

β -lactamases-dependent antimicrobial resistance in enterobacteria isolated from commercial poultry farms in the Makkah province, Saudi Arabia

Tariq Alpakistany¹, Taher M. Taha^{2,3}, Khaled S. Gazi³, Mohammed A. Thabet³, Ali A Hroobi³, Mohammad Melebari^{3*}

¹Ministry of Health, King Faisal Medical Complex, Taif, Saudi Arabia.

²Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Assiut 71524, Egypt.

³Department of Biology, Faculty of Science, Al-Baha University, Al-Baha 65431, Saudi Arabia.

ARTICLE INFO

Article history:

Received on: October 23, 2023

Accepted on: January 10, 2024

Available online: February 20, 2024

Key words:

Antibiotic resistant,
Enterobacteriaceae,
Extended spectrum β -lactamase,
Multidrug-resistant,
Mobile genetic elements.

ABSTRACT

The emergence of antibiotic-resistant bacterial isolates is one of the intractable problems in the health-care sector that threatens human and livestock health. β -lactamases are among the most common enzymes involved in antibiotic-resistance mechanisms. Enterobacterial isolates were isolated from commercial poultry farms from the Makkah region. A collection of 40 *Enterobacteriaceae* isolates resistant to one or more third-generation cephalosporins was examined for the existence of β -lactamases, including extended-spectrum β -lactamase (ESBL), AmpC β -lactamase, and metallo- β -lactamase (MBL), both phenotypically and genotypically. Based on the phenotypic examinations, 97.5% of the isolates were ESBL, 5% were AmpC, and only 2.5% were MBL. Out of these 40 resistant isolates, 9 (22.5%) were multidrug-resistant (MDR). Based on genotypic examinations, five resistance genes were detected, with the most prevailing gene being TEM (10, 25%), followed by CMY-2 (5, 12.5%), FOX (5, 12.5%), SHV (1, 2.5%), and CTX-M universal (1, 2.5%). The gene mobile factors of Class 1 integrons, transposons, and plasmids were also detected in 5 (12.5%), 5 (12.5%), and 2 (5%) of the examined isolates, respectively. An interesting ESBL MDR isolate was identified which includes genetic elements (transposon and plasmid). In conclusion, the data presented in this study indicated that commercial farm poultry in the Makkah province, Saudi Arabia, is colonized by β -lactamases producing *Enterobacteriaceae*. This supports the hypothesis that non-human sources could be a source of ESBL/AmpC-producing bacteria in humans.

1. INTRODUCTION

Antimicrobial resistance is among the greatest threats and challenges facing human health worldwide, including the Kingdom of Saudi Arabia. The number of annual deaths due to antibiotic-resistant pathogens is expected to rise to up to 10 million by 2050 [1]. The number of recorded antibiotic-resistant isolates (including important human pathogens) is increasing worldwide [2]. *Enterobacteriaceae*, considered to be normal flora in the intestine of food-producing livestock [3-5], make up a large proportion of these resistant isolates.

The emergence and spreading proliferation of resistance among *Enterobacteriaceae* are serious threats to public health [6]. Extended-spectrum β -lactamase (ESBLs) and AmpC β -lactamases both have epidemiological and clinical importance since they are capable of inactivating broad-spectrum cephalosporins and penicillin. The emergence of metallo-beta-lactamase (MBL) producers makes the treatment of ESBL and AmpC producers more complicated. The ideal

treatment of ESBL and AmpC producers was carbapenems which can be hydrolyzed by MBL producers [7]. The most significant reservoir is the intestine of humans and animals, especially those who frequently take antibiotics, particularly β -lactams, in sub-therapeutic doses, as in prophylaxis and growth promotion, and/or therapeutic doses, as in the treatment of bacterial diseases in livestock [8].

The most common antimicrobial resistance in *Enterobacteriaceae* is observed against β -lactams, fluoroquinolones, and aminoglycosides. β -lactam resistance in *Enterobacteriaceae* is mainly conferred by β -lactamases capable of hydrolyzing β -lactam antibiotics. The mechanisms of resistance are genetically diverse since they can be present on chromosomes, plasmids, integrons, and transposons [9]. The most important β -lactamases are ESBLs, AmpCs, and MBLs [10]. ESBLs are β -lactamases that show resistance to penicillins, cephalosporins, and aztreonam (but not to cephamycins or carbapenems) by hydrolyzing these antibiotics and can be inhibited by clavulanic acid [11]. AmpC β -lactamases can hydrolyze cephalosporins, aminopenicillins, cephamycins, and monobactams but are not inhibited by clavulanic acid [12]. MBL producers are resistant to all β -lactam antibiotics with the exception of monobactams and can be inhibited by ethylene diamine tetraacetic acid (EDTA) [13]. The epidemiology of ESBLs/AmpCs is complex: There are several reservoirs, including

*Corresponding Author:

Mohammad Melebari,

Department of Biology, Faculty of Science, Al-Baha University, Al-Baha 65431, Saudi Arabia.

E-mail: melebari@bu.edu.sa.

the environment (e.g., water and soil), wild animals, pets, and farm animals. The contamination of food and the environment is a crucial route for its spread, whether from humans or livestock, and is therefore an important area for control [14]. Thereby, they serve as spreaders of β -lactam-resistance genes. β -lactam-resistance genes can be acquired by pathogenic human bacteria through horizontal transfer and, consequently, complicating infections and antibacterial therapy [15].

The transmission of ESBL-producing pathogens or ESBL genes between animals and owners/raisers/consumers is currently a subject of intense, controversial discussion. Transmission of the resistance might occur through either direct contact with animals harboring the resistant bacteria or consumption of food [16,17]. There are many reports with evidence of zoonotic spread of β -lactamase genes [18-21].

