genes were predominantly detected in strains from suppliers A and C, with statistically significant differences, indicating their notable distribution in specific sources.

The current study reports carbapenemase genes $bla_{\text{OXA-48}}$ and bla_{IMP} in *E. coli* isolated from broiler chicken for commercial purpose. The $bla_{\text{OXA-48}}$ gene was identified in 13% of isolates and was most prevalent in strains from suppliers B and C ($P = 0.008$). These findings highlight the dissemination of carbapenems resistance among poultry-associated bacteria, posing a potential public health risk (Loqman et al. 2021). Carbapenem resistance in common bacterial pathogens has emerged as a major global public health concern, as carbapenem antibiotics are considered critically important for treating infections in humans. Despite the absence of use of these antibiotics in animals, the presence of carbapenem resistance genes in bacteria from animals has been reported in Tunisia, including food-producing animals such as chicken and rabbits (Lengliz et al. 2021; Ben Haj Yahia et al. 2023) and wild animals (Selmi et al. 2022).

In conclusion, the elevated incidence of antimicrobial resistance and the emergence of MDR *E. coli* in poultry intended for human consumption, along with the potential dissemination of these bacteria to humans and environment, is a matter of concern. This study confirms the high prevalence of ESBL-producing *E. coli* in chickens, along with a high rate of MDR isolates. Notably, $bla_{\text{CTX-M-G1}}$-ESBL and bla_{TEM} genes were highly prevalent, particularly in strains from specific suppliers, underscoring their important role in resistance dissemination. Poultry farms could represent a major reservoir of ESBL-producing bacteria, contributing to challenging-to-treat illnesses in humans. This arises from the potential spread of these bacteria (or their antimicrobial resistance genes) in both the environment and humans, further complicating the issue of antibiotic resistance. Restricting the utilization of antimicrobials in chicken production may contribute to minimizing the emergenceof antimicrobial resistance issue and reducing the risk of human infection.

Conflict of interest

The authors report no conflicts of interest in this work.

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