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FOOD MICROBIOLOGY - RESEARCH PAPER

Prevalence of AmpC, ESBL, and colistin resistance genes in *Enterobacterales* isolated from ready-to-eat food in Algeria

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Abstract

Antimicrobial resistance among bacteria present in ready-to-eat foods is an emerging concern. Hence, this study investigated the presence of extended-spectrum and AmpC β-lactamases (ESBL/AmpC)-producing *Enterobacterales* (ESBL-E) and the dissemination of *mcr-1 in ESBL-E from ready-to-eat food samples (RTE) in Algeria*. RTE food samples ($n = 204$) were aseptically collected and selectively cultured using MacConkey agar. The isolates were screened for ESBL production using the DDST test, confirmed ESBL-E isolates were identified using different conventional methods and MALDI-TOF MS, antibiotic susceptibility was determined using the disc diffusion and broth microdilution assay, ESBL-E isolates were analyzed for colistin and ESBL/AmpC encoding genes by PCR, and food samples were analyzed by univariate and multiple logistic regression. Overall, 48 (17.4%) of the 276 *Enterobacterales* were confirmed as ESBL producers, with a high prevalence in soups (40%), salads (25%), and cream-filled pastries (23.8%). Antibiotic susceptibility testing revealed that all the ESBL-E isolates were found multi-drug resistant. PCR revealed that bla_{TEM}, $bla_{\text{CTX-M}}$, $bla_{\text{CMY-2}}$, $bla_{\text{OXA-1}}$, and bla_{SHV} were the most frequently detected. $bla_{\text{CTX-M-9}}$ and $bla_{\text{CTX-M-1}}$ were the predominant CTX-M types. Furthermore, four isolates were positive for *mcr-1*; three of them harbored the colistin resistance gene and ESBL/AmpC genes (2 *E. cloacae* and 1 *S. enterica*). To the best of our knowledge, this is the first report that detects the presence of *the mcr-1* gene in ESBL-E strains isolated from RTE foods in Algeria. These findings suggest an urgent need for strict policies that prevent the spread and transmission of ESBL-E in food.

Keywords ESBL · Colistin · Ready-to-eat food · *Enterobacterales* · Antibiotic susceptibility · Algeria

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Introduction

Foodborne diseases are one of the most common health problems in the world, and they are a major cause of low economic productivity and morbidity [1, 2]. Ready-to-eat foods (RTE) are responsible for causing many foodborne diseases because they are eaten *without further cooking, washing, or additional preparation* before consumption. And they could be contaminated with different pathogens during the preparation process, commercial distribution, or marketing [3–5]. According to the World Health Organization (WHO), foodborne infections kill 420,000 people each year [6].

The severity of foodborne infections can be partly caused by the presence of multi-drug-resistant bacteria, and one of the reasons for the rise of antimicrobial resistance is the excessive use of antibiotics in humans, in veterinary medicine, and, more importantly, in food production chains [7–9]. Hence, resistant bacteria use food as an ideal medium for

growth and thus causing foodborne illnesses following the consumption of contaminated food [10].

Enterobacterales are the most prevalent bacteria incriminated in foodborne infections [11, 12]. They are responsible for causing community- and nosocomial-acquired infections in humans [13]. Their resistance to beta-lactams, which are the primary therapeutic choices used to treat infections, has become a public health concern [14–16]. This is due to their ability to produce a variety of β-lactamases, such as cephalosporinases (AmpC) and extended-spectrum β-lactamases (ESBLs) [17, 18]. ESBLs can dismantle first-, second-, and third-generation cephalosporins and penicillins. And some of them can also break down fourth-generation cephalosporins [19–21]. Therefore, the dissemination of extended-spectrum β-lactamases-producing *Enterobacterales* (ESBL-E) in the community outside the hospital could affect the future use of expanded-spectrum beta-lactam antibiotics to treat some severe infections, such as bacteraemia and urinary tract infections. Hence, healthcare professionals could be obligated to use last-resort antibiotics such as colistin. However, the effectiveness of colistin has been *questioned* due to the emergence of the colistin resistance gene, *mcr-1* [22]. This gene has been found recently in food samples of different origins [12, 23, 24].

Information is scarce regarding the presence and characteristics of ESBL-E in RTE foods in Algeria. Earlier, Yaici et al. [8] reported the presence of ESBL/AmpC genes in *Escherichia coli* and *Klebsiella pneumoniae* in sandwiches. Hence, to the best of our knowledge, this study is the first to screen AmpC/ESBL and colistin resistance on a large variety of RTE foods. Thus, we evaluated the presence of ESBL/AmpC genes and the dissemination of *mcr-1* in extended-spectrum β-lactamases-producing *Enterobacterales* isolated from ready-to-eat foods in Algeria.

Material and methods

Study area and the sampling

The study was carried out from November 2021 to March 2022, in which a total of 204 samples of RTE foods were collected from four provinces in Algeria: Batna (n =160), Algiers (n = 20), Setif (n = 14), and Biskra province (n = 10). Food samples were randomly collected from restaurants (n = 30), bakeries (n =13), and supermarkets (n = 5), and each vendor was visited only once. Ten categories of the high-circulating RTE food categories were chosen: cream-filled pastries (n = 42), rice dishes (n = 31), chicken dishes (n = 29), salads (n = 25), cooked potato (n = 22), meat dishes (n = 14), cheese (n =11), fish dishes (n = 8), soups (n = 7), and 15 mixed dishes (they usually contain a gratin with pasta, potatoes, and chicken/meat). The samples were stored in sterile bags and immediately transported to the laboratory in a cooler box for analysis within 4 h after the collection.

