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Research Note

Carbapenemases and Extended-Spectrum β-Lactamase–Producing Multidrug-Resistant *Escherichia coli* Isolated from Retail Chicken in Peshawar: First Report from Pakistan

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ABSTRACT

We report the prevalence of extended-spectrum β-lactamases and carbapenemases in *Escherichia coli* isolated from retail chicken in Peshawar, Pakistan. One hundred *E. coli* isolates were recovered from retail chicken. Antibiotic susceptibility testing was carried out against ampicillin, chloramphenicol, kanamycin, nalidixic acid, cephalothin, gentamicin, sulfamethoxazole-trimethoprim, and streptomycin. Phenotypic detection of β-lactamase production was analyzed through double disc synergy test using the antibiotics amoxicillin-clavulanate, cefotaxime, ceftazidime, cefepime, and aztreonam. Fifty multidrug-resistant isolates were screened for detection of *sul1, aadA, cmlA, int,* bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, $bla_{\text{OXA-10}}$, bla_{VIM}, bla_{IMP}, and $bla_{\text{NDM-1}}$ genes. Resistance to ampicillin, nalidixic acid, kanamycin, streptomycin, cephalothin, sulfamethoxazole-trimethoprim, gentamicin, cefotaxime, ceftazidime, aztreonam, cefepime, amoxicillin-clavulanate, and chloramphenicol was 92, 91, 84, 73, 70, 67, 53, 48, 40, 39, 37, 36, and 23% respectively. Prevalence of *sul1, aadA, cmlA, int,* bla_{TEM}, $bla_{\text{CTX-M}}$, bla_{IMP}, and $bla_{\text{NDM-1}}$ was 78% ($n = 39$), 76% ($n = 38$), 20% ($n = 10$), 90% ($n = 45$), 74% ($n = 37$), 94% ($n = 47$), 22% ($n = 11$), and 4% ($n = 2$), respectively. bla_{SHV}, $bla_{\text{OXA-10}}$, and bla_{VIM} were not detected. The coexistence of multiple antibiotic resistance genes in multidrug-resistant strains of *E. coli* is alarming. Hence, robust surveillance strategies should be developed with a focus on controlling the spread of antibiotic resistance genes via the food chain.

Key words: $bla_{\text{NDM-1}}$; Carbapenemase; Extended-spectrum β-lactamases; Multidrug resistant

Antibiotics are frequently used in poultry farming, and bacteria could develop resistance against them by evolving various mechanisms *(17)*: changes in the antibiotic target sites, acquisition of antibiotic inactivating enzymes, evolution of drug efflux systems, or making the cell less permeable to antibiotics. Antibiotic resistance genes could be transferred among different bacterial species through transposons, integrons, and plasmids *(17)*. Foods contaminated with resistant bacteria could have a role in the spread of resistance genes. These resistant bacteria could disrupt the normal body flora, making the host more vulnerable to infections *(4)*. *Escherichia coli* can act as a vehicle for transfer of antibiotic resistance genes. These bacteria are diverse and are normally present in the gastrointestinal tract of humans, poultry, and other animals. However, certain pathogenic strains of *E. coli* cause both nosocomial and community-acquired infections, leading to deadly outbreaks *(37)*. Resistant *E. coli* strains could be transmitted to humans through the food chain, causing serious health-related issues *(29)*.

The major classes of antibiotics include quinolones, oxazolidinones, β-lactams, glycopeptides, β-lactamase inhibitors, lipopeptides, treptogramins, sulfonamides, macrolides, and rifamycins. β-Lactamase enzymes are produced by bacteria to neutralize the effect of β-lactam antibiotics. Production of β-lactamases is the main resistance mechanism found in *Enterobacteriaceae*. Extended-spectrum β-lactamases (ESBLs) are β-lactamases that provide resistance against a number of β-lactam antibiotics *(8)*. Many of the ESBLs are derivatives of bla_{TEM} or bla_{SHV} enzymes *(9)*. So far, more than 600 ESBLs have been identified *(7)*. The most important of them are bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$ *(21)*.

