Kafkas Univ Vet Fak Derg
24 (4): 613-617, 2018
DOI: 10.9775/kvfd.2018.19731

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
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Short Communication

# Isolation and Molecular Characterization of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* from Cage Birds in Adana Region, Turkey

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**Article Code: KVFD-2018-19731 Received: 10.03.2018 Accepted: 03.06.2018 Published Online: 03.06.2018**

**How to Cite This Article**

**Sakin F, Müjde C, Aslantaş Ö:** Isolation and molecular characterization of extended spectrum beta-lactamase (ESBL) producing *Escherichia coli* from cage birds in Adana region, Turkey. *Kafkas Univ Vet Fak Derg,* 24 (4): 613-617, 2018. DOI: 10.9775/kvfd.2018.19731

## Abstract

In this study, it was aimed to investigate the presence of extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* in caged birds. A total of 139 fecal swab samples were collected from cage birds from 14 different aviaries in Adana. ESBL producing *E. coli* was detected in 4.3% (n=6) of the fecal samples and these isolates harbored $bla_{CTX\text{-}M\text{-}15}$ gene. ERIC-PCR analysis revealed three different band patterns among the isolates. These results indicate that cage birds are carrier of ESBL-producing *E. coli*. Therefore, further epidemiological studies are needed to determine the presence of resistant bacteria including ESBL producing *E. coli* in various animal species.

***Keywords:*** *Cage birds, Extended spectrum beta-lactamase (ESBL), Escherichia coli, ERIC-PCR*

# Adana Yöresinde Kafes Kuşlarından Geniş Spektrumlu Beta-Laktamaz (GSBL) Sentezleyen *Escherichia coli* İzolasyonu ve Moleküler Karakterizasyonu

## Öz

Bu çalışmada, kafes kuşlarında geniş spektrumlu beta-laktamaz (ESBL) sentezleyen *Escherichia coli* varlığının araştırılması amaçlandı. Bu amaçla Adana'daki 14 farklı kafes kuşu satışı yapan işyerinden toplam 139 fekal svab örneği toplandı. İncelenen örneklerin %4.3'ünden (n=6) GSBL sentezleyen *E. coli* saptandı ve izolatların tamamında $bla_{CTX\text{-}M\text{-}15}$ geni belirlendi. ERIC-PCR analizi izolatlar arasında üç farklı band patterni gösterdi. Bu sonuçlar kafes kuşlarının ESBL sentezleyen *E. coli* için taşıyıcı olduğunu göstermektedir. Bu nedenle, çeşitli hayvan türlerinde ve ortamlarda ESBL sentezleyen *E. coli* dahil dirençli bakterilerin varlığını belirlemek için daha fazla epidemiyolojik çalışmalara ihtiyaç vardır.

***Anahtar sözcükler:*** *Kafes kuşları, Geniş spektrumlu beta laktamaz (GSBL), Escherichia coli, ERIC-PCR*

## INTRODUCTION

Antimicrobial resistant microorganisms are globally major concern for animal and public health due to limited therapy options [1]. One of the important resistance mechanism encountered in the members of Enterobactericae family is resistance to extended-spectrum cephalosporins mediated by extended-spectrum beta-lactamase (ESBL) synthesis. ESBLs have high hydrolytic activity against the majority of beta-lactams, including cephalosporins but not against carbapenems (e.g. meropenem, imipenem, ertapenem) and cephamycins (e.g. cefoxitin, cefotetan). Their activities are completely or partially inhibited by beta-lactamase inhibitors such as clavulanic acid, tazobactam, sulbactam [2]. ESBL are mainly mediated by CTX-M, SHV and TEM enzymes. The genes encoding these enzymes are frequently localized on plasmids, leading these genes to spread easily within species as well as within genera. Prior to 2000, TEM and SHV were dominant ESBLs types, but after this date CTX-M became dominant ESBL type all over

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the world [3]. In recent years, presence of ESBL-producing bacteria have started to receive more attention in different bird species (e.g. seagulls, migratory birds, birds of prey, pigeon) and it has been shown that these bacteria were found in these animals with a various prevalence rates [4-7]. However, the data has been very scarce on the presence of these bacteria in cage birds [8].

The aim of this study was (i) to investigate the potential role of cage birds as carriers of ESBL producing *E. coli* and (ii) to determine antimicrobial susceptibilities and clonal relationship of the isolates.