Isolation and phenotypic identification of ESBL-producing *Enterobacterales* isolates (ESBL-E)

Food samples (25 g) were homogenized with a mixer in 225 mL of trypticase soy broth (TSB, Sigma, Germany), followed by incubation at 37 °C for 24 h. The samples were then streaked on MacConkey agar plates and incubated for 24 h at 37 °C. MacConkey agar is a *selective and differential medium used to isolate non-fastidious Gram-negative rods*, presumptive *Enterobacterales* colonies per morphology/color were picked from each sample, and they were identified using Gram staining, biochemical characterization (TSI test), the API 20E Galeries, and MALDI-TOF MS.

Enterobacterales isolates were then used for the initial screening of ESBL production; they were streaked on MacConkey agar (Sigma, Germany) supplemented with 2 mg/L cefotaxime as previously described by Costa et al. [25]. Isolates showing growth on MacConkey agar were then screened for susceptibility to aztreonam (AZT; 30 µg), cefotaxime (CTX; 30 µg), and ceftazidime (CAZ; 30 µg) by the disc diffusion method. *E. coli* ATCC 25922 was used as a control strain. The isolates showing reduced susceptibility to one or all antibiotics used were confirmed using the double disc synergy test [26].

The double disc synergy test (DDST)

The double-disc synergy test was used to confirm the ESBL-resistance phenotype. Discs containing cefotaxime, cefoxitin, aztreonam, and ceftazidime were used with and without amoxicillin–clavulanic acid (AMC, 20/10 µg) on the same plate containing Muller-Hinton agar (Oxoid, UK). A positive test result was determined when a 5 mm increase in the zone diameter was observed compared to that of a disc without clavulanic acid [26].

Phenotypic antimicrobial resistance characterization of ESBL-E isolates

Disc diffusion method

The antimicrobial susceptibility of ESBL-E isolates was tested using the Kirby-Bauer disc diffusion method according to the European Committee on Antimicrobial Susceptibility Testing guidelines [27]. Thirteen antibiotics (Oxoid, UK) were used in this study, and they included cefotaxime (CTX; 30 µg), aztreonam (AZT; 30 µg), ceftazidime (CAZ; 30 µg), amoxicillin-clavulanic acid (AMC; 20/10 µg), tetracycline (TET; 30 µg), gentamicin (GN; 10 µg), cefazolin (CZ; 30 µg), amikacin (AK; 30 µg), kanamycin (KAN;

30µg), chloramphenicol (C; 30 µg), trimethoprim-sulfamethoxazole (SXT; 1.25/23.75 µg), imipenem (IMI; 10 µg), and ciprofloxacin (CIP; 5 µg). Plates were incubated for 18 h at 35 ± 1 °C. The inhibition zone was measured, and the breakpoints were interpreted according to EUCAST (2022). The *E. coli* strain ATCC 25922 was used as a control strain.

Broth microdilution assay (BMD)

ESBL-E isolates ($n = 48$) were also screened for their other antibiotics using the broth microdilution assay (BMD). The EURGNCOL sensititre (Thermo Fisher, Vantaa, Finland) plates were used for the BMD assay, and it consisted of a panel of five antibiotics [colistin (0.25–8 µL; COL); piperacillin/tazobactam (1/4–32/4 µL; P/T4); ceftolozane/tazobactam (0.25/4–8/4 µL; C/T; ceftazidime/avibactam (1/4–16/4 µL; *CZA)*; Meropenem (0.12–16 µL; MER)]. The sensititre plates were automatically read using the Sensititre Vizion Digital MIC Viewing System (Thermo Scientific, Vantaa, Finland). All interpretations were based on the epidemiological cut-off (ECOFF) values established by the EUCAST (2022).

DNA extraction

All the phenotypically confirmed ESBL-E isolates were genotyped using multiplex polymerase chain reaction (mPCR) to identify the ESBL/AmpC genes that confer resistance to beta-lactam antibiotics and *mcr-1* gene that confers resistance to colistin. Genomic DNA was extracted using the QIACUBE connect system (Qiagen GmBH, Qiagen Strasse 1, Hilden, Germany) using the Qiagen blood and tissue kit, following the manufacturer's instructions. The extracted genomic DNA was quantified with a Qubit fluorometer 4.0 (Invitrogen, Singapore).

Detection of ESBL/AmpC genes and the colistin resistance gene mcr-1

Each mPCR was conducted using a 25 µL reaction mix. The reaction mix was made up of 17 µL of the Hyclone PCR grade water (Fischer Scientific, USA), 5 µL of the 10× Dream Taq buffer (including 20mM of MgCl2), 1 µL of the dNTP mix (10mM), 0.5 µL of the Taq DNA polymerase (VWR Life Science, Helsinki, Finland), 1 µL of primers (Metabion, München, Germany), and 0.5 µL of the DNA template [28]. The mPCR was performed in a Maxygene II thermocycler (Corning, USA) using the primer sequences, and the amplification parameters are shown in Table 1. The genetic targets were: multiplex I (bla_{TEM}, bla_{SHV}, and $bla_{\text{OXA-1}}$), multiplex II ($bla_{\text{CTX-M}}$ groups 1, 2, and 9), and $bla_{\text{CTX-M}}$ groups 8/25 as well as simplex PCR to screen for the mobile colistin resistance gene (*mcr-1*). The PCR amplicons were loaded onto 2% Tris-acetate-EDTA (TAE) gel, and electrophoresis was performed at 100 V for 1 h. The separated bands were viewed on a gel imager (Alpha Innotech, California, USA).