The β-lactam antibiotics called carbapenems are used as the last therapeutic option against resistant gram-negative bacteria; however, some strains of *Klebsiella pneumoniae* and *E. coli* have evolved resistance mechanisms against them *(32, 47)*. Resistance to carbapenems was first observed in the United States in 1996 and, later, globally *(32)*. The most important resistance mechanisms against carbapenems involve production of carbapenemase enzymes such as *K.*

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pneumoniae carbapenemase (KPC), oxacillinase (OXA-48), Verona integron-encoded metallo-β-lactamase (VIM), imipenemase metallo-β-lactamase (IMP), and New Delhi metallo-β-lactamase (NDM) *(31)*. Bacteria that produce these enzymes have been isolated from hospital-acquired infections and domestic animals *(47)*. The exact origin of carbapenemases is not completely understood; however, environmental bacteria are considered to be potential sources *(44)*. NDM is an important carbapenemase that hydrolyzes β-lactams and last therapeutic option carbapenems. Only a few antibiotics, such as colistin, fosfomycin, and tigecycline, are effective against NDM-producing bacteria *(14)*.

Chicken is a main source of protein in Pakistan. The emergence of resistant bacteria in poultry is a major health issue that could lead to spread of these bacteria to the human population, causing noncurable infections. This spread of resistance bacteria could occur through consumption of undercooked meat or meat products, cross-contamination, direct contact with contaminated meat, or through the environment, e.g., the release of contaminated poultry carcasses into fresh water. Few data are available on the prevalence of antibiotic resistance genes in chicken-borne pathogens in the region. Hence, for proper management, it is relevant to analyze the occurrence of chicken-related resistant bacteria and their development mechanisms. This study analyzed the occurrence of antibiotic resistance genes in *E. coli* isolated from chicken in Peshawar, Pakistan. To our knowledge, this is the first report on the prevalance of antibiotic resistance genes (and the first ever on the existence of NDM-1) in *E. coli* isolated from chicken.

MATERIALS AND METHODS

Sampling and isolation. One hundred raw chicken meat samples (leg and breast) and intestinal samples (small intestine) were collected from retail shops in different locations of Peshawar during 2015 and 2016. Isolation and identification were carried out following standard procedures *(13, 35)*. Briefly, samples were collected in sterile bags and transferred to a laboratory immediately. Samples were prepared in peptone water, and serial dilutions were made. Each serial dilution (100 μL) was inoculated onto MacConkey agar (Oxoid, Basingstoke, UK), followed by incubation at 37°C for 24 h. A total of 100 isolates were selected. The isolates were identified through morphological screening (Gram staining) and biochemical tests, e.g., catalase, oxidase, citrate, indole, motility, triple sugar iron, methyl red, and Voges-Proskauer tests and through the results of growth on eosin methylene blue agar *(35)*. One isolate was selected from each sample for analysis.

Antibiotic susceptibility. Antibiotic susceptibility was performed using the Kirby Bauer disk diffusion method, according to the recommendations of the Clinical and Laboratory Standards Institute *(11, 12)*. Antibiotic susceptibility was performed against chloramphenicol (CAM; 30 μg), ampicillin (AMP; 10 μg), kanamycin (KAN; 30 μg), cephalothin (CET; 30 μg), nalidixic acid (NX; 30 μg), gentamicin (GEN; 10 μg), streptomycin (STR; 10 μg), and sulfamethoxazole-trimethoprim (TMP-SMX; 25 μg).

Phenotypic ESBL detection. Phenotypic detection of ESBLs was carried out using the double disc synergy test *(22)*. The antibiotics amoxicillin-clavulanate (AMC; 30 μg), cefotaxime (CTX; 30 μg), ceftazidime (CAZ; 30 μg), cefepime (FEP; 30 μg), and aztreonam (ATM; 30 μg) were used for ESBL assays. Bacterial innoculum was spread on Mueller-Hinton agar (Oxoid). A disc of AMC was placed in the center. The remaining antibiotics were placed 15 to 20 mm apart from the AMC disc, followed by incubation at 37°C overnight and measurement of the inhibition zones.

Detection of non–β-lactam antibiotic resistance genes and class 1 integrase gene. Fifty multidrug-resistant (resistant to three or more antibiotics) *E. coli* isolates were selected for detection of antibiotic resistance genes. Genomic and plasmid DNA was isolated as reported *(46)*. Three non–β-lactam antibiotic resistance genes, i.e., *sul1* (sulfonamide), *aadA* (aminoglycoside), and *cmlA* (chloramphenicol), and *int* (class 1 integrase) were detected using specific primers for each gene *(45)*. A 25-μL PCR reaction volume consisted of 12.5 μL of 2× SuperHot master mix (cat. no. 119102, Bioron, http://www.mountbio.com/pdf%20files/BIORON%20E-Catalogue.pdf), 1 μL of template DNA, 1 μL of primer (0.5 μM), and 9.5 μL of molecular-grade water. PCR reaction conditions consisted of initial denaturation (95°C, 5 min) followed by 30 cycles of denaturation (94°C, 30 s), annealing (50°C, 30 s), and extension (72°C, 1 min), followed by final extension (72°C, 7 min). Gel electrophoresis of PCR products was carried out using 1.5% agarose gel and a 100-bp DNA ladder (cat. no. 304105, Bioron) as the size marker.