# MATERIAL and METHODS

### *Sample Collection*

Sampling was randomly performed in 14 different aviaries in Adana, Turkey between March 2017 and June 2017. The birds were frequently cage-bred alone or/and in groups. The aviaries also housed imported birds. A total of 139 fresh faecal samples were collected from each cage using Stuart Transport Medium (LP Italiana, 118898, Italy). The samples were taken from fresh stool droplets of the same type of birds held in each cage and were considered as a single sample. Distribution of cage birds species was given in *Table 1*.

### *Selective Isolation and Phenotypic Confirmation of ESBL/pAmpC Producing E. Coli*

Faecal swab samples were incubated in buffered peptone water (BPW) (Merck, Germany, 107228) at 37°C for overnight under aerobic conditions. Then, 100 µL of culture was inoculated onto Eosin Methylen Blue (EMB) agar (Merck, Germany, 101347) supplemented with 2 µl/mL cefotaxime (Sigma, Germany) and incubated 37°C for 24 h under aerobic conditions. One colony per plate with typical metallic sheen appearance was randomly selected and subcultured onto Blood agar (Merck, Germany, 110886) supplemented with 7% defibrinated sheep blood in order to obtain pure culture. Identification was performed based on biochemical tests (Gram staining, IMVIC tests, oxidase, catalase) [9] and confirmed by polymerase chain reaction (PCR) for amplification of *E. coli* specific 16S RNA using E16Sa-CCCCCTGGACGAAGACTGAC and E16Sb-ACCGCTGGCAACAAAGGATA primers [10]. Phenotypic confirmation of ESBL producers was done by disc combination method [11] and double disc synergy method [12].

### *Antimicrobial Susceptibility Testing*

Antimicrobial susceptibility of ESBL producing *E. coli* isolates was determined by disc diffusion method as per Clinical Laboratory Standards Institute (CLSI) guidelines [11]. The antimicrobials (Bioanalyse, Turkey) tested were: ampicillin (AM, 10 µg), amoxicillin-clavulanic acid (AMC, 10/20 µg), nalidixic acid (NA, 30 µg), ciprofloxacin (CIP, 5 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg) cefepime (FEB, 30 µg), cefoxitin (FOX, 30 µg), aztreonam (ATM, 30 µg), imipenem (IMP, 10 µg), chloramphenicol (30 µg), streptomycin (S, 10 µg), gentamicin (CN, 10 µg), tobramycin (TOB, 10 µg), amikacin (AK, 10 µg), kanamycin (K, 30 µg), tetracycline (TE, 30 µg) and sulfametoxazole-trimethoprim (SXT, 1.25/23.75 µg). *E. coli*

***Table 1.*** *Distribution of cage birds sampled in the study*

| Aviary Code | Number of Collected Samples | Species of Cage Birds (Sample Number) |
|---|---|---|
| A | 25 | *Melopsittacus undulatus* (11), *Taeniopygia guttata* (5), *Erythrura gouldiae* (4), *Nymphicus hollandicus* (3), *Agapornis roseicollis* (1), *Serinus canaria* (1) |
| B | 7 | *Melopsittacus undulatus* (2), *Hippolais icterina* (1), *Psittacus erithacus* (1), *Cacatua galerita* (1), *Serinus canaria* (1), *Agapornis roseicollis* (1) |
| C | 3 | *Melopsittacus undulatus* (2), *Fringilla coelebs* (1) |
| D | 8 | *Melopsittacus undulatus* (7), *Serinus canaria* (1) |
| E | 45 | *Serinus canaria* (45) |
| F | 3 | *Melopsittacus undulatus* (3) |
| G | 3 | *Melopsittacus undulatus* (3) |
| H | 2 | *Melopsittacus undulatus* (1), *Serinus canaria* (1) |
| I | 5 | *Melopsittacus undulatus* (3), *Serinus canaria* (1), *Agapornis roseicollis* (1) |
| J | 4 | *Melopsittacus undulatus* (2), *Agapornis roseicollis* (1), *Nymphicus hollandicus* (1) |
| K | 10 | *Melopsittacus undulatus* (4), *Taeniopygia guttata* (1), *Psittacus erithacus* (1), *Serinus canaria* (1), *Poicephalus senegalus* (1), *Agapornis roseicollis* (1), *Fringilla coelebs* (1) |
| L | 6 | *Melopsittacus undulatus* (4), *Nymphicus hollandicus* (2) |
| M | 3 | *Melopsittacus undulatus* (1), *Psittacus erithacus* (1), *Taeniopygia guttata* (1) |
| N | 15 | *Melopsittacus undulatus* (7), *Psittacus erithacus* (1), *Fringilla coelebs* (1), *Serinus canaria* (1), *Platycercus Flaveolus* (1), *Taeniopygia guttata* (1), *Geopelia cuneata* (1), *Psittacus erithacus* (1), *Nymphicus hollandicus* (1) |
| Total | 139 | |