Table 1 The primer sequences used in the detection of ESBL, AmpC, and colistin genes

Gene targeted	Sequence	Amplification parameters	Expected band size (pb)	Reference
TEM-1 and 2 and its variants	CATTTCCGTGTCGCCCTTATTC CGTTCATCCATAGTTGCCTGAC	98 °C, 2 min; 32 cycles of 98 °C, 10 s, 56 °C, 30 s, 72 °C, 75 s, 72 °C, 6 min	800	[29]
SHV-1 and its variants	AGCCGCTTGAGCAAATTAAAC ATCCCGCAGATAAATCACCAC		713	
OXA-1, OXA-4, and OXA-30	GGCACCAGATTCAACTTTCAAG GACCCCAAGTTTCCTGTAAGTG		564	
CTX-M-1 including 3 and 15	TTAGGAARTGTGCCGCTGYA[a] CGATATCGTTGGTGGTRCCAT[a]	94 °C, 10 min; 32 cycles of 94 °C, 40 s, 60 °C, 40 s, 72 °C, 75 s, 72 °C, 6 min	688	
CTX-M-2 and its variants	CGTTAACGGCACGATGAC CGATATCGTTGGTGGTRCCAT[a]		404	
CTX-M-9 and its variants	TCAAGCCTGCCGATCTGGT TGATTCTCGCCGCTGAAG		561	
CTX-M-8, 25, 26, and 39	AACRCRCAGACGCTCTAC[a] TCGAGCCGGAASGTGTYAT[a]		326	
CMY-2 to CMY-7	CGAAGAGGCAATGACCAGAC ACGGACAGGGTTAGGATAGY[a]		538	
MCR-1	AGTCCGTTTGTTCTTGTGGC AGATCCTTGGTCTCGGCTTG	94 °C, 15 min; 25 cycles of 94 °C, 30 s, 58 °C, 90 s, 72 °C, 60 s, 72 °C, 10 min	320	[30]

[a] $Y = T$ or C, $R = A$ or G, $S = G$ or C, $D = A$ or G or T

Statistical analysis

Data were analyzed using IBM SPSS version 20.0. Descriptive analysis was done to analyze the prevalence of ESBL-E isolated from different food samples. Chi-square tests with Bonferroni-adjusted *p* values were performed to compare differences. *Spearman's rank* correlation *test* was used to study the correlation between *phenotypic and genotypic resistance* profiles. Descriptive statistics are presented as a comparison of ESBL-*Enterobacterales* carriers and non-carriers. Potential statistical associations between the study variables and the acquisition of ESBL-E were explored in univariate analyses performed with logistic regression, and the following variables were used: vendor types, the city, cream-filled pastries, cooked chicken, salads, cooked potato, mixed dishes, meat dishes, cheese, fish dishes, and soups. Factors that were statistically significant in univariate analysis ($p < 0.05$) were included in multiple logistic regression analysis to determine variables associated with the ESBL-E carriage. p value < 0.05 was considered significant unless otherwise specified.

Results

Prevalence and identification of ESBL-E

Of the two hundred and four (204) samples screened, a total of 276 *Enterobacterales* were isolated (Table S1). ESBL-producing *Enterobacterales were detected in* 17.4% (n = 48/276) of the samples from 30 shops out of the 48 visited (n = 62.5%).

The highest percentage of ESBL-E was found in soups (n = 4/48, 40%), and the highest number of ESBL-E was found in cream-filled pastries (n = 20/48, 23.8%) (Table 2). Moreover, according to the univariate analysis, three variables (vendor types, city, and soups) were associated with ESBL-*Enterobacterales* carriage (Table 3). All statistically significant variables from the univariate analysis were included in the multiple regression analysis to exclude possible cofounders. The multiple logistic regression model showed that the only independent predictor of colonization with ESBL-*Enterobacterales* was the variable "soups" (OR: 0.58; 95%CI 0.05–0.73, p = 0.02) (Table S2).

Among the ESBL-E isolates, *Enterobacter cloacae* were the most isolated species (n = 29/48, 60.4%), followed by *Enterobacter sakazakii* (n = 6/48, 12.5%), *Citrobacter freundii* (n = 6/48, 12.5%), *Enterobacter cancerogenus* (n = 2/48, 4.1%), *Salmonella enterica* (n = 2/48, 4.1%), *Enterobacter aerogenes* (n = 2/48, 4.1%), and single isolates of *Citrobacter youngae* (n = 1/48, 2.9%) and *Hafnia alvei* (n = 1/48, 2.9%).

Table 2 Distribution of ESBL-E in ready-to-eat food samples

Category	N° samples	N° *Enterobacterales*	N° ESBL-E isolates	ESBL isolates (%)							
				E. cloacae	*E. sakazakii*	*C. freundii*	*E. cancerogenus*	*S. enterica*	*E. aerogenes*	*C. youngae*	*H. alvei*
Cream-filled pastries	42	84	20	14 (70.0)	2 (10.0)	2 (10.0)	2 (10)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Rice dishes	31	55	4	0 (0.0)	2 (50.0)	1 (25.0)	0 (0)	0 (0.0)	0 (0.0)	1 (25.0)	0 (0.0)
Cooked chicken	29	30	7	4 (57.1)	0 (0.0)	0 (0.0)	0 (0.0)	1 (14.3)	1 (14.3)	0 (0.0)	1 (14.3)
Salads	25	12	3	2 (66.6)	0 (0.0)	0 (0.0)	0 (0.0)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)
Cooked potato	22	22	4	2 (50.0)	0 (0.0)	2 (50.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Mixed dishes	15	24	1	0 (0.0)	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Meat dishes	14	21	3	2 (66.6)	1 (33.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Cheese	11	9	1	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Fish dishes	8	9	1	0 (0.0)	1 (100.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Soups	7	10	4	4 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	204	276	48	29 (60.4)	6 (12.5)	6 (12.5)	2 (4.1)	2 (4.1)	1 (2.1)	1 (4.8)	1 (4.8)

