Multidrug Resistant Carbapenemase-Producing *Escherichia coli* from Chicken Meat Reveals Diversity and Co-Existence of Carbapenemase Encoding Genes

Muhammad Younas¹⁸, Sadeeq ur Rahman²¹ *, Sumaira Shams¹, Mian Muhammad Salman² and Imad Khan²

¹Department of Zoology, Abdul Wali Khan University, Mardan, Pakistan; ²College of Veterinary Sciences and Animal Husbandry, section microbiology, Abdul Wali Khan University, Mardan, Pakistan

*Corresponding author: sadeeq@awkum.edu.pk

**ARTICLE HISTORY** (18-364)

Received: October 02, 2018

Revised: February 03, 2019

Accepted: February 08, 2019

Published online: April 06, 2019

**Key words:**
Antimicrobial resistance

*bla*<sub>NDM</sub>

*bla*<sub>OXA-48</sub>

*bla*<sub>VIM</sub>

Carbapenemase-encoding genes

Mardan-Khyber

Pakhtunkhwa

Pakistan

**ABSTRACT**

Literature regarding multidrug resistant (MDR) carbapenemase-producing *Escherichia coli* (CPE) recovered from food-producing animals is lacking from Pakistan. Here, we report on the isolation and characterization of MDR-CPE recovered from retail-poultry meat in Khyber Pakhtunkhwa Pakistan. A total of 101 retail poultry-meat samples were screened for *Escherichia coli* and tested against 17 different antibiotics through disc diffusion test to define MDRs. MDR isolates were genotyped by PCR for carbapenemase-encoding elements. Finally, integron typing, insertion sequence common region 1 (ISCR1) and its association with carbapenemase-encoding genes was established through PCR. Of the 33 *E. coli* isolates, 28 (Phylogroup B2=39.2% (11/28), A=32.1 % (9/28) and group D=28.5% (8/28) were MDR displaying resistance to cefotaxime, tetracycline, ampicillin and trimethoprim-sulfamethoxazole, while, 96.4, 92.8, 89.2 and 85.7% were also found resistant to colistin-sulphate, enrofloxacin, doxycycline and ciprofloxacin, respectively. The *bla*<sub>VIM</sub> was predominantly identified in 8 (28.5%) isolates followed by *bla*<sub>NDM</sub> 4/28 (14.2%) and 2 isolates were carrying a combination of *bla*<sub>NDM</sub>+*bla*<sub>VIM</sub>. Class-1 integron was detected in 21 (75%), class-2 and class-3 integrons were detected in 25% and 7.1%, respectively. ISCR1 element was identified among 11 isolates (39.28%) that was found associated with *bla*<sub>VIM</sub> gene among 3 isolates (27.25%) suggesting involvement in its mobilization. We report the incidence of *E. coli* carrying carbapenemase-encoding genes located on the plasmids carrying integron 1 recovered from retail-poultry-meat in Mardan, Pakistan.

©2019 PVJ. All rights reserved

**To Cite This Article:** Younas M, ur Rahman S, Shams S, Salman MM and Khan I, 2019. Multidrug resistant carbapenemase-producing *Escherichia coli* from chicken meat reveals diversity and co-existence of carbapenemase encoding genes. Pak Vet J, 39(2): 241-245. http://dx.doi.org/10.29261/pakvetj/2019.047