Surveillance studies to assess animals and food of animal origin as potential sources and disseminators of β -lactamases-producing bacteria have been done in different countries [22-24]. Unfortunately, there is scarce information on β -lactamase-producing bacteria in animals and food in Saudi Arabia. The focus of this study was to investigate the prevalence and characterization of β -lactamases-producing *Enterobacteriaceae* isolates obtained from poultry farms in the Makkah Province, Saudi Arabia, using phenotypic methods in addition to molecular techniques.

2. MATERIALS AND METHODS

2.1. Sample Collection

Fecal samples of poultry were aseptically collected from different commercial poultry farms in the Makkah Province, including Makkah, Jeddah, and Taif cities. A total of 60 samples were collected (20 samples from each city). Random farms from the three different cities covering the study region were selected for sampling. The fecal samples were directly transported within 2h to the laboratory on ice and then directly subjected to further tests.

2.2. Isolation and Identification of Enterobacteria

A loopful of the semisolid fecal sample was inoculated onto MacConkey agar and then incubated aerobically for 24 h at 37°C. Bacterial colonies were streaked several times until obtaining completely purified colonies, and then a single colony from the purified isolates was selected and identified. In addition to culturing on MacConkey, the purified colonies were also subcultured on Eosin methylene blue agar. Identification of enterobacteria was done based on colony characteristics on MacConkey agar and Eosin methylene blue agar. Confirmation of enterobacteria identification was done by VITEK 2 (bioMérieux, France).

2.3. Antimicrobial Susceptibility Test

Antibiotic susceptibility was determined by the disk diffusion method on Mueller-Hinton agar [25]. Briefly, a single colony from each bacterial isolate was cultured on Mueller-Hinton agar for 18h. These bacteria were used for the preparation of bacterial suspension with an optical density equivalent to 0.5 McFarland standards by suspending bacteria in sterile saline solution (0.85% NaCl aqueous solution). A volume of 100 μ L of bacterial suspension was spread on Mueller-Hinton agar plates, and then antibiotic disks were applied on the prepared plats. After incubation for 24 h at 37°C, the inhibition zones were measured and the bacterial isolates classified as sensitive or resistant based on the Clinical and Laboratory Clinical and Laboratory

Standards Institute [6]. The susceptibility test was conducted against 28 antibiotics [Table 1] (Oxoid, USA, and Biomerieux, France).

2.4. Phenotypic Detection of β -lactamases Production

2.4.1. Detection of ESBLs production

Isolates that showed cephalosporin resistance were subjected to the double-disk synergy test (DDST). The synergy between cefotaxime and clavulanate was detected by placing a disk containing a combination of amoxicillin (20 μ g) and clavulanate (10 μ g) between cefotaxime 30 μ g (up) and ceftazidime 30 μ g (down) at a distance of 20 mm. A clear-cut extension of the edge of the cefotaxime and ceftazidime inhibition zone toward the disk containing clavulanate. The DDST was considered positive when the decreased susceptibility to cefotaxime was combined with the synergy between ceftazidime and clavulanate. We detect the ESBL enzymes by several methods (DDST), determination of minimum inhibitory concentration, VITEK®2 system, and E-test) [26].

Table 1: Antibiotic resistance pattern of the isolated *Enterobacteriaceae* isolates against selected antibiotics.

Antibiotics	No. of resistant enterobacterial isolates	% of resistant strains
Ampicillin	36	90
Amoxicillin/Clavulanic acid	35	87.50
Cefotaxime	28	70
Ceftazidime	35	87.50
Cefepime	11	27.50
Cefuroxime	21	52.50
Ceftriaxone	22	55
Cefoxitin	3	7.50
Cefazolin	0	0
Cephalothin	0	0
Imipenem	19	47.50
Ertapenem	2	5
Aztreonam	21	52.50
Meropenem	11	27.50
Piperacillin	0	0
Mezlocillin	0	0
Piperacillin/tazobactam	10	25
Tetracycline	0	0
Moxifloxacin	0	0
Ciprofloxacin	2	5
Levofloxacin	0	0
Norfloxacin	10	25
Tobramycin	0	0
Gentamycin	8	20
Fosfomycin	3	7.50
Nitrofurantoin	0	0
Trimethoprim/sulfamethoxazole	23	57.50
Amikacin	2	5



2.4.2. Detection of AmpC production

Detection of AmpC β -lactamase production was done using the disk antagonism test. Volumes of 100 μ L of the isolates with an optical density equivalent to that of 0.5 McFarland standards were spread out over Mueller-Hinton agar plates. Antibiotic disks contained a combination of ceftiofur-cloxacillin disk diffusion tests and ceftiofur-EDTA disk diffusion tests. The appearance of an inhibition zone around a disk indicates a positive result [27].

2.4.3. Detection of metallo- β -lactamase production

This method is based on the synergy between inhibitors like EDTA and imipenem for metallo- β -lactamase detections. This method takes advantage of metalloenzymes' dependence on zinc ions using chelating agents like EDTA to inhibit zinc-dependent hydrolysis of antibiotics. The appearance of an inhibition zone around a disk indicates a positive result [28].

2.5. Detection of β -lactamases Genes

2.5.1. Extraction of genomic DNA

Gram-negative bacteria whose plasmid DNA was to be extracted were grown overnight at 37°C in tryptic soy broth. The bacterial cells were harvested using a centrifuge at 4000 rpm for 10 min. Pelleted cells were collected directly for the extraction step. Extraction of genomic DNA was performed using the boiling method. A volume of 250 μ L Q water was added to an Eppendorf tube, followed by one or two fresh colonies of bacteria which were completely dissolved by vortex for 1 min. All samples were loaded into a thermal block device and heated to 100°C for 15 min. The tubes were then transferred to an ice block for 10 min and then centrifuged at 1300 rpm for 5 min. A volume of 1.5 μ L of the supernatant was transferred to another Eppendorf tube and stored at -20°C.