Table 3 Univariate logistic regression analyses of determinants for ESBL/pAmpC-producing *Enterobacterales* carriage isolated from ready-to-eat food samples

Variables	ESBL-*Enterobacterales* carriage		Univariate analysis	
Vendor	Negative (n = 226)	Positive (n = 48)	OR[a] (95%CI[b])	p value
Restaurents	140	25	-	-
Bakeries	66	21	0.56 (0.32–0.97)	0.040*
Supermarkets	20	2	3.18 (0.68–14.7)	0.139
City				
Batna	195	36	-	-
Algiers	15	0	3.32 (1.23–5.43)	0.453
Biskra	5	6	1.23 (0.56–8.09)	0.830
Setif	11	6	0.33 (0.21–0.50)	0.008*
Source of food				
Cream-filled pastries	67	20	0.59 (0.31–1.12)	0.107
Cooked chicken	36	7	1.10 (0.46–2.66)	0.816
Meat dishes	15	3	1.06 (0.29–3.83)	0.922
Fish dishes	5	1	1.06 (0.12–9.31)	0.956
Rice dishes	38	4	2.22 (0.75–6.55)	0.148
Cheese	10	1	2.17 (0.27–17.4)	0.464
Mixed dishes	6	1	1.28 (0.15–10.8)	0.820
Salads	16	3	1.14 (0.32–4.08)	0.837
Soups	4	1	0.04 (0.005–0.44)	0.008*
Cooked potato	32	4	1.81 (0.61–5.39)	0.284

[a]Odds ratio (OR)

[b]Confidence interval of odds ratio

Antibiotic susceptibility patterns

The frequency of antimicrobial resistance of ESBL-E isolates in general and based on the food samples is presented in Fig. 1 and Table 4, respectively. All the ESBL isolates (n = 48) were found resistant to ceftazidime (100%), cefazolin (100%), cefotaxime (100%), and amoxicillin-clavulanic acid (100%), followed by tetracycline (46/48, 95.8%) and aztreonam (37/48, 77.1%), while resistance to ciprofloxacin and trimethoprim-sulfamethoxazole was 2.1% each (1/48). However, no resistance was observed against the other antibiotics used in the disc diffusion method (amikacin, kanamycin, chloramphenicol, and imipenem).

Furthermore, results from the BMD assay showed that 50% of the isolates were resistant to the fifth-generation Cephalosporin, the ceftolozane-tazobactam combination (n = 24/48), followed by the piperacillin-tazobactam combination (n = 17/48, 35.4%), and ceftazidime-avibactam (n = 8/48, 16.6%), and interestingly, 12.5% of the isolates were resistant to colistin (n = 6/48). This polymyxin E antibiotic is considered a last resort (reserve) antibiotic. Moreover, all the ESBL-E isolates were found multi-drug resistant (MDR) [31], and 7 (14.5%) isolates were resistant to ≥ 5 classes of antimicrobials, indicating extensive multi-drug resistant phenotype (Table S3).

Molecular characterization of colistin resistance and beta-lactamase genes

The details of the phenotypic and genotypic antimicrobial resistance of ESBL-E isolates are described in Table 5. Among the 48 ESBL-producing *Enterobacterales*, the mobile colistin resistance gene (*mcr-1*) was detected in 8.3% of the isolates (4/48), two isolates were recovered from cream-filled pastries (2 *E. cloacae* isolates), one from chicken dishes (1 *S.*

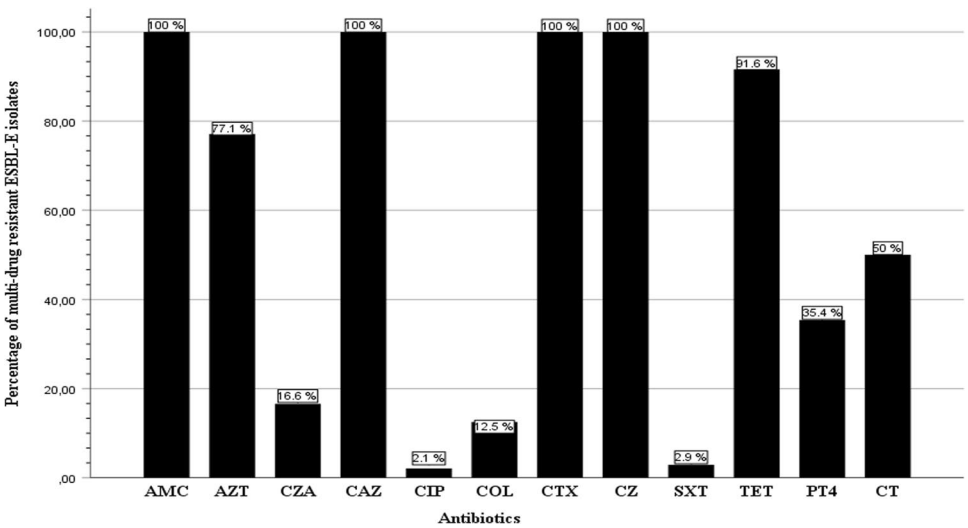

Fig. 1 Resistance phenotypes of ESBL-E isolated from ready-to-eat food in Algeria. CTX: cefotaxime, AZT: aztreonam, CAZ: ceftazidime, AMC: amoxicillin–clavulanic acid, TET: tetracycline, CZ: cefazolin, SXT: trimethoprim sulfamethoxazole, CIP: ciprofloxacin, P/T4: piperacillin/tazobactam, COL: colistin, CZA: ceftazidime/avibactam, C/T: ceftolozane/tazobactam

Table 4 Frequency of antimicrobial resistance of ESBL-E isolates depending on the food origin