Detection of ESBL genes. Prevalence of four ESBL genes, i.e., bla_{TEM}, bla_{SHV}, bla_{CTX-M}, and bla_{OXA-10}, was analyzed using specific primers *(16, 36, 45)*. PCR reaction mixture for bla_{TEM}, bla_{CTX-M}, and bla_{OXA-10} consisted of similar composition as used for non–β-lactam genes. A 20-μL PCR reaction volume for bla_{SHV} consisted of 4 μL of 5× FIREPol master mix (cat. no. 04-12-00125, Solis BioDyne, Tartu, Estonia), 1 μL of DNA, 1 μL of primer (0.5 μM), and 13 μL of molecular-grade water. The amplification profile for bla_{TEM} was the same as used for non–β-lactam genes. Amplification profile for bla_{SHV}, bla_{CTX-M}, and bla_{OXA-10} consisted of initial denaturation (95°C, 5 min) followed by 30 cycles of denaturation (94°C, 1 min), annealing (60°C, 1 min for bla_{SHV}; 55°C, 1 min for bla_{CTX-M}; and 57°C, 1 min for bla_{OXA-10}), and extension (72°C, 1 min), followed by final extension (72°C, 5 min). *Pseudomonas aeruginosa* clinical isolates positive for these genes were used as controls.

Detection of carbapenemases genes. Prevalence of three carbapenemase genes, i.e., bla_{VIM}, bla_{IMP}, and bla_{NDM-1}, was analyzed using specific primers *(36, 42)*. PCR reaction mix composition was the same as used for non–β-lactam genes. Amplification conditions for bla_{VIM} and bla_{IMP} included initial denaturation (95°C, 5 min) followed by 30 cycles of denaturation (94°C, 1 min), annealing (56°C, 1 min for bla_{VIM}; 54°C, 1 min for bla_{IMP}), and extension (72°C, 1 min), followed by final extension (72°C, 5 min). The amplification profile for bla_{NDM-1} consisted of initial denaturation (95°C, 5 min) followed by 35 cycles of denaturation (94°C, 30 s), annealing (55°C, 40 s), and extension (72°C, 50 s), followed by final extension (72°C, 5 min).

RESULTS

Phenotypic resistance to ampicillin, nalidixic acid, kanamycin, streptomycin, cephalothin, sulfamethoxazole-trimethoprim, gentamicin, cefotaxime, ceftazidime, aztreonam, cefepime, amoxicillin-clavulanate, and chloramphenicol was 92, 91, 84, 73, 70, 67, 53, 48, 40, 39, 37, 36, and

23%, respectively. Phenotypically, ESBL production was confirmed in 32 isolates. Eight antibiotic resistance genes (*sul1, aadA, cmlA, int,* bla_{TEM}, bla_{CTX-M}, bla_{IMP}, and bla_{NDM-1}) were detected in the isolates (Table 1). The sizes of amplified products corresponding to *sul1, aadA, cmlA, int,* bla_{TEM}, bla_{CTX-M}, bla_{NDM-1}, and bla_{IMP} genes were 822, 284, 698, 250, 857, 552, 475, and 361 bp, respectively. Multiple antibiotic resistance genes were present in all the isolates. The prevalence of *sul1* gene was 78% ($n = 39$) (Table 1). The *aadA* gene was present in 76% ($n = 38$) of isolates, the *cmlA* gene in 20% ($n = 10$) of isolates, and the *int* gene in 90% ($n = 45$) of isolates (Table 1). The frequency of bla_{TEM}, bla_{CTX-M}, bla_{IMP}, and bla_{NDM-1} genes was 74% ($n = 37$), 94% ($n = 47$), 22% ($n = 11$), and 4% ($n = 2$), respectively (Table 1). The genes bla_{SHV}, bla_{OXA-10}, and bla_{VIM} were not detected. One isolate (I56) from intestinal samples harbored all eight genes detected in the current study and showed phenotypic resistance to 11 different antibiotics (Table 1). One isolate (I44) possessed seven different antibiotic genes and had resistance to 10 different antibiotics (Table 1). Three isolates (F16, I3, and I11) possessed six different antibiotic resistance genes, and 23 isolates carried five different antibiotic genes (Table 1). The occurrence of bla_{NDM-1} was observed for the first time in 2 chicken intestinal isolates (I53 and I56).