**INTRODUCTION**

Antimicrobial resistance (AMR) is challenging our current progress in health. AMR is increasingly reported from all over the world (Nordmann et al., 2011; Mohsin et al., 2017; Khattak et al., 2018; ur Rahman et al., 2018a). The scenario seems even more challenging for developing countries mainly due to unrestricted use of antimicrobials and lack of surveillance programs to monitor emergence of drug resistance (Khan et al., 2010; Mitema 2010; ur Rahman et al., 2018a). Particularly, resistance to carbapenem drugs is quite worrisome as these drugs are thought to be the last resort against MDR bacteria (Meletis, 2016). Carbapenem drugs are very important for those who develop infections due to bacteria that can inactivate extended spectrum cephalosporins. Resistance to carbapenem drugs is presented by carbapenemase enzymes. At present more than hundred types of carbapenemase genes have been reported (Meletis, 2016). Ambler classification divides these enzymes into four major categories such as A, B, C and D. Class A carbapenemases contain KPC type, class B Metallo-β-Lactamases are comprised of VIM, New Delhi metallo-β-lactamase-1 (NDM-1) and IMP types etc., and class D is represented by OXA-48 etc. Of the above mentioned carbapenemases, particularly NDM, though initially reported from a Swedish patient who travelled to subcontinent (Yong et al., 2009), has now been reported widely from many other countries (Nordmann et al., 2011).

---

*These authors contribute equally to this work.*
Co-occurrence of different carbapenemases or combination of carbapenemase with other resistance conferring elements has been associated with potentially increased spectrum of resistance in Enterobacteriaceae (Sattar et al., 2016) including increased minimum inhibition concentration levels against carbapenems (Meletis, 2016). Elements such as insertion sequence common region 1 (ISCR1), conjugative plasmids and integrons have been found involved in mobilization and fast dissemination of resistance conferring elements including carbapenemase encoding genes (Ali et al., 2016).

Despite the fact that antimicrobials are not strictly regulated in Pakistan and generous availability of antibiotics cheering its misuse and pumping selective pressure for the emergence of antimicrobial resistance, literature data regarding overall antimicrobial resistance surveillance is totally missing in the country with random reports suggesting widespread and high level of resistance against cephalosporins, sulfonamides and carbapenem drugs (Perry et al., 2011; Sattar et al., 2016; Adnan et al., 2017; ur Rahman et al., 2018b). To the best of our knowledge, no signal report is published from Khyber Pakhtunkhwa, Pakistan regarding the status of carbapenemase-producing Enterobacteriaceae associated with poultry meat in Pakistan.

MATERIALS AND METHODS

Ethics: This work was reviewed and approved by the ethical committee of Abdul Wali Khan University Mardan Pakistan and work was carried out according to local institutional and national guidelines.

Collection of samples and location: Samples from poultry meat (n=101) were collected from butcher shops at live bird market of chicken sales points during December 2017 to March 2018. Different parts of chicken were collected including 50g of liver, whole spleen and heart. All samples were transported in a sterile bag in ice box and streaked immediately for culturing.

Isolation and screening of E. coli: Samples were streaked onto MacConkey agar (Difco™ Becton Dickinson, Sparks, MD USA) supplemented with meropenem 0.5mg/L and incubated for 24 hours at 37 °C. Pink candidate colonies were further purified and streaked onto eosin methylene blue agar (EMB) and presumably E. coli colonies were Gram stained, and further confirmed by specie specific PCR assay as described earlier (Tantawiwat et al., 2005).

Antibiotic susceptibility testing and MDR identification: Mueller-Hinton agar (Difco™) has been used for the antibiotic susceptibility against 17 different antibiotics following the standard Kirby-Bauer disk diffusion method and results were interpreted according to the guidelines of the CLSI (CLSI, 2014). Minimum inhibitory concentration (MIC) of selected antibiotic was performed by microdilution method and interpreted as per CLSI guidelines.

Genotypic screening of carbapenemase-encoding enzymes: Bacterial DNA from MDR E. coli was isolated by boiling method as described earlier (Ali et al., 2016). PCR assay was used for detection of NDM-, KPC-, OXA-48-, VIM- and IMP-encoding genes as described previously using specific primers and cited in Sup. Table 1.

Phylogenetic grouping: A triplex PCR was performed targeting yjA, chuA and the TspE4 for classification of isolates into specific phylogroups as reported previously by (Clermont et al., 2000).