2.5.2. Polymerase chain reaction (PCR) assays for β -lactamase genes

β -lactamase-producing isolates were tested for β -lactamases genes — namely, TEM, SHV, CMY-2, DHA, OXA, FOX, and CTX-M 1,2,4 and universal by PCR using the primers listed in Table 2 under amplification conditions specified in Table 3. PCR was performed using 25 μ L PCR reaction tubes, 12.5 μ L of 2 $\times$ master mix (0.05 U/ μ L Taq DNA polymerase, reaction buffer, 4 mM MgCl₂, 0.4 mM of each dNTP (dATP, dCTP, dGTP, and dTTP) (Thermo Fisher Scientific, USA), primer (2 μ L), the sample (3 μ L), and Q water (7.5 μ L) were added to the reaction tubes. The DNA template was prepared using a simple boiling method.

2.5.3. Electrophoresis of amplified products

Agarose (1.4 g) was added to 100 mL of 10 $\times$ TBE buffer (40 mM tris-acetate, 20 mM acetic acid, 1 mM EDTA, pH 8.0). The agarose was solubilized by heating it in a microwave oven for about 10 min, after which 20 μ L of ethidium bromide was added. The gel mixture was then poured into an electrophoresis mold and allowed to cool to room temperature. Then, 5 μ L of each of the PCR product samples was put into the gel alongside 5 μ L of a suitable molecular weight marker; in each case, this was applied after mixing with 1 μ L loading buffer on a piece of parafilm. Each mixture was applied to a slot prepared in the gel using a 10 μ L micropipette. The electrophoresis gel was covered, and the power supply was switched on and adjusted to 10 V/cm. After running, the gel was visualized in a UV transilluminator.

2.6. Extraction of Plasmid DNA

Gram-negative bacteria whose plasmid DNA was to be extracted were grown overnight at 37°C in tryptic soy broth. The bacterial cells were harvested using a centrifuge at 4000 rpm for 10 min. Pelleted cells were

Table 2: Primers used for the detection of all genes in this study.

Assay	Gene name	Primer sequence	Size of pattern (bp)
Set1	CTX-M 4	F*-GACAAAGAGAGTGCAACGGATG R*-TCAGTGCGATCCAGACGAAA	501
	TEM	F-AGTGCTGCCATAACCATGAGTG R-CTGACTCCCCGTCGTGTAGATA	431
	OXA	F-ATTATCTACAGCAGCGCCAGTG R-TGCATCCACGTCTTTGGTG	296
	SHV	F-GATGAACGCTTTCCCATGATG R-CGCTGTTATCGCTCATGGTAA	214
Set2	CMY-2	F-AGCGATCCGGTCACGAAATA R-CCCGTTTTATG CACCCATGA	695
	CTX-M 1	F-TCCAGAATAAGGAATCCCATGG R-TGCTTTACCCAGCGTCAGAT	612
	CTX-M 2	F-ACCGCCGATAATTTCGCAGAT R-GATATCGTTGGTGTTGCCATAA	588
	DHA	F-GTGGTGGACAGCACCATTA R-CCTGCGGTATAGGTAGCCAGAT	314
Set3	CTX-M Universal	F-CCGCTGRTTCTGGTSACYTAYTTYACCA R-GGCGACYAAGACCASTRATRAARTGGGT	591
Set4	FOX	F-AACATGGGGTATCAGGGAGAT R-CAAAGCGCGTAACCGGATTGG	191
Set5	merA	F-TCCGCAAGTNGCVACBGTTGG) R-ACCATCGTCAGRTARGGRAAVA)	288
Set6	intI 1	F-GGCATCCAAGCAGCAAG R-AAGCAGACTTGACCTGA	491

F*: Forward, R*: Reverse.

Table 3: PCR conditions used for the detection of ESBL genes.

Assay	PCR reaction	Target gene	PCR conditions
Set1	Multiplex	CTX-M 4 TEM OXA SHV	Initiation for 5 min at 94°C; 30 cycles of 94°C for 60 s, 55°C for 60s, 72°C for 60 s; and final extension of 72°C for 5 min.
Set2	Multiplex	CMY-2 CTX-M 1 CTX-M 2 DHA	Initiation for 5 min at 94°C; 30 cycles of 94°C for 60 s, 55°C for 60 s, 72°C for 60 s; and final extension of 72°C for 5 min.
Set3	Uniplex	CTX-M UNIVERSAL	Initiation for 15 min at 95°C; 30 cycles of 94°C for 30 s, 62°C for 90 s, 72°C for 60 s; and final extension of 72°C for 10 min.
Set4	Uniplex	FOX	Initiation for 90 s at 94°C; 30 cycles of 94°C for 90 s, 57°C for 630 s, 72°C for 60 s; and final extension of 72°C for 10 min.
Set5	Uniplex	merA	Initial DNA denaturation step at 94°C for 5 min, followed by 35 cycles, beginning with 1 min of denaturation at 94°C, 30 s of primer annealing at 62°C, and 30 s of extension at 72°C. The final extension step was performed at 72°C for 7 min; final storage at 4°C.
Set6	Uniplex	intI 1	PCR was performed for 30 cycles of 94°C for 30 s, 55°C for 30 s, and extension at 72°C for 45 s for amplification of the integrase genes or 4 min for amplification of the cassette region.

PCR: Polymerase chain reaction, ESBL: Extended-spectrum beta-lactamase.

collected directly for the extraction step. The protocol for purifying large plasmid DNA is presented in [Table 4](#), according to the manufacture protocol (Thermo Fisher Scientific, USA). Agarose gel electrophoresis was performed in a tris-acetate buffer containing 40 mM Tris, 20 mM acetic acid, and 2 mM Na₂EDTA (pH 8.1). The gels contained 0.6% agarose, and electrophoresis was performed at 100V (3.6 V/cm) for five hours. Gels were stained with 0.5 µg/mL of ethidium bromide and photographed.