Antimicrobial class	Antibiotic	Cream-filled pastries	Rice dishes	Cooked chicken	Meat dishes	Cooked potato	Fish dishes	Salads	Cheese	Mixed dishes	Soups
Cephalosporins	CAZ	20 (100)	4 (100)	7 (100)	3 (100)	4 (100)	1 (100)	3 (100)	1 (100)	1 (100)	4 (100)
	CXT	19 (95.0)	4 (100)	6 (85.7)	3 (100)	4 (100)	1 (100)	3 (100)	1 (100)	1 (100)	4 (100)
	CZ	20 (100)	4 (100)	7 (100)	3 (100)	4 (100)	1 (100)	3 (100)	1 (100)	1 (100)	4 (100)
Quinolones	CIP	0 (0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Monobactams	AZT	17 (85.0)	3 (75.0)	3 (42.8)	2 (66.6)	4 (100)	0 (0)	1 (33.3)	0 (0)	1 (100)	4 (100)
Aminoglycosides	AKA	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	KAN	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	GEN	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
β-Lactamase inhibitors	AMC	20 (100)	4 (100)	7 (100)	3 (100)	4 (100)	1 (100)	3 (100)	1 (100)	1 (100)	4 (100)
	P/T4	9 (45.0)	2 (50.0)	1 (14.9)	1 (33.3)	1 (25.0)	0 (0)	1 (33.3)	0 (0)	1 (100)	1 (25.0)
	CZA	2 (10.0)	1 (25.0)	1 (14.9)	1 (33.3)	1 (25.0)	0 (0)	1 (33.3)	0 (0)	0 (0)	1 (25.0)
	C/T	12 (60.0)	3 (75.0)	2 (28.5)	1 (33.3)	1 (25.0)	0 (0)	2 (66.6)	0 (0)	1 (100)	3 (75.0)
Carbapenems	MER	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	IPI	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Tetracyclines	TET	19 (95.0)	3 (75.0)	7 (100)	3 (100)	4 (100)	1 (100)	3 (100)	1 (100)	1 (100)	4 (100)
Others	COL	2 (10.0)	0 (0)	1 (14.3)	1 (33.3)	1 (25.0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)
	SXT	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	CHL	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Extensive MDR		2 (10.0)	0 (0)	2 (28.5)	1 (33.3)	1 (25.0)	0 (0)	1 (33.3)	0 (0)	0 (0)	0 (0)

Table 5 Phenotypic and genotypic characteristics of ESBL-E isolated from different food

Strain ID	Food	Type of vendor[a]	City	Isolated strain	Antibiotic resistance profile[b]	Extended-spectrum beta-lactamases genes							AmpC	Colistin
						CTXM1	CTXM2	CTXM8	CTXM9	SHV	TEM	OXA1	CMY-2	mcr-1
E75	Cream-filled pastries	BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	-	-	+	-	-	-	-	-
E80		BK	Batna	E. cancerogenus	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T	-	-	-	-	-	+	-	-	-
E81		BK	Batna	E. cancerogenus	CAZ, TET, AMC, AZT, FOX, CZ, C/T, PIP-TAZ	+	-	-	-	-	-	-	+	-
E90		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, CZA, C/T, PIP TAZ	-	-	-	-	-	+	-	+	-
E119		BK	Batna	E. sakazakii	CAZ, TET, AMC, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	+	-	-	-	+	-
E120		BK	Batna	C. freundii	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	-	-	+	-	+	-
E122		BK	Batna	C. freundii	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	-	-	-	-	-	-	-	-
E129		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	+	-	-	-	-	+	-	+	-
E132		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	+	-	-	-	-	+	-	-	-
E144		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T	-	-	-	-	-	-	-	+	-
E145		BK	Batna	E. sakazakii	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	+	-	-	-	+	-
E164		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	+	-	+	-	-	-	-	-
E168		BK	Batna	E. cloacae	CAZ, TET, AMC, CXT, FOX, CZ, C/T, PIP-TAZ	-	+	-	-	-	-	-	-	-
E178		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, CZA, C/T, PIP-TAZ	-	-	-	+	-	-	-	-	-
E173		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	-	-	-	-	-	-
E174		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	-	-	-	-	-	-	+	-
E184		BK	Batna	E. cloacae	CAZ, AMC, AZT, CXT, FOX, CZ	-	+	-	-	-	-	-	+	-
E204		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	-	-	+	-	-	-	-	-
E221		BK	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, COL, PIP-TAZ	+	-	-	+	-	-	-	-	+
E216		BK	Batna	E. cloacae	CAZ, TET, AMC, CXT, FOX, CZ, C/T, COL, PIP-TAZ	-	-	-	-	-	-	-	-	+

Table 5 (continued)

Strain ID	Food	Type of vendor[a]	City	Isolated strain	Antibiotic resistance profile[b]	Extended-spectrum beta-lactamases genes							AmpC	Colistin
						CTXM1	CTXM2	CTXM8	CTXM9	SHV	TEM	OXA1	CMY-2	mcr-1
E229	Meat dishes	R	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	-	-	-	-	-	-
E269		R	Batna	E. cloacae	CAZ, TET, SXT, AMC, AZT, CXT, FOX, CZ, CZA, COL	-	-	-	+	-	-	-	-	-
E385		R	Biskra	E. sakazakii	CAZ, TET, AMC, CXT, FOX, CZ	-	-	-	+	-	-	-	+	-
E278	Cooked potato	R	Setif	C. freundii	CAZ, TET, AMC, AZT, CXT, FOX, CZ, CZA, C/T, PIP-TAZ	-	-	-	-	-	-	-	-	-
E290		R	Setif	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, COL	-	-	-	-	-	-	+	-	+
E305		R	Setif	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	+	-	+	-	-	-	-	-
E375		R	Biskra	C. freundii	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	-	-	+	-	-	-	-	-
E106	Rice dishes	R	Batna	E. sakazakii	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	-	-	-	-	+	+	+	-
E160		R	Setif	E. sakazakii	CAZ, TET, AMC, CXT, FOX, CZ, CZA, C/T	-	-	-	-	-	+	-	+	-
E351		R	Batna	C. freundii	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	-	-	+	-	+	-
E382		R	Setif	C. youngae	CAZ, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	-	-	+	-	+	-
E319	Soups	R	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, CZA, C/T	-	-	-	-	-	+	-	-	-
E329		R	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	-	-	-	-	-	-	-	-
E330		R	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	-	-	+	-	-	-
E334		R	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T	+	-	-	-	-	-	-	-	-
E12	Salads	R	Batna	E. cloacae	CAZ, TET, AMC, CXT, FOX, CZ, CZA, C/T, COL	-	-	-	+	-	+	-	+	-
E111		R	Setif	S. enterica	CAZ, TET, AMC, AZT, CXT, FOX, CZ	-	-	+	-	-	+	-	+	-
E311		R	Batna	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	-	-	+	-	-	-