ATCC 25922 was used as quality control strain. The isolates showing resistance to three or more different class of antimicrobials were considered as multi-drug resistant (MDR).

***DNA Isolation***

DNA isolation was done using boiling method as previously reported by Ahmed *et al*.[13].

***PCR Detection of ESBL Genes and Sequencing***

Presence of ESBL genes ($bla_{TEM}$, $bla_{OXA}$, $bla_{SHV}$ and $bla_{CTX-M}$) were screened by PCR as previously described [13]. Amplified PCR products in ESBL positive isolates were sequenced from both ends using sequencing primers for the determination of the exact subtypes of beta-lactamase genes (Medsantek, İstanbul, Turkey) and compared with deposited sequences in the GenBank database (NCBI) (https://www.ncbi.nlm.nih.gov).

***Determination of Plasmid Mediated Quinolone Resistance (PMQR) Genes***

PMQR genes *qnr*A, *qnr*B and *qnr*C genes were investigated as previously suggested by Kim *et al.*[14].

***Enterobacterial Repetitive Intergenic Consensus* (ERIC)-PCR**

The clonal relationship among ESBL producing *E. coli* isolates was determined as described previously by Versalovic *et al*.[15], using hsdR+2758-5′-CAGCCATGAACAACTGGTGGCG-3′ and hsdR+3235R-5′-TGCTTTGCGCAGGGAAGATTCC-3′ primers.

***Computer-Assisted ERIC-PCR Fingerprint Analysis***

ERIC-PCR data were recorded to generate a binary matrix as either (1) for the presence of a band and (0) for its absence and analyzed using NTSYS-pc software (version 2.02 K, Applied Biostatistics, Inc., NY, USA). The similarity between the strains was determined on the basis of the Jaccard coefficients. The dendrogram was constructed using the UPGMA (Unweighted Pair Group Method Arithmetic Average) method.

# RESULTS

ESBL producing *E. coli* was isolated from six (4.3%) of 139 fecal samples. ESBL producing *E. coli* was isolated from three *Melopsittacus undulatus*, one in aviary D and two in aviary L, two *Serinus canaria* in aviary E, and one *Fringilla coelebs* in aviary K. Isolated isolates were also confirmed to be as *E. coli* by PCR amplification of 16S rRNA gene *(Fig. 1)*.

All isolates were positive for $bla_{CTX-M-15}$. *qnr* genes were detected in 5 (83.3%) isolates and of which three isolates carried *qnr*S, one isolate carried *qnr*B and one isolate carried both *qnr*B and *qnr*S *(Fig. 2)*.

Antimicrobial susceptibility testing results revealed that all isolates were resistant to ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, but susceptible to imipenem, nalidixic acid, ciprofloxacin, cefepime, amikacin. Various resistance rates to sulfametoxazole-trimethoprim (5; 83.3%), streptomycin (5; 83.3%), tetracycline (5; 83.3%), aztreonam