Detection of integrons and ISCR1: Integron typing was performed as described earlier (Dillon et al., 2005a). A PCR assay was also performed to detect insertion sequence ISCR1 and its association with carbapenemase enzymes as we optimized and reported earlier (Ali et al., 2016).

RESULTS

Isolation, antibiotic susceptibility and MDR E. coli from poultry meat: Of the total 101 sample, a total of 33 E. coli isolates were randomly screened initially against a panel of 17 different antibiotics. Briefly, 17 different antimicrobials including β-lactams (ampicillin), cephalosporins (cefotaxime, cefazidime, ceftipime etc.), carbapenems (meropenem, imipenem), tetracycline (tetracycline, doxycycline), quinolones (ciprofloxacin), floroquinolones (ciprofloxacin, norfloxacin), aminoglycosides (gentamycin), monobactams (aztreonam), sulfonamides (Trimethoprim-sulfamethoxazole, fosfomycin), phenicol (chloramphenicol) and polymyxin B (Colistin). Our results indicated that most of the isolates were found to be MDR displaying resistance against two or more classes of antimicrobials tested (Table 2). All isolates were found resistant to cefotaxime, tetracycline, ampicillin and trimethoprim-sulfamethoxazole. Isolates (96.4%) were also found resistant against colistin-sulphate, 92.8% were resistant to enrofloxacin, 89.2% to doxycycline and 85.7% to ciprofloxacin. Isolates that were found resistant against at least two classes of antibiotics were termed as MDR and detail is listed in Table 2. MIC value for ampicillin and cefotaxime was >64µg/ml indicated highly resistant phenotypes. 6 isolates (21.4%) displayed a MIC of ≥4 µg/ml against meropenem suggesting resistance, while, 8 isolates (28.5%) showed a MIC of ≥4 µg/ml against imipenem showing resistance. Finally, 26 isolates (92.8%) isolates showed resistance against doxycycline displaying a MIC of ≥16µg/ml.

Carbapenemase genotyping of E. coli from poultry meat: All 28 MDR isolates were further characterized for the presence of carbapenemase-encoding genes including blaNDM and blaOXA-48, blaTEM, blaIMP and blaKPC and results are shown in Table 3. Of these, 11 were found to harbor at least one type of the carbapenemase-encoding gene with 3 isolates carrying more than one type of carbapenemase family. Briefly, our results revealed that blaTEM was predominant and identified in 8 (28.5%) isolates. This was followed by blaNDM carried by 4/28 (14.2%) isolates. Two isolates were carrying KPC while one was harboring OXA-48 gene. Of note, no IMP type of genotype could be confirmed among all 28 under study E. coli isolates.
Table 1: Antimicrobial resistance profile of E. coli isolates (n=28) recovered from poultry meat

| S. No | Antimicrobial agent | Abbreviation | Conc. (µg) | Susceptible (%) | Intermediate (%) | Resistance (%) |
|-------|---------------------|--------------|------------|-----------------|-----------------|---------------|
| 1     | Ciprofloxacin       | CIP          | 5 µg       | 4/28(14.2)      | 0/28(0)         | 24/28(85.7)   |
| 2     | Tetracycline        | TE           | 30 µg      | 0/28(0)         | 0/28(0)         | 28/28(100)    |
| 3     | Meropenem           | MEM          | 10 µg      | 15/28(53.5)     | 8/28(28.5)      | 5/28(17.8)    |
| 4     | Doxycycline         | DO           | 30 µg      | 2/28(7.1)       | 1/28(3.5)       | 25/28(89.2)   |
| 5     | Gentamycin          | CN           | 10 µg      | 18/28(64.2)     | 5/28(17.8)      | 5/28(17.8)    |
| 6     | Imipenem            | IPM          | 10 µg      | 8/28(28.5)      | 12/28(42.8)     | 8/28(28.5)    |
| 7     | Aztreonam           | ATM          | 30 µg      | 5/28(17.8)      | 12/28(42.8)     | 11/28(39.2)   |
| 8     | Cefazidime          | CAZ          | 30 µg      | 7/28(25)        | 13/28(46.2)     | 8/28(28.5)    |
| 9     | Cefepime            | FEP          | 30 µg      | 19/28(67.5)     | 4/28(14.2)      | 5/28(17.8)    |
| 10    | Ampicillin          | AMP          | 10 µg      | 0/28(0)         | 0/28(0)         | 28/28(100)    |
| 11    | Norfloxacin         | NQR          | 10 µg      | 4/28(14.2)      | 5/28(17.8)      | 19/28(67.5)   |
| 12    | Trimethoprim-sulfamethoxazole | SXT | 1.25/23.75 µg | 0/28(0) | 0/28(0) | 28/28(100) |
| 13    | Colistin sulphate   | CT           | 10 µg      | 0/28(0)         | 1/28(3.5)       | 27/28(96.4)   |
| 14    | Chloramphenicol     | C            | 30 µg      | 7/28(25)        | 0/28(0)         | 21/28(75)     |
| 15    | Enrofloxacin        | ENR          | 5 µg       | 2/28(7.1)       | 0/28(0)         | 26/28(92.8)   |
| 16    | Cefoxazime          | CTX          | 10 µg      | 0/28(0)         | 0/28(0)         | 28/28(100)    |