Table 5 (continued)

Strain ID	Food	Type of vendor[a]	City	Isolated strain	Antibiotic resistance profile[b]	Extended-spectrum beta-lactamases genes							AmpC	Colistin
						CTXM1	CTXM2	CTXM8	CTXM9	SHV	TEM	OXA1	CMY-2	mcr-1
E09	Cooked chicken	R	Batna	H. alvei	CAZ, TET, AMC, CZ	+	-	-	-	+	-	+	+	-
E152		R	Batna	E. cloacae	CAZ, TET, AMC, CXT, FOX, CZ	-	-	-	+	-	-	-	-	-
E256		SM	Batna	S. enterica	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, COL, PIP-TAZ	-	-	+	-	-	-	-	+	+
E310		R	Batna	E. aerogenes	CAZ, TET, AMC, CXT, FOX, CZ, CZA	-	-	-	-	-	+	-	+	-
E389		R	Biskra	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, CIP	+	-	-	-	-	+	-	+	-
E364		R	Biskra	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T	+	-	-	-	-	-	+	+	-
E368		R	Biskra	E. cloacae	CAZ, TET, AMC, AZT, CXT, FOX, CZ	+	-	-	-	-	-	-	-	-
E356	Fish dishes	SM	Batna	E. sakazakii	CAZ, TET, AMC, CXT, FOX, CZ	-	-	-	-	-	-	-	-	-
E369	Cheese	R	Biskra	E. cloacae	CAZ, TET, AMC, CXT, FOX, CZ	-	-	-	-	-	+	-	-	-
E374	Mixed dishes	R	Biskra	C. freundii	CAZ, TET, AMC, AZT, CXT, FOX, CZ, C/T, PIP-TAZ	-	-	-	-	-	+	-	-	-

[a]*BK*, bakery; *R*, restaurant; *SM*, supermarket

[b]*CTX*, cefotaxime; *FOX*, cefoxitine; *AZT*, aztreonam; *CAZ*, ceftazidime; *AMC*, amoxicillin–clavulanic acid; *TET*, tetracycline; *CZ*, cefazolin; *SXT*, trimethoprim sulfamethoxazole; *CIP*, ciprofloxacin; *P/T4*, piperacillin/tazobactam; *COL*, colistin; *CZA*, ceftazidime/avibactam; *C/T*, ceftolozane/tazobactam

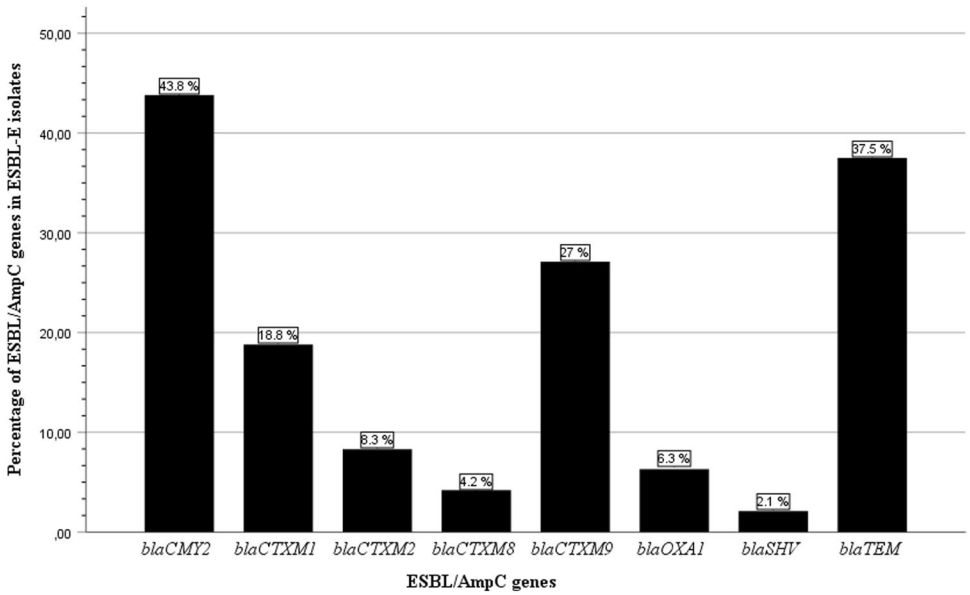

Fig. 2 Percentage of ESBL/AmpC genes in ESBL-E isolates from ready-to-eat food

enterica isolate), and one from cooked potato (1 *E. cloacae* isolate). Moreover, a statistically significant positive correlation was found between the *colistin resistance phenotype* and the *mcr-1* gene ($r = 0.798$, $p < 0.01$).