Among the 50 isolates analyzed for detection of antibiotic resistance genes, sulfonamide resistance was detected in 39 isolates (78%) phenotypically, and the *sul1* gene was also detected in 39 isolates, which showed a good correlation between phenotypic and genotypic data (Table 1). However, among the 39 genotypically resistant isolates, 5 were phenotypically sensitive. Similarly, 6 isolates showed phenotypic resistance and no genotypic resistance that could be due to involvement of different resistance genes. Phenotypic resistance to aminoglycosides also corelated well with genotypic resistance (*aadA* gene) because 38 isolates showed both phenotypic and genotypic resistance (Table 1). However, 12 isolates showed phenotypic resistance, but not genotypic resistance that could be due to the presence of other resistance genes. Correlation between the presence of the *cmlA* gene and phenotypic resistance to chloramphenicol was weak: only 5 of 10 genotypically positive isolates showed phenotypic resistance, whereas 12 other isolates showed only phenotypic resistance (Table 1). Phenotypic resistance against β-lactam antibiotics correlated well with the occurrence of β-lactamase genes because all phenotypically resistant isolates showed the presence of at least one β-lactamase gene (Table 1).

DISCUSSION

Foodborne diseases are a global health issue. In the current study, a high prevalence of multidrug resistance was observed in *E. coli* isolates of chicken origin. The prevalence of antibiotic-resistant microorganisms in food and animal samples has been reported across the world *(2, 3)*. Akbar et al. *(1)* reported 92% resistance to ampicillin, as in the current study. Another study from Bangladesh reported a high frequency of streptomycin (70%) and kanamycin (76%) resistance in *E. coli* of poultry origin *(30)*. The findings of Salehi and Bonab *(41)* were strikingly similar to those in this study: i.e., 100% resistance to nalidixic acid, 81% resistance to streptomycin, 80% resistance to trimethoprim-sulphamethoxazole, and 77% resistance to kanamycin.

In the current study, the occurrence of non–β-lactam antibiotic resistance genes (*sul1, aadA,* and *cmlA*) and the integrase gene (*int*) was observed. Existence of these genes in *E. coli* of chicken origin has been reported by other authors *(3, 27)*. A previous study reported 36.84% prevalence of *cmlA* and 47.36% prevalence of *sul1* gene in *E. coli* isolated from chicken *(30)*. In the current study, the *int* gene was found in 90% of isolates. These results are in agreement with the findings of Altalhi et al. *(3)*. Integrons play an important role in the spread of resistance genes among bacteria *(49)*.

Resitance to β-lactam antibiotics was observed in the current study. Similar results have been reported previously *(29)*. A study from Turkey reported a low prevalence of ESBL and AmpC β-lactamases among *E. coli* isolates from chicken meat and fecal samples *(24)*. In the current study, the prevalence of bla_{TEM} was 72% and that of bla_{CTX-M} was 94%, findings similar to those of a previous study *(3)*. Another study reported 24.3% prevalence of bla_{TEM} among *E. coli* isolates *(27)*. In Gemany, 43.9% prevalence of ESBLs in enterobacteria isolated from retail chicken meat was noticed *(39)*. Presence of bla_{CTX-M} in *E. coli* from chicken was reported for the first time in Spain in 2001 *(6)*. Since then, various studies have reported the occurrence of bla_{CTX-M}–producing *E. coli* from chicken *(20, 25, 50)*. A study from France reported 10.7% occurrence of bla_{CTX-M} in *E. coli* collected from chicken *(19)*. Another study from China reported 12.3% prevalence of bla_{CTX-M} in chicken *E. coli* isolates *(50)*. In Hong Kong, a higher prevalence (68.5%) of bla_{CTX-M} in *E. coli* and other *Enterobacteriaceae* isolates from fecal chicken samples was observed *(20)*. In Germany, 40% prevalence of bla_{CTX-M} was observed in retail chicken meat *(25)*. Another study from Germany reported 60% prevalence of ESBL-producing *E. coli,* mainly possessing bla_{CTX-M} and bla_{SHV} genes *(10)*. In Spain, the prevalence of ESBL-producing *E. coli* was found to have significantly increased from 62.5% in 2007 to 93.5% in 2010, with bla_{SHV} as the dominant type of ESBL detected *(15)*. Stuart et al. *(43)* carried out a comparative study of ESBL prevalence in organic and conventional retail chicken meat. Both the meat samples (organic and conventional) had ESBL-producing bacteria, although organic meat samples had comparatively lower prevalence of bla_{CTX-M}, bla_{SHV}, and bla_{TEM}. Leverstein-van Hall et al. *(26)* conducted a comparative study of *E. coli* strains, ESBL genes, and plasmids in Dutch patients, chicken meat, and poultry that pointed to a possible dissemination of resistant bacteria, ESBL genes, and plasmids from poultry to human via the food chain. They found poultry-associated ESBL genes in 35% of human isolates. Interestingly, 19% of these ESBL genes were located on the same plasmids in both poultry and human isolates. The dominant ESBL genes were bla_{CTX-M} and bla_{TEM} in isolates from all the three sources (Dutch patients, chicken meat, and poultry). The occurrence of