2.7. Detection of Integrons and Transposons

Class 1 integrons and transposons were scanned in chromosomal DNA using intI 1 and merA primers [[Table 2](#)], respectively. The uniplex PCR technique was used under amplification conditions specified in [Table 3](#).

3. RESULTS

3.1. Prevalence of *Enterobacteriaceae*

From the collected 60 poultry fecal samples, 40 different bacterial isolates belonging to *Enterobacteriaceae* were recovered [[Figure 1](#)].

Table 4: Protocol of plasmid extraction from Gram-negative bacteria.

Steps	Procedure
1	Pelleted cells were resuspended in 379 µL of 6.7% sucrose, 50 mM Tris, 1 mM EDTA (pH 8.0), and warmed to 37°C.
2	96.5 µL of lysozyme was added (10 mg/mL in 25 mM Tris, pH 8.0). Then, it was incubated for 5 min at 37°C.
3	48.2 µL of 0.25M EDTA, 50 mM Tris (pH 8.0) was added.
4	27.6 µL of sodium dodecyl sulfate was added (20% [wt/vol] in 50 mM Tris, 20 mM EDTA, pH 8.0) and immediately mixed.
5	The solution was incubated in 1.5 mL Eppendorf for 5–10 min at 37°C to complete lysis, and then vortexed at the highest setting for 30 s in an appropriate tube.
6	27.6 µL Fresh 3.0N NaOH was added and mixed gently by intermittent inversion for 10 min.
7	49.6 µL of 2.0M tris-hydrochloride (pH 7.0) was added and mixed for 3 min.
8	71.7 µL of 5.0M NaCl was added.
9	700 µL Phenol saturated with 3% NaCl was added, and then mixed thoroughly and centrifuged for 5 min.

EDTA: Ethylene diamine tetraacetic acid.

The isolated bacteria were identified as *Escherichia coli* (*n* = 36), *Klebsiella pneumoniae* (*n* = 2), *Enterobacter cloacae* (*n* = 1), and *Providencia alcalifaciens* (*n* = 1).

3.2. Prevalence of Antibiotic Resistance

The results of antibiotic resistance tests of the isolated bacteria against 28 antibiotics are summarized in [Table 1](#). Thirty-six bacterial isolates (90%) were resistant against ampicillin, while 35 bacterial isolates (87.5%) were resistant against amoxicillin/clavulanic acid and ceftazidime. All the studied bacterial isolates were sensitive to nine antibiotics — namely, cefazolin, cephalothin, piperacillin, mezlocillin, tetracycline, moxifloxacin, levofloxacin, tobramycin, and nitrofurantoin. The number of resistant bacterial isolates to the rest of the used antibiotics ranged from 2 to 28.

3.3. Phenotypic Detection of β-lactamases Producers and Multidrug-resistant (MDR) Isolates

This work was designed to detect β-lactamase-producing *Enterobacteriaceae*, including ESBL-, AmpC-, and MBL-producing isolates using phenotypic tests. Out of the studied 40 isolates, 39 (97.5%) were ESBL-positive; the only ESBL-negative isolate was *P. alcalifaciens*. Of the 39 ESBL-positive isolates, 36 (90%) were *E. coli*, 2 (5%) were *K. pneumoniae*, and 1 (2.5%) was *E. cloacae* [[Figure 2](#)].

Out of the 40 isolates, two isolates (5%) were AmpC producers, while only one isolate (2.5%) was an MBL producer. In this study, we considered any isolates that showed resistance to three or more antibiotic groups to be MDR. Based on this criterion, nine isolates (22.5%) were potentially MDR [[Table 5](#)]. Of these potential MDR isolates, eight were *E. coli* and one was *K. pneumoniae*.

3.4. Genotypic Distribution of β-lactamases Genes

The existence of 10 β-lactamase genes was tested in the chromosomal DNA of bacterial isolates. Of these 10 genes, eight genes — TEM, SHV, CMY-2, OXA, CTX-M-1, CTX-M-2, CTX-M-4, and

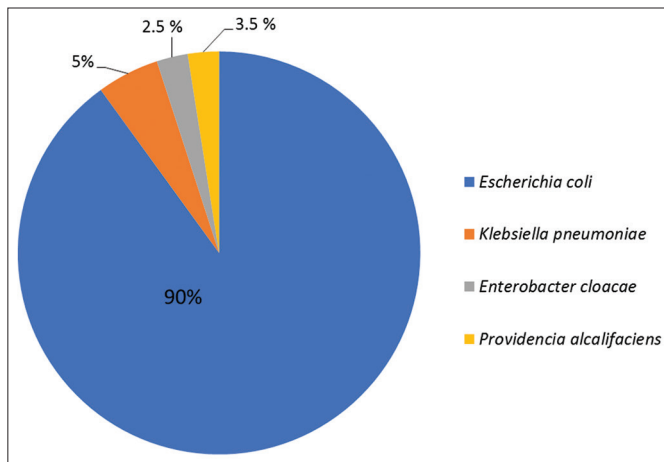


Figure 1: Distribution of isolated bacterial species and their ESBL production results. Only *Providencia alcalifaciens* is ESBL negative. ESBL: Extended-spectrum beta-lactamase.