Regarding the ESBL/AmpC genes, the $bla_{\text{CTX-M}}$ gene was the most abundant, as it was detected in 58.3% (28/48) of the isolates, followed by $bla_{\text{CMY-2}}$ (43.8%, 21/48), bla_{TEM} (37.5%, 18/48), $bla_{\text{OXA-1}}$ (6.3%, 3/48), and bla_{SHV} (2.1%, 1/48). Moreover, 19 isolates (39.6%) produced both an ESBL and a plasmid-mediated AmpC beta-lactamase, and 7 isolates (14.6%) did not harbor any ESBL/AmpC genes (Fig. 2).

In the 28 $bla_{\text{CTX-M}}$ isolates, 13 were clustered as $bla_{\text{CTX-M-9 group}}$ (27.0%); 9 were clustered as $bla_{\text{CTX-M-1}}$ (18.8%), which is comprised of $bla_{\text{CTX-M-1}}$, $bla_{\text{CTX-M-3}}$, and $bla_{\text{CTX-M 15}}$; 4 were clustered as $bla_{\text{CTX-M-2}}$ (8.3%); and 2 were clustered as $bla_{\text{CTX-M-8}}$ (4.2%). Moreover, the co-carriage of at least two beta-lactamase genes was detected in 22.9% (11/48) of the isolates, and three *E. cloacae* isolates presented the same combination ($bla_{\text{CTX-M1}}$/bla_{TEM}/$bla_{\text{CMY-2}}$).

The correlation between antibiotic resistance phenotypes and ESBL/AmpC genes showed a positive correlation between the presence of $bla_{\text{CTX-M1}}$ and resistance to ciprofloxacin ($r = 0.304$, $p = 0.036$). However, a negative correlation was observed between the presence of $bla_{\text{CTX-M1}}$ and resistance to cefoxitin ($r = -0.304$, $p = 0.036$).

Of the *E. cloacae* isolates, 58.6% ($n = 17$), 31.0% ($n = 9$), and 27.6% ($n = 8$) were positive for $bla_{\text{CTX-M}}$, bla_{TEM}, and $bla_{\text{CMY-2}}$, respectively. Among the *E. sakazakii* isolates, 83.3% ($n = 5$), 50% ($n = 3$), and 33.3% ($n = 2$) harbored $bla_{\text{CMY-2}}$, $bla_{\text{CTX-M}}$, and bla_{TEM}, respectively. While among *C. freundii* isolates, 50% ($n = 3$), 33.3% ($n = 2$), and 16.7% ($n = 1$) harbored bla_{TEM}, $bla_{\text{CMY-2}}$, and $bla_{\text{CTX-M}}$, respectively (Table S4). Moreover, based on the food samples, 55.0% ($n = 11$), 45.0% ($n = 9$), and 25.0% ($n = 5$) of cream-filled pastries samples harbored $bla_{\text{CTX-M}}$, $bla_{\text{CMY-2}}$, and bla_{TEM} genes, respectively; 85.7% ($n = 6$), 71.4% ($n = 5$), and 28.6% ($n = 2$) of cooked chicken samples harbored $bla_{\text{CTX-M}}$, $bla_{\text{CMY-2}}$, and bla_{TEM}, respectively.

Furthermore, it was found that there is a significant positive association between the presence of *S. enterica* and $bla_{\text{CTX-M8}}$ ($p \leq 0.001$), *H. alvei* and bla_{SHV} ($p \leq 0.001$), and a significant negative association between the presence of *E. cloacae* and $bla_{\text{CMY-2}}$ ($p \leq 0.001$). And interestingly, it was found that there is a significant positive association between rice and the presence of $bla_{\text{CMY-2}}$ and bla_{TEM} in ESBL-E isolates ($p = 0.006$).

Discussion

Beta-lactamase-producing *Enterobacterales* are no longer solely associated with the healthcare system. Thus, it is essential to investigate their presence in other possible reservoirs, such as food. A total of 204 ready-to-eat foods were analyzed, and 48 isolates were found to be ESBL-producing *Enterobacterales* with a prevalence of 17.4%. These results are similar to the 19.7% prevalence reported in India [32], lower than the 42.4% that was reported in Saudi Arabia [33], but higher than the 5% and 1.9% reported in Switzerland [34] and China [12], respectively. The different prevalence rates observed in these studies may be due to differences in the methodology, sample size, food origin, and ESBL isolation techniques.

Cream-filled pastries were the most analyzed samples in this study, and they harbored a high number of ESBL-E

($n = 20$); these products are rich in milk, eggs, and raw constituents, making them rich nutrient media for microbial growth [35]. We could not find any study that reported the presence of ESBL-E in cream-filled pastries. Hence, this is the first study to the best of our knowledge that analyzes the presence of ESBL-E isolates in cream-filled pastries. Moreover, based on the multiple logistic regression analysis, the variable "soups" was the only independent predictor of colonization with ESBL-*Enterobacterales*; soups are usually cooked in large quantities, and kept unrefrigerated for some time, and then processed again; this way of cooking and dealing with soups can facilitate their contamination by ESBL-E isolates.

While several studies had reported the occurrence of ESBL in *E. coli* as the most prevalent ESBL-E in food [8, 36–38], we detected the highest prevalence of ESBL in *E. cloacae*. These bacteria are capable of causing a wide variety of infections, such as septicemia and pneumonia [39], and they have been found in some studies on RTE food [32, 38, 40]. The large diversity of ESBL-E in food may be related to the enormous diversity of RTE food and the ease by which food items can be contaminated during processing, packaging, or storing [41].