TABLE 1. *Antibiotic susceptibility pattern and occurrence of antibiotic resistance genes among* E. coli *isolates*

Isolate	Resistance pattern to antibiotics	*sul1*	*aadA*	*cmlA*	*int*	bla_{TEM}	bla_{SHV}	bla_{CTX-M}	bla_{OXA-10}	bla_{VIM}	bla_{IMP}	bla_{NDM-1}
F24	AMP, NX, STR	−	+	−	+	+	−	+	−	−	−	−
F15	AMP, CAM, KAN, NX, CET, GEN	−	+	−	−	+	−	+	−	−	−	−
F45	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	−	+	−	−	+	−	+	−	−	−	−
F44	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	+	−	+	−	−	−	−
F43	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	−	−	+	−	−	−	−
F62	AMP, CAM, KAN, NX, CET, GEN, STR, AMC, ATM, CTX, CAZ	−	+	+	+	+	−	+	−	−	−	−
F20	AMP, NX, CET, GEN, TMP-SMX, STR	−	+	+	+	+	−	−	−	−	−	−
F60	AMP, CAM, KAN, NX, CET, STR, ATM, FEP, CTX, CAZ	+	+	−	+	+	−	+	−	−	−	−
F16	AMP, CAM, KAN, NX, GEN, TMP-SMX, STR, AMC, CTX	+	+	+	+	+	−	+	−	−	−	−
F7	AMP, CAM, KAN, NX, CET, TMP-SMX, STR	−	+	+	+	+	−	+	−	−	−	−
F30	AMP, CAM, KAN, NX, GEN, TMP-SMX, STR	+	+	−	+	+	−	+	−	−	−	−
F8	AMP, CAM, KAN, NX, CET, TMP-SMX, STR	+	+	−	+	+	−	+	−	−	−	−
F14	AMP, CAM, KAN, NX, CET, GEN, TMP-SMX, STR	+	+	−	+	+	−	+	−	−	−	−
F12	AMP, CAM, KAN, NX, TMP-SMX, STR, AMC, CTX, CAZ	−	+	−	+	+	−	+	−	−	−	−
F19	AMP, NX, CET, GEN, TMP-SMX, STR	+	+	−	+	+		+	−	−	−	−
F27	AMP, CAM, NX, GEN, TMP-SMX, STR	−	+	−	+	+	−	+	−	−	−	−
F13	AMP, CAM, KAN, NX, CET, GEN, TMP-SMX, ST, AMC, CTX, CAZ	+	+	+	+	+	−	−	−	−	−	−
F18	AMP, NX, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	−	+	−	−	+	−	+	−	−	−	−
F9	AMP, CAM, KAN, NX, CET, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	+	−	+	−	−	−	−
F28	AMP, CAM, KAN, NX, GEN, TMP-SMX, STR	+	+	−	+	+	−	+	−	−	−	−
F61	AMP, CAM, KAN, NX, CET, STR, ATM, FEP, CTX	+	+	−	+	+	−	+	−	−	−	−
F29	AMP, CAM, KAN, NX, GEN, TMP-SMX, STR	+	+	−	+	−	−	+	−	−	−	−
F11	AMP, CAM, KAN, NX, CET, STR	+	+	−	+	−	−	+	−	−	−	−
F25	AMP, NX, TMP-SMX, STR	−	+	−	+	+	−	+	−	−	−	−
I34	AMP, NX, CET, TMP-SMX, STR, CTX	+	+	−	+	+	−	+	−	−	−	−
I46	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	+	−	+	−	−	−	−
I53	AMP, NX, CET, GEN, TMP-SMX, STR, ATM, FEP, CTX, CAZ	+	−	−	+	−	−	+	−	−	−	+
I8	AMP, CAM, NX, CET, GEN, AMC, CTX, CAZ	+	−	−	+	+	−	+	−	−	−	−
I33	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX	+	−	−	+	+	−	+	−	−	+	−
I57	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	+	−	+	−	−	−	−
I56	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	+	+	+	−	+	−	−	+	+
I30	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	+	−	+	−	+	−	−	−	−