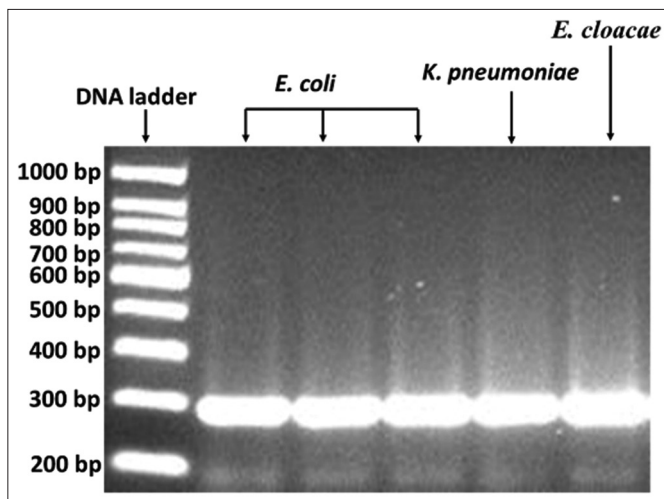


Figure 2: Uniplex PCR shows the positive FOX isolates with a marker (1kb). PCR: Polymerase chain reaction.

DHA — were tested using the multiplex PCR technique, while the FOX and CTX-M-universal genes were tested using the uniplex PCR technique [Figure 3]. The detected chromosomal genes were TEM, FOX, CMY-2, SHV, and CTX-M universal [Table 6]. The TEM gene was detected in ten isolates [Figure 3]. Eight of them were *E. coli* and the other two were *K. pneumoniae* and *E. cloacae*. The FOX gene was detected in three *E. coli* isolates, one *K. pneumoniae* isolates, and one *E. cloacae* isolates. The other genes were detected only in *E. coli* isolates: CMY-2 (5), SHV (1), and CTX-M- universal (1).

3.5. Detection of Gene Transfer Factors of Antibiotic Resistance

The detection of three gene transfer factors — Class I integrons (intI gene), transposon (merA), and plasmids — was carried out. Class I integrons were detected in five *E. coli* isolates (12.5%). Interestingly, all the isolates that contained the intI gene also contained the TEM gene. Four of them contained the CMY-2 gene, three of them contained the FOX gene, one of them contained the CTX-M universal gene, and one contained the SHV gene. The transposable element merA was detected in two isolates of *E. coli*, both of which had the TEM, CMY-2, and FOX genes. Five *E. coli* isolates were

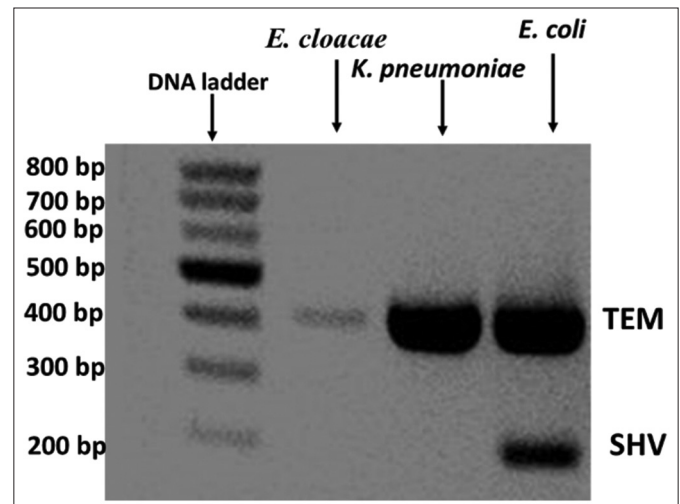


Figure 3: Multiplex PCR shows the positive isolates for TEM and SHV with a marker (1kb). PCR: Polymerase chain reaction.

Table 5: Antimicrobial resistance pattern of MDR ESBL-producing isolates.

Bacteria	Quinolone resistance	Other resistance
<i>E. coli</i>	Ciprofloxacin-Norfloxacin	Trimethoprim/Sulfamethoxazole, Gentamycin
<i>E. coli</i> (3)	Norfloxacin	Trimethoprim/Sulfamethoxazole
<i>E. coli</i> (2)	Norfloxacin	Trimethoprim/Sulfamethoxazole, Amikacin
<i>E. coli</i> (2)	Norfloxacin	Trimethoprim/Sulfamethoxazole, Gentamycin
<i>K. Pneumoniae</i>	Norfloxacin	Trimethoprim/Sulfamethoxazole

E. coli: *Escherichia coli*, *K. pneumoniae*: *Klebsiella pneumoniae*, MDR: Multidrug resistant, ESBL: Extended-spectrum beta-lactamase.

found to possess plasmids. The genotypes of these isolates were CMY-2 (5), TEM (5), FOX (3), SHV (1), and CTX-M UNIVERSAL (1). Interestingly, an MDR *E. coli* isolates that contained two gene transfer factors (transposon and plasmid) also contained TEM, CMY-2, and FOX genes.

4. DISCUSSION

This study investigates the distribution of β -lactamases, including ESBL-, AmpC-, and MBL-producing *Enterobacteriaceae* in fecal collected from poultry farms in the Makkah Region. A total of 40 bacterial isolates were isolated from the collected samples ($n=60$). These bacterial isolates were identified as *E. coli*, ($n=36$), *K. pneumoniae* ($n=2$), *E. cloacae* ($n=1$), and *P. alcalifaciens* ($n=1$). Based on phenotypic tests, out of these 40 isolates, 39 (97.5%) were ESBL producers, while AmpC and MBL production was detected in two isolates (5%) and one isolate (2.5%), respectively. Concerning the prevalence of ESBL-producing *Enterobacteriaceae* in poultry, our results are in accordance with many records from different countries, such as Germany (100%) [29], Denmark (93%) [30], Finland (94.5%) [31], and the Netherlands (94%) [32]. However, other studies have reported a lower prevalence of ESBL producers in poultry farms. For example, Nossair *et al.* [33] reported 25% of ESBL producers in poultry farms in Egypt, and Falgenhauer reported 29% of ESBL producers in poultry in Ghana [34]. In this study, our results show that *E. coli* was most prevalent among the isolates (90%), while

Table 6: Distribution of β -lactamase genes in the bacterial isolates.