Table 2: Frequency of MDR E. coli isolates from retail poultry meat

| Source of samples | Total samples | No of E. coli isolates (%) | MDR phenotypes |
|-------------------|---------------|---------------------------|----------------|
| Spleen            | 43            | (21(48.8)                | 19(40.93)       |
| Liver             | 20            | 6(30.0)                   | 3(15.00)        |
| Heart             | 38            | 6(15.8)                   | 2(6.81)         |
| Total             | 101           | 33(33.0)                  | 28(84.8)        |

Table 3: Frequency of carbapenemase genotypes from retail poultry meat

| S.No | Genes | Number of samples | Frequency | Percentage |
|------|-------|-------------------|-----------|------------|
|      |       | positive          |           |            |
| 1    | bloVIM | 1                  | 5         | 28/28(92.8) |
| 2    | bloNMD | 1                  | 3         | 4/28(14.2)  |
| 3    | bloKPC | 1                  | 0         | 2/28(7.1)   |
| 4    | bloOXA-48 | 0          | 0         | 1/28(3.5)   |
| 5    | bloIMP | 0                  | 0         | 0/28(0)     |
|      | Co-existence of multiple carbapenemase encoding genes | 2     | 0.00      |
| 6    | bloVIM+bloNMD | 2     | 2/24(8.3) |
| 7    | bloVIM+bKC | 1     | 1/24(4.1) |

Strikingly, 8 isolates were harboring singular type of the carbapenemase encoding family tested, while 3 were carrying more than one type (Table 3). The most predominant combination of co-occurrence was of blaVIM+blaOXA (8.3%). One isolate was also carrying a combination of blaVIM+blaKPC (1/24=4.1%).

Phylogenetic classification indicates B2 as predominant group: Phylo- group B2 was the most prevalent (11/28, 39.2%) followed by group A (9/28, 32.1%), and group D (8/28, 28.5%), respectively (Table 4).

Integron typing of E. coli isolates from poultry meat: Our results showed that class-1 integron was detected in 21 (75%) isolates of E. coli, while, class-2 and class-3 integrons were detected in 25 and 7.1%, respectively (Table 4). Class 1 integron was identified among E. coli isolated from all different sources suggesting random distribution.

Insertion sequence ISCR1 and its association with carbapenemase encoding genes: A total of 11 samples (39.28%) were carrying ISCR1, of which 3 isolates (27.25%) were found associated with blaVIM revealed by the PCR product size (Table 4). We could not amplify any desired size product from combination of ISCR1 and OXA-48 or NDM suggesting absence of association.