TABLE 1. *Continued*

Isolate	Resistance pattern to antibiotics	*sul1*	*aadA*	*cmlA*	*int*	bla_{TEM}	bla_{SHV}	bla_{CTX-M}	bla_{OXA-10}	bla_{VIM}	bla_{IMP}	bla_{NDM-1}
I36	AMP, NX, CET, GEN, TMP-SMX, STR, ATM, FEP, CTX, CAZ	+	−	−	+	−	−	+	−	−	−	−
I49	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	−	−	−	+	−	−	+	−	−	−	−
I43	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	−	−	+	−	−	−	−
I42	AMP, NX, CET, GEN, TMP-SMX, STR, ATM, FEP, CTX, CAZ	+	−	−	+	−	−	+	−	−	+	−
I52	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	+	−	+	−	−	−	−
I32	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	−	−	+	+	−	+	−	−	+	−
I3	AMP, CAM, KAN, NX, CET, TMP-SMX, STR	+	+	+	+	+	−	+	−	−	−	−
I44	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX	+	+	+	+	+	−	+	−	−	+	−
I50	AMP, NX, CET, GEN, TMP-SMX, STR, ATM, FEP, CTX, CAZ	+	+	−	+	−	−	−	−	−	+	−
I47	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	−	−	+	−	−	+	−	−	−	−
I35	AMP, NX, CET, TMP-SMX, STR, CTX	+	−	−	+	+	−	+	−	−	+	−
I41	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX	+	−	+	−	+	−	+	−	−	−	−
I45	AMP, NX, CET, GEN, TMP-SMX, STR, ATM, FEP, CTX, CAZ	+	−	−	+	+	−	+	−	−	−	−
I18	AMP, NX, CET	+	+	−	+	−	−	+	−	−	−	−
I11	AMP, NX, CET	+	+	−	+	+	−	+	−	−	+	−
I38	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	−	−	+	−	−	+	−
I10	AMP, NX, CET	+	−	−	+	+	−	+	−	−	+	−
I29	AMP, NX, CET, GEN, TMP-SMX, STR, AMC, ATM, FEP, CTX, CAZ	+	+	−	+	−	−	+	−	−	+	−

[a] +, presence of gene; −, absence of gene. $n = 50$.

ESBL-producing *E. coli* was 94%; of these, 39% belonged to the same genotypes that were present in human samples.

In the current study, two carbapenemases, bla_{IMP} and bla_{NDM-1}, were also detected with 10 and 4% prevalence. Carbapenem resistance in *Enterobacteriaceae* has been reported worldwide, with bla_{KPC-2} and bla_{NDM-1} as important resistance genes *(23, 33)*. NDM was first reported in *E. coli* and *K. pneumoniae* that were isolated from a Swedish patient who was once hospitalized in New Delhi *(48)*. The enzyme has been reported in members of *Enterobacteriaceae, Pseudomonas,* and *Acinetobacter* species across the world *(5, 23, 40)*. However, to our knowledge, bla_{NDM-1} has not been previously reported in *E. coli* from chicken. Carbapenems are not routinely used in poultry farming; however, bla_{NDM-1} production could have coevolved along with resistance to other antibiotics such as tetracyclines, cephalosporins, and quinolones, which are commonly used in the poultry industry. These resistant strains could be further disseminated through direct contact, insect vectors, and other animals, as has been suggested previously *(18, 28, 38)*. The reverse scenario, i.e., human to chicken transmission, could spread through infected human carriers or wastewater discharge. The results of this study clearly show that raw chicken sold at markets in Pakistan is contaminated with *E. coli* that possesses multiple resistance genes. These bacteria could have a role in the spread of infections upon consumption of contaminated chicken meat and meat products. These findings are alarming and highlight serious health risks to both workers and consumers. The spread of these resistant bacteria could lead to deadly infections and epidemics *(34)*. Currently, no antibiotic resistance monitoring program in animals is in place in Pakistan. Such monitoring systems should be established for routine surveillance of resistance-associated issues to prevent spread of this resistance to the human population.

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