Genotype	Bacterial spp.			Total
	<i>Escherichia coli</i>	<i>Klebsiella pneumoniae</i>	<i>Enterobacter cloacae</i>	
CMY (CMY II)	5	0	0	5
CTX-M	1	0	0	1
UNIVERSAL				
TEM	8	1	1	10
SHV	1	0	0	1
AmpC (FOX)	3	1	1	5
Total	18	2	2	22

K. pneumoniae and *E. cloacae* were the least prevalent (5% and 2.5%, respectively). These results are similar to those obtained by Chenouf et al. [35], who recorded ESBL-producing *E. coli* isolates (73) and *K. pneumoniae* isolates (5) among 78 isolates (93.6% and 6.4%, respectively) in poultry livers. *E. coli* is prevalent in poultry farms as well as the surrounding environments [24,36].

Out of the 40 bacterial isolates, 9 (22.5%) MDR isolates were detected; eight of them were *E. coli*, while the ninth isolate was *K. pneumoniae*. These results are lower than that obtained by Bushen et al. [37], who found that MDR enterobacterial isolates from droppings of farm chickens in Ethiopia were 52.5% of the total isolates. Higher percentages of MDR isolates were also reported from Bangladesh (39%) [38] and China (88.2%) [39]. The disagreement of results may be due to the use of different types and concentrations of antibiotics in the poultry industry in the different countries. Following are some examples explaining that. In France, using of tetracyclines and penicillins is allowed in the poultry industry, penicillins are stably used from 2006 to 2016 while there is a decrease in using of tetracyclines during this period. The results of antibiotic resistance showed the existence of around 40% of amoxicillin (belonging to penicillins) resisting *E. coli* accompanied with a decrease in tetracycline resistant *E. coli* during this period [40]. Another example, in the USA the resistance rates of *E. coli* against gentamicin (allowed) and ampicillin (not allowed) are 40% and 20%, respectively [40].

In this study, out of the 10 genes that were checked for, only five were detected. The most prevailing gene was TEM (25%), followed by CMY-2 and FOX (12.5%), and CTX-M universal, and SHV (2.5%). These genes were previously detected in several reports; TEM and CMY-2 genes were detected in antimicrobial-resistant enterobacterial isolates in Egypt from septicemic broilers [41] and healthy broilers [24], while the SHV gene was detected in 96% of 180 *E. coli* isolates from healthy farm chickens in Al-Taif, Saudi Arabia [42]. The TEM gene was also detected in *E. coli* isolates isolated from chicken meat in Al-Taif, Saudi Arabia [43]. Another study detected many genes, including TEM, SHV, and FOX, in *E. coli* isolated from poultry hatcheries in Egypt, with TEM being the most prevailing gene [44]. In our study, the most prevailing resistance gene was TEM, which is in complete accordance with the report by Moawad et al., [24], who detected the TEM gene in 85.7% of ESBL and AmpC β -lactamase-producing isolates. Broadly, the conflictions in the detected resistance genes in different studies may be due to the targeting of different genes from different bacterial species in different studies, the use of different sources for isolating resistant bacterial isolates, and the conducting of these studies in different countries that may use different antimicrobial regimes in poultry industry.

The gene-transferring factors integrons, plasmids, and transposons were detected in 5 (12.5%), 5 (12.5%), and 2 (5%) *E. coli* isolates,

respectively. In this study, we targeted the Class 1 integron using the *intI* 1 marker, as it was the most common integron in ESBL-producing *E. coli* isolated from different sources [45,46]. The Class 1 integron is also detected in all multi-resistance *E. coli* isolates from chicken meat in Taif, Saudi Arabia [43], and in 29.3% of avian pathogenic *E. coli* [45]. Plasmids carrying antibiotic-resistant genes are also detected in many studies: Abbassi [47] detected plasmids in 35% of MDR *E. coli* isolated from healthy broilers in Tunisia, which is higher than our results. It is worth mentioning that mobile genetic elements, including integrons, transposons, and plasmids, have central roles in the horizontal spread of antibiotic resistance among bacteria in the environment [47].

The complex epidemiology of ESBLs/AmpC is quite complicated since there are several reservoirs, containing the environment (soil and water), wild animals, domestic animals, and pets, making it an essential region for control. Horizontal transfer of beta-lactam resistance genes from human disease-causing bacteria to discharged enterobacteria could be the initial reason. Nowadays, the main subject of deep and debatable discussion is the spread of ESBL genes or ESBL-producing pathogens among animals and people (owner/caretaker/consumer). Transmission of the resistance may arise either through direct connection with animal sheltering the resistant bacteria or by food [48].

5. CONCLUSION

This study has shown that healthy chickens in commercial poultry farms in the Makkah Province, Saudi Arabia, are colonized by β -lactamase-producing *Enterobacteriaceae*, including the β -lactamases ESBL, AmpC, and MBL. Some of these β -lactamase producers were MDR. The genotypic analysis of these isolates showed the existence of important antibiotic-resistance genes such as TEM, CMY-2, and FOX. Gene transferring factors, including Class 1 integrons, transposons, and plasmids, were also detected in some of the isolates. Extended research is required to prove the transfer of antibiotic resistance genes to the environment and humans.

6. AUTHORS' CONTRIBUTIONS

All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agreed to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FUNDING

There is no funding to report.

8. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

10. DATA AVAILABILITY

All the data is available with the authors and shall be provided upon request.