DISCUSSION

The current uprise in bacterial resistance against antimicrobials is worrisome. No country of the world could escape of bacterial pathogens offering resistance to at least commonly used antibiotics. Consistent and misuse of antibiotics is known to energize selective pressure for the emergence of antibiotic resistance just like when bacteria secretes its toxins in response to external signals in the form of molecules available in its surrounding (ur Rahman and van Ulsen 2013; ur Rahman et al., 2014; van Ulsen et al., 2014; Piet et al., 2016). Most of the use of antimicrobials goes for agriculture and livestock sector, and that too, not for treatment, but mainly for prevention of diseases and as growth promoter. Situation gets worsen as use of antimicrobials in developing countries like Pakistan is not strictly monitored mainly in agriculture and livestock. Furthermore, absence of regular surveillance data also makes situation further complicated. Poultry industry in Pakistan is a dynamic industry that contributes to 28% of total meat produced in the country (Pakistan, 2016). Besides meat, table eggs of poultry layer birds also contribute in daily breakfast of majority people in Pakistan. However, use of antibiotics in poultry industry is also not strictly regulated (Mitema, 2010) raising concern of emergence of AMR. Antimicrobial resistant E. coli have been increasingly reported from food producing animals (Ali et al., 2017; ur Rahman et al., 2018a; ur Rahman et al., 2018b). The current study reports on the CPE recovered from retail poultry meat raising concern of its dissemination.

Among 33 E. coli isolates, 28 displayed the phenotype of MDR. Among these, 11 were found to produce at least one carbapenemase encoding gene suggesting MDR phenotype is possibly due to carbapenemases enzymes. Although, literature study suggests that NDM has been increasingly reported from clinical (Perry et al., 2011; Hussain 2015; Sattar et al., 2016) and community settings in Pakistan (Sartor et al., 2014), current data demonstrated that blaVIM is instead a predominant carbapenemase encoding gene followed by blaNDM. Identification of NDM in isolates of E. coli recovered from poultry meat is alarming suggesting that NDM has been equally widespread both among animal- as well as human. NDM gene is considered to be originated from Indian continent particularly India, Pakistan and...
**Table 4**: Characterization of MDR E. coli isolated from retail poultry meat