The present study shows that all the isolates were resistant to ceftazidime, cefazolin, cefotaxime, and amoxicillin-clavulanic acid, as well as high resistance to ceftolozane (a fifth-generation cephalosporin). Moreover, all the ESBL-E isolates were found multi-drug resistant [31], and 14.6% were found to be extensively multi-drug resistant [31]. Many factors could explain the rise of antibiotic resistance in the food industry, such as the use of antibiotics in animal feeds as growth promoters *to improve the performance of food animals* [42], the irrigation of plants and crops through sewage water contaminated with fecal material from the effluent of surrounding farms [43, 44], and the use of preservatives and disinfectants for food protection, that can create pressure on the microbial populations, which can trigger them to adapt by developing transient resistances [45, 46].

Interestingly, 95.8% of the isolates were found resistant to tetracycline; this antibiotic is widely used against Gram-negative bacteria, especially in livestock production, because of its few side effects and low cost [47]. However, its extended use in the food and agriculture industry appears to be the reason behind the observed high resistance in our study.

Furthermore, 12.5% of the isolates were resistant to colistin (a reserve antibiotic). This is close to the 19.5% colistin resistance rate reported in RTE foods in Brazil [48], but higher than those found in China (3.2%, [12]) and Spain (2.0%, [49]), respectively. The prevalence of *mcr-1* was 8.3%, which is higher than those found in Germany (3.8%, [50]) but lower than those found in Brazil (19.5%) [48]. The presence of ESBL-E harboring *the mcr-1* gene in RTE food is a cause for public health concern since this could contribute to the acceleration of the spread of the *mcr-1* gene in the environment. In this study, we report for the first time, to our knowledge, the identification of colistin-resistant ESBL-producing *Enterobacterales* strains carrying the *mcr-1* gene in ready-to-eat foods in Algeria.

Screening for the presence of ESBL/AmpC genes revealed the presence of $bla_{\text{CMY-2}}$, $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM}, and $bla_{\text{OXA-1}}$ genes. The presence of the plasmid-mediated AmpC cephalosporinase gene $bla_{\text{CMY-2}}$ has been previously reported across the globe [8, 51–53]; its high prevalence in this study is because it is considered the most common type of plasmid AmpC β-lactamase in the world [54]. The predominance of $bla_{\text{CTX-M}}$ in RTE foods (52.0%) is in accordance with a previous study analyzing sandwiches in Algeria [8] and studies from other countries [32, 55–58]. Moreover, 14.6% of the ESBL isolates that were positive in the double disc synergy test did not harbor any ESBL/AmpC genes. These isolates could carry other ESBL/AmpC genes not investigated in the present study, such as $bla_{\text{ACT/MIR}}$, bla_{DHA}, and bla_{MOX}. Hence, these findings highlight a new avenue that *could* be explored *in future studies*.

Based on food samples, cream-filled pastries were predominately contaminated with ESBL-E isolates harboring $bla_{\text{CTX-M9}}$, bla_{TEM}, and $bla_{\text{CTX-M-1}}$. Interestingly, $bla_{\text{CTX-M-1}}$ is regularly found in animal sources [59], and its presence in pastries proves that the origin of ESBL-E contaminating RTE food is highly diverse. In cooked chicken samples, the presence of most of the ESBL genes was detected ($bla_{\text{CTX-M1}}$, $bla_{\text{CTX-M8}}$, $bla_{\text{CTX-M9}}$, bla_{TEM}, $bla_{\text{OXA-1}}$, bla_{SHV}), with the predominance of $bla_{\text{CTX-M-1}}$, which is in accordance with previous studies on RTE food of animal origin, who found that $bla_{\text{CTX-M-1}}$ was the most prevalent subtype [32, 40, 49, 60].

The combination $bla_{\text{CTX-M-1}}/bla_{\text{TEM}}/bla_{\text{CMY-2}}$ was the most prevalent in this study, which is in line with previous studies [37, 61, 62], and interestingly, the co-carriage of ESBL genes and colistin resistance gene, *mcr-1,* was observed in three isolates (E221, E256, E305). Zhang et al. (2021) [12] also reported ESBL and *mcr-1* gene co-carriage in RTE foods in China. Rhouma and Letellier [63] reported that there is a possible historical link between the presence of the *mcr-1* gene with ESBL genes. However, more studies are needed to confirm this hypothesis.

Moreover, in this study, analyzing the phenotype–genotype correlations showed that a positive correlation between $bla_{\text{CTX-M1}}$ and ciprofloxacin was observed. Colodner [64] suggested that ESBL-producing bacteria often show cross-resistance with other groups of antibiotics, such as fluoroquinolones, which may explain in part these findings. Furthermore, a negative correlation between $bla_{\text{CTX-M1}}$ and cefoxitin was observed. This may indicate that the presence of $bla_{\text{CTX-M1}}$ gene is a bad biomarker for resistance to cefoxitin which is not in accordance with the study of Ramadan

et al. [65], who found that the presence of a $bla_{\text{CTX-M}}$ gene might be leveraged as a good indicator for resistance to beta-lactam antibiotics. Long et al. [66] found that the $bla_{CTX\text{-}M}$ gene has a substantial correlation with the phenotypic resistance of ceftriaxone and cefepime.

In conclusion, our findings showed that RTE foods such as filled cream pastries, salads, and soups are important ESBL/AmpC reservoirs, and we report, for the first time, the presence of colistin-resistant ESBL-E strains carrying the *mcr-1* gene in ready-to-eat foods in Algeria. *Moreover,* we report the co-carriage of an ESBL- and an *mcr-1* gene with zoonotic implications. Hence, strict policies and active surveillance of RTE foods should be implemented to control the spread of these resistant genes and to ensure food safety.

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Author contributions ZN and AIA conducted laboratory analysis of samples, ZN wrote the manuscript, BA and OR edited the paper, and AH revised the article. All authors read and approved the final manuscript.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Competing Interests The authors declare no competing interests.

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