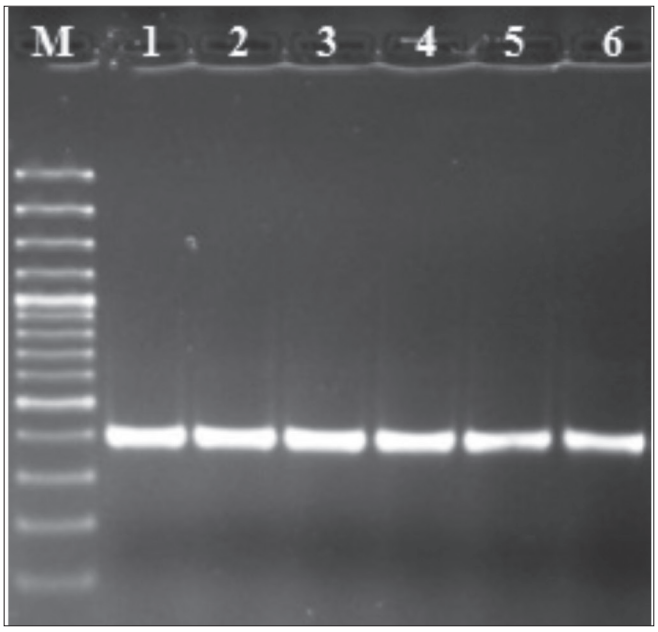



**Fig 1.** PCR amplification of *E. coli* spesific 16S rRNA gene (401 bp). Lane M: 100 bp plus molecular marker, Lane 1-6: 16S rRNA positive isolates

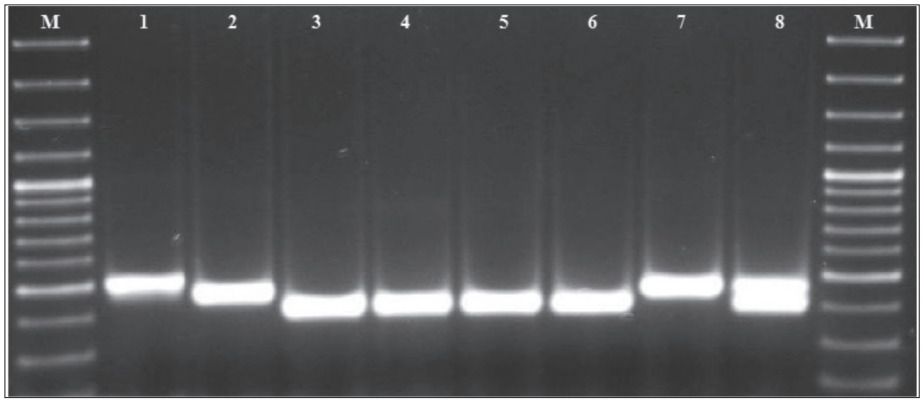
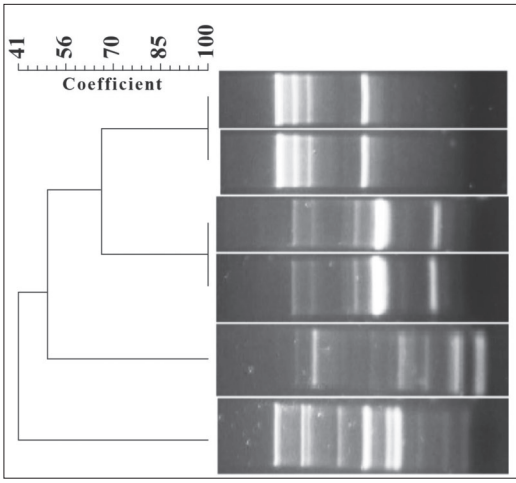



**Fig 2.** Agarose gel electrophoresis of PMQR genes. Lane M: 100 bp plus molecular marker, Lane 1: *qnr*A (516 bp) positive control, Lane 2: *qnr*B (476 bp) positive control, Lane 3: *qnr*S (428 bp) positive control, Lane 4-6: *qnr*S positive samples, Lane 7: *qnr*B positive sample and Lane 8: *qnr*B and *qnr*S positive sample




| Isolate ID (Aviary) | ERIC-PCR Profile | ESBL Type | PMQR Genes | Resistance to non-β-lactams |
|---|---|---|---|---|
| 26 (K) | A | CTX-M-15 | *qnr*S | SXT, TE, S |
| 27 (L) | A | CTX-M-15 | *qnr*S | SXT, TE, S |
| 29 (E) | B | CTX-M-15 | *qnr*S | TOB, SXT, S, CN |
| 41 (E) | B | CTX-M-15 | *qnr*B | TOB, SXT, TE, C, S, CN, K |
| 17 (L) | C | CTX-M-15 | *qnr*S, *qnr*B | SXT, TE, C, S, K |
| 14 (D) | D | CTX-M-15 | - | TE, C |

**Fig 3.** Dendogram showing the results of ERIC-PCR of six ESBL producing *E. coli* isolates. ERIC-PCR profiles are indicated as capital letters. ERIC-PCR groups A, B, C and D consisted of the isolates having a similarity coefficient ≥85%. The scale bar given on the top indicates similarity percentages detected for ERIC-PCR types

(4; 66.7%), chloramphenicol (3; 50%), cefoxitin (3; 50%), gentamicin (2; 33.3%), tobramycin (2; 33.3%), and kanamycin (2; 33.3%) were determined *(Fig. 3)*. According to ERIC-PCR analysis, four different band patterns were detected among the isolates *(Fig. 3)*.