| #   | ID       | Phylo-Place of isolation | Carbapenemase genes | Integron typing | R/I Phenotypes to the Antibiotics |
|-----|----------|--------------------------|----------------------|-----------------|-----------------------------------|
| 1   | ss3c1    | B2 Malankad chalk        | VIM, NDM             | +               | CAZ, MEM, CIP, AMP, NOR, SXT, TE, ATM, DO, C, EF |
| 2   | ss26sc1  | B2 Gajju khan            | KPC, VIM            | -               | CIP, AMP, SXT, TE, CN, ATM, DO, C, MEM, IPM |
| 3   | ls5sc1   | D Colleg chalk           | -                    | +               | CAZ, CIP, AMP, NOR, SXT, TE, ATM, DO, C |
| 4   | ss16sc1  | D Colleg chalk           | KPC                | -               | CAZ, CIP, AMP, NOR, SXT, TE, CN, ATM, DO, C, MEM, IPM |
| 5   | ss12sc2  | B2 Shaikh Malton         |                      | -               | CIP, AMP, NOR, SXT, TE, MEM, ATM, DO, C, MEM |
| 6   | ss3sc2   | D Malankad chalk         | VIM                | -               | CAZ, CIP, AMP, NOR, SXT, TE, ATM, DO, C, EF |
| 7   | ss10sc1  | A Malankad chalk         | +                    | +               | CAZ, CIP, AMP, NOR, SXT, TE, ATM, DO, C, IPM |
| 8   | Hs19sc1  | A Shaikh Malton          | -                    | -               | CAZ, CIP, AMP, SXT, TE, ATM, MEM, IPM, DO |
| 9   | ss12sc1  | D Shaikh Malton          | -                    | -               | CAZ, CIP, AMP, NOR, SXT, TE, CN, DO, C, EF, IPM |
| 10  | Hs9sc2   | A Malankad chalk         | -                    | -               | CAZ, CIP, AMP, NOR, SXT, TE, C |
| 11  | ss15sc1  | A Colleg chalk           | +                    | +               | CIP, AMP, NOR, SXT, TE, ATM, DO, C, EF |
| 12  | Hs11sc1  | B2 Colleg chalk          | -                    | -               | CAZ, CIP, AMP, NOR, SXT, TE, ATM, DO |
| 13  | ss30sc1  | B2 Gajju khan            | VIM                | -               | CAZ, CIP, AMP, NOR, SXT, TE, ATM, DO, C, EF, IPM |
| 14  | ls5sc4   | B2 Colleg chalk          | NDM, VIM           | -               | CAZ, CIP, AMP, NOR, SXT, TE, CN, DO, C, IPM |
| 15  | ss22sc1  | B2 Gajju khan            | VIM                | +               | CAZ, MEM, CIP, AMP, NOR, SXT, TE, CN, DO, C |
| 16  | ss14sc1  | B2 Colleg chalk          | NDM                | -               | CIP, AMP, NOR, SXT, TE, CN, ATM, DO, C, MEM, IPM |
| 17  | Hs12sc1  | B2 Shaikh Malton         | +                    | -               | CAZ, CIP, AMP, NOR, SXT, TE, CN, DO, C |
| 18  | ss13sc1  | A Shaikh Malton          | +                    | +               | CAZ, CIP, AMP, NOR, SXT, TE, DO, IPM |
| 19  | ss26sc2  | B2 Gajju khan            | VIM                | -               | CAZ, CIP, AMP, NOR, SXT, TE, CN, DO, C |
| 20  | ss18sc1  | D Colleg chalk           | -                    | -               | CAZ, CIP, AMP, SXT, TE, ATM, MEM, IPM |
| 21  | Hs13sc1  | D Shaikh Malton          | -                    | -               | CAZ, CIP, AMP, NOR, SXT, TE, DO, IPM |
| 22  | ss85sc4 | D Malankad chalk         | VIM                | -               | CAZ, CIP, AMP, NOR, SXT, TE, CN, DO, C, EF, IPM |
| 23  | ss21sc1  | A Gajju khan             | -                    | -               | CAZ, CIP, AMP, NOR, SXT, CN, DO, C, EF |
| 24  | ss25sc1  | A Shaikh Malton          | -                    | -               | CAZ, AMP, SXT, TE, DO, MEM, IPM |
| 25  | ls2sc1   | A Shaikh Malton          | +                    | -               | CAZ, CIP, AMP, NOR, SXT, TE, CN, DO, C, EF |
| 26  | Hs38sc1  | A Shaikh Malton          | OXA-48             | -               | CAZ, CIP, AMP, NOR, SXT, TE, CN, DO, C |
| 27  | ss36sc1  | B2 Gajju khan            | -                    | -               | CAZ, CIP, AMP, SXT, TE, CN, DO, C, EF, IPM |
| 28  | ls26sc1  | D Gajju khan             |                      | +               | CAZ, MEM, CIP, AMP, NOR, SXT, TE, CN, MEM, ATM |

**Supplementary Table 1**: Primers, Targeted Genes and Amplicon size

| Primer | Sequence (5’ to 3’) | Target gene | Anneling temperature | Amplicon size | References |
|--------|---------------------|-------------|----------------------|---------------|------------|
| IMP-F  | GGAATAGATGGGCTTTAAATC | blazw      | 55°C                 | 232           | (Poirel et al, 2011) |
| IMP-R  | GTTTTAAYAAACAAACACAC |            |                      |               |            |
| VIM-F  | GATGGTGTTTTGGTGCGATTA | blazw      | 39°C                 |               |            |
| VIM-R  | CGAATGGCCGACCCAG     |            |                      |               |            |
| OXA-48-B | GCGTGTTAAGGATGAAACAC | blazw, ox48 | 55°C                 | 438           |            |
| OXA-48-R | CATCAAGTTCACAACCCAGG |            |                      |               |            |
| NDM-R  | GGGATGGCGATCGTGTGTTTC | bladme     | 55°C                 | 621           |            |
| NDM-F  | GGGATGGCGATCGTGTGTTTC | bladme     | 55°C                 | 621           |            |
| KPC-F  | CGTCTAGATCGGTCTCTTG  | blavc      | 55°C                 | 798           |            |
| KPC-R  | CTGTGCTTGAGGTGAGG    |            |                      |               |            |