11. PUBLISHER'S NOTE

This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

REFERENCES

- Praveenkumarreddy Y, Akiba M, Guruge KS, Balakrishna K, Vandana KE, Kumar V. Occurrence of antimicrobial-resistant *Escherichia coli* in sewage treatment plants of south India. *J Water Sanit Hyg Dev* 2020;10:48-55.
- Serwecińska L. Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. *Water* 2020;12:3313.
- Carattoli A, Lovari S, Franco A. Extended-spectrum β -lactamases in *Escherichia coli* isolated from dogs and cats in Rome, Italy, from 2001 to 2003. *Antimicrob Agents Chemother* 2005;49:833-5.
- Ben Sallem R, Ben Slama K, Saenz Y, Rojo-Bezares B, Estepa V, Jouini A, *et al.* Prevalence and characterization of extended-spectrum beta-lactamase (ESBL)- and CMY-2-producing *Escherichia coli* isolates from healthy food-producing animals in Tunisia. *Foodborne Path Dis* 2012;12:1137-42.
- Bannon J, Melebari M, Jordao C Jr., Leon-Velarde CG, Warriner K. Incidence of top 6 shiga toxigenic *Escherichia coli* within two Ontario beef processing facilities: Challenges in screening and confirmation testing. *Aims Microbiol* 2016;2:278-91.
- Pitout JD, Laupland KB. Extended-spectrum β -lactamase producing *Enterobacteriaceae*; an emerging public health concern. *Lancet Infect Dis* 2008;8:159-66.
- Salvia T, Dolma KG, Dhakal OP, Khandelwal B, Singh LS. Phenotypic detection of ESBL, AmpC, MBL, and their co-occurrence among MDR *Enterobacteriaceae* isolates. *J Lab Physicians* 2022;14:329-35.
- Waters AE, Contente-Cuomo T, Buchhagen J, Liu CM, Watson L, Pearce K, *et al.* Multidrug-resistant *Staphylococcus aureus* in US meat and poultry. *Arch Clin Infect Dis* 2011;10:1227-30.
- Bradford PA. Extended-spectrum β -lactamases in the 21st century: Characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev* 2001;14:933-51.
- Batchelor M, Threlfall EJ, Liebana E. Cephalosporin resistance among animal-associated Enterobacteria: A current perspective. *Expert Rev Anti Infect Ther* 2005;3:403-17.
- Wadekar MD, Anuradha K, Venkatesha D. Phenotypic detection of ESBL and MBL in clinical isolates of *Enterobacteriaceae*. *Int J Curr Res Acad Rev* 2013;1:89-95.
- Conen A, Frei R, Adler H, Dangel M, Fux CA, Widmer AF. Microbiological screening is necessary to distinguish carriers of plasmid-mediated AmpC beta-lactamase-producing *Enterobacteriaceae* and extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* because of clinical Similarity. *PLoS One* 2015;10:e0120688.
- Kali A, Srirangaraj S, Kumar S, Divya HA, Kalyani A, Umadevi S. Detection of metallo-beta-lactamase producing *Pseudomonas aeruginosa* in intensive care units. *Australas Med J* 2013;6:686-93.
- Pitout JD, Nordmann P, Laupland KB, Poirer L. Emergence of *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs) in the community. *J Antimicrob Chemother* 2005;56:52-9.
- Mancuso G, Midiri A, Gerace E, Biondo C. Bacterial antibiotic resistance: The most critical pathogens. *Pathogens* 2021;10:1310.
- Geser N, Stephan R, Kuhnert P, Zbinden R, Kaeppli U, Cernela N, *et al.* Fecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in swine and cattle at slaughter in Switzerland. *J Food Prot* 2011;34:446-9.
- Marshall BM, Levy SB. Food animals and antimicrobials: Impacts on human health. *Clin Microbiol Rev* 2011;24:718-33.
- Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum β -lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: A global perspective. *Clin Microbiol Infect* 2012;18:646-55.
- Schmiedel J, Falgenhauer L, Domann E, Bauerfeind R, Prenger-Berninghoff E, Imirzalioglu C, *et al.* Multiresistant extended-spectrum β -lactamase-producing *Enterobacteriaceae* from humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol* 2012;12:187.
- Davis GS, Price LB. Recent research examining links among *Klebsiella pneumoniae* from food, food animals, and human extraintestinal infections. *Curr Environ Health Rep* 2016;2:128-35.
- Robinson TP, Wertheim HF, Kakkar M, Kariuki S, Bu D, Price LB. Animal production and antimicrobial resistance in the clinic. *Lancet* 2016;387:e1-3.
- Levy SB, FitzGerald GB, Maccone AB. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. *N Engl J Med* 1976;295:583-8.
- Cardozo MV, Liakopoulos A, Brouwer M, Kant A, Pizauro LJ, Borzi MM, *et al.* Occurrence and molecular characteristics of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* recovered from chicken, chicken meat, and human infections in Sao Paulo State, Brazil. *Front Microbiol* 2021;12:628-738.
- Moawad AA, Hotzel H, Neubauer H, Ehrlich R, Monecke S, Tomaso H, *et al.* Antimicrobial resistance in *Enterobacteriaceae* from healthy broilers in Egypt: Emergence of colistin-resistant and extended-spectrum β -lactamase-producing *Escherichia coli*. *Gut Pathog* 2018;10:39.
- CLSI. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard. 12th ed., Vol. M02-A12. Wayne, PA: Clinical and Laboratory Standards Institute; 2015.
- Drieux L, Brossier F, Sougakoff W, Jarlier V. Phenotypic detection of extended-spectrum β -lactamase production in *Enterobacteriaceae*: Review and bench guide. *Clin Microbiol Infect* 2008;14:90-103.
- Adler H, Fenner L, Walter P, Hohler D, Schultheiss E, Oezcan S, *et al.* Plasmid-mediated AmpC beta-lactamases in *Enterobacteriaceae* lacking inducible chromosomal ampC genes: Prevalence at a Swiss university hospital and occurrence of the different molecular types in Switzerland. *J Antimicrob Chemother* 2008;61:457-8.
- Lee K, Chong Y, Shin H, Kim Y, Yong D, Yum J. Modified hodge and EDTA-disk synergy tests to screen metallo- β -lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 2001;7:88-91.
- Geser N, Stephan R, Hachler H. Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* in food producing animals, minced meat and raw milk. *BMC Vet Res* 2012;8:21.
- Agerso Y, Jensen JD, Hasman H, Pedersen K. Spread of extended spectrum cephalosporinase-producing *Escherichia coli* clones and plasmids from parent animals to broilers and to broiler meat in a production without use of cephalosporins. *Foodborne Pathog Dis* 2014;11:740-6.
- Lyhs U, Ikonen I, Pohjanvirta T, Raninen K, Perko-Makela P, Pelkonen S. Extraintestinal pathogenic *Escherichia coli* in poultry meat products on the Finnish retail market. *Acta Vet Scand* 2012;54:64.
- Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, *et al.* Dutch patients, retail chicken meat and poultry share the same ESBL genes,