## DISCUSSION

In previous studies [4-7], carriage of ESBL producing *E. coli* has been well documented in various wild avian species including migratory birds, birds of prey and waterfowl. However, there is a paucity of the carriage of ESBL producing *E. coli* in cage birds both in the world and in Turkey. We therefore investigated the occurence of ESBL producing *E. coli* in cage birds. The present study revealed low carriage rate 4.3% (6/139) ESBL producing *E. coli* in cage birds. Recently, a low carriage rate of 2.7% (4/148) in cage birds was also reported by Yılmaz and Dolar [8]. In contrast, higher carrige rates have been reported in migratory birds from Pakistan (17.3%) [6], in birds of prey from Portugal 26.9% [7], and in waterbirds from Poland (10.5%) [5]. However, in Germany, Guenther *et al.*[4] reported a low carriage rate (2.3%) in wild birds. Various prevalence rates of ESBL producing *E. coli* in different bird population might be explained by different ecological niches, human influence and antibiotic exposure.

CTX-M-15 was only the ESBL genotype detected among six ESBL producing *E. coli* isolates in the study. This finding is in agreement with previous study carried out by Yılmaz and Dolar [8], who detected $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-1}}$ together with $bla_{\text{TEM-1}}$ among the isolates. Similarly, CTX-M genotype has been reported to be the dominant genotype in ESBL producing *E. coli* from different wild avian species [4-7]. These data are also in coherence with human cases in Turkey, where CTX-M-15 is the most common genotype reported in ESBL producing *E. coli* isolates from various clinical settings [16,17]. Despite the lack of spesific molecular data, it might be speculated that current carriage status of ESBL producing *E. coli* in cage birds is of anthropogenic nature. Worldwide distribution of CTX-M is explained by localization these genes on plasmid, which facilitates the dissemination of these genes within species and among genera [3].

Another important cause of concern encountered in ESBL strains is that these isolates are frequently resistant to different class of antimicrobials. Because ESBL genes are localized on plasmids and these plasmids also carry other resistance genes to other antimicrobials such as aminoglycosides, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline and quinolone. Infectious diseases that may arise from multiresistant ESBL strains is a major cause of concern for both human and animal health because due to limited treatment options. Indeed, all isolates showed a MDR phenotype. Similarly, Yılmaz and Dolar [8] found all ESBL producing *E. coli* isolates as MDR. The authors also stressed that usage of non-beta lactam antibiotics may lead selection of ESBL genes.

Plasmid-mediated resistance is of growing clinical concern due to transfer resistance genes to other species via horizontal gene transfer, conferring resistance quinolones. In addition, co-existence of ESBL and qnr genes on the same plasmid has been reported [18]. The *qnr* proteins are capable to protect DNA gyrase from quinolones by binding this gene. Although all isolates were found to be susceptible to ciprofloxacin and nalidixic acid, *qnr* genes were detected in 83.3% of the isolates in the study. The *qnr* genes have been shown to be responsible for *in vitro* low-level quinolone resistance, but facilitate the emergence of high-level resistance in the presence of quinolones in therapeutic levels [19].

ERIC-PCR has been shown to be a useful method for investigating clonal relationship among multi-resistant

*Enterobactericeae* isolates [20]. In the study, ERIC-PCR analysis revealed four different pattern among the isolates. In a previous study, Yılmaz and Dolar [8] found two identical pattern among four ESBL isolates using pulsed field gel electrophoresis (PFGE) analysis.

In conclusion, this study shows that different cage bird species are colonized with ESBL-producing *E. coli* strains, even at low rates. Since cage birds are close contact with humans, these bacteria might be transmitted from birds to humans or vice versa. Therefore, there is a need to further epidemiologic studies to gain better understanding of transmission route of this bacterium among birds, humans and environment.

## Conflicts of Interest

The authors declare no conflict of interest.

## Acknowledgement

The authors greatfully thank Prof. George Jacoby (Lahey Clinic, Burlington, USA) for supplying *qnr* positive reference strains.

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