Integrons

| intI1-F | CTC CCC GCA GCA TGA TGC | intI1 | 54°C | 280-bp | Dillon et al., 2005 |
| intI2-F | TCC AGT CAT CGT CAG GC | intI2 | 54°C | 439-bp | Dillan et al., 2005 |
| intI3-F | ATG TCT AAG AGT CCA TTT TTA AAT TCT A | intI3 | 54°C | 599-bp | Dillan et al., 2005 |

E. coli-Specific

| UAL | TGT TAA TTA TCC GCG AAA ACG GC | udaA | 62°C | 147-bp | Tantawiwat et al, 2005 |

**References**

- Forward, R- reverse.
Bangladesh and spread to other parts of the world quite speedily (Poirel et al., 2010; Habeeb et al., 2013). Isolates that produce NDM have shown resistance to most of the cephalosporins and carbapenems. However, our results were quite interesting as few of the isolates were still found susceptible to meropenem raising further curiosity. Possibly, other co-expressing resistance encoding elements or promoter sequences might have role in such phenotypic characteristics.

Co-existence of multiple carbapenemase encoding genes has been reported previously in Pakistan (Sattar et al., 2016). Sattar et al reported the co-existence of KPC and NDM-1 encoding genes in clinical isolates of Klebsiella pneumoniae isolates, while we identified VIM and NDM combination. Although, sources of samples of E. coli isolation were different, co-existence of similar combination of two different carbapenemase encoding genes is alarming. It would be interested to further investigate the genetic background of these genes, plasmid types, and plasmid sizes, and whether all these genes are carried on the same or different plasmids. Altogether, our data report on the presence MDR-CPE suggesting that poultry meat might be a source of these genes or CPE.

Conclusions: We report the occurrence of MDR-CPE recovered from poultry meat suggesting that poultry meat could be a source for the tested carbapenemase-encoding genes, which may further be disseminated to environment and human. An urgent intervention is required including awareness regarding prudent use of antimicrobials in poultry production systems. Furthermore, Sequence typing by whole genome sequencing of few candidate isolates should be carried out to analyze them further.

Funding: No funding was available specifically for this work.

Acknowledgements: Authors acknowledge technical support of Mr. Ali Murtaza and Dr. Attaur Rahman during collection and processing of samples.

Authors contribution: SUR designed and conceive the project. MY collected all the samples and processed them. MMS and IK helped in culturing and processing. SS and SUR wrote the draft. All authors approved the final manuscript.

REFERENCES

Adnan M, Khan H, Kashif J, et al., 2017. Clonal expansion of sulfonamide resistant Escherichia coli isolates recovered from diarrheic calves. Pak Vet J 37:230-2.

Ali T, ur Rahman S, Zhang L, et al., 2017. Characteristics and genetic diversity of multi-drug resistant extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli isolated from bovine mastitis. Oncoarget 8:90144.

Ali T. Zhang L, Shahid M, et al., 2016. ESBL-producing Escherichia coli from cows suffering mastitis in China contain clinical class I integrases with CTX-M linked to ISCR1. Front Microbiol 7:1931.

Clermont O, Bonacorsi S and Bingen E, 2000. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl Environ Microbiol 66:4555-8.

CLSI, 2014. Performance standards for antimicrobial susceptibility testing. Clinical and Laboratory Standard Institute. CLSI