- plasmids and strains. Clin Microbiol Infect 2011;6:873-80.
33. Nossair MA, Abd El Baqy FA, Rizk MS, Elaadli H, Mansour AM, El-Aziz AH, *et al*. Prevalence and molecular characterization of extended-spectrum β -lactamases and AmpC β -lactamase producing *Enterobacteriaceae* among human, cattle, and poultry. Pathogens 2022;11:852.
 34. Falgenhauer L, Imirzalioglu C, Oppong K, Akenten CW, Hogan B, Krumkamp R, Poppert S, *et al*. Detection and characterization of *esbl*-producing *Escherichia coli* from humans and poultry in Ghana. Front Microbiol 2019;9:3358.
 35. Chenouf NS, Carvalho I, Messaï CR, Ruiz-Ripa L, Mama OM, Titouche Y, *et al*. Extended spectrum β -lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* from broiler liver in the center of Algeria, with detection of CTX-M-55 and B2/ST131-CTX-M-15 in *Escherichia coli*. Microb Drug Resist 2021;2:268-76.
 36. Dahshan H, Abd-Elall AM, Megahed AM, Abd-El-Kader MA, Nabawy EE. Veterinary antibiotic resistance, residues, and ecological risks in environmental samples obtained from poultry farms. Egypt Environ Monit Assess 2015;187:2.
 37. Bushen A, Tekalign E, Abayneh M. Drug- and multidrug-resistance pattern of *Enterobacteriaceae* isolated from droppings of healthy chickens on a poultry farm in Southwest Ethiopia. Infect Drug Resist 2021;14:2051-8.
 38. Nahar A, Siddiquee M, Nahar S, Anwar KS, Islam S. Multidrug resistant-*Proteus mirabilis* isolated from chicken droppings in commercial poultry farms: Bio-security concern and emerging public health threat in Bangladesh. J Biosafety Health Educ 2014;2:120.
 39. Yassin AK, Gong J, Kelly P, Lu G, Guardabassi L, Wei L, *et al*. Antimicrobial resistance in clinical *Escherichia coli* isolates from poultry and livestock, China. PLoS One 2017;12:e0185326.
 40. Roth N, Käsbohrer A, Mayrhofer S, Zitz U, Hofacre C, Domig KJ. The application of antibiotics in broiler production and the resulting antibiotic resistance in *Escherichia coli*: A global overview. Poult Sci 2019;98:1791-804.
 41. Ahmed AM, Shimamoto T, Shimamoto T. Molecular characterization of multidrug-resistant avian pathogenic *Escherichia coli* isolated from septicemic broilers. Int J Med Microbiol 2013;30:475-83.
 42. 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.
 43. Altalhi AD, Gherbawy YA, Hassan SA. Antibiotic resistance in *Escherichia coli* isolated from retail raw chicken meat in Taif, Saudi Arabia. Foodborne Pathog Dis 2010;7:281-5.
 44. Osman KM, Kappell AD, Elhadidy M, ElMougy F, El-Ghany WA, Orabi A, *et al*. Poultry hatcheries as potential reservoirs for antimicrobial-resistant *Escherichia coli*: A risk to public health and food safety. Sci Rep 2018;1:5859.
 45. Awad A, Arafat N, Elhadidy M. Genetic elements associated with antimicrobial resistance among avian pathogenic *Escherichia coli*. Ann Clin Microbiol Antimicrob 2016;15:59.
 46. Pérez-Etayo L, Berzosa M, González D, Vitas AI. Prevalence of integrons and insertion sequences in ESBL-producing *E. coli* isolated from different sources in Navarra, Spain. Int J Environ Res Public Health 2018;10:2308.
 47. Abbassi MS, Kilani H, Zouari M, Mansouri R, El Fekih O, Hammami S, *et al*. Antimicrobial resistance in *Escherichia coli* isolates from healthy poultry, bovine and ovine in Tunisia: A real animal and human health threat. J Clin Microbiol Biochem Technol 2017;3:19-23.
 48. Khedkar S, Smyshlyaev G, Letunic I, Maistrenko OM, Coelho LP, Orakov A, *et al*. Landscape of mobile genetic elements and their antibiotic resistance cargo in prokaryotic genomes. Nucleic Acids Res 2022;6:3155-68.

How to cite this article:

Alpakistany T, Taha TM, Gazi KS, Thabet MA, Hroobi AA, Melebari M. β -lactamases-dependent antimicrobial resistance in enterobacteria isolated from commercial poultry farms in the Makkah province, Saudi Arabia. J App Biol Biotech. 2024;12(2):231-238. DOI: 10.7324/JABB.2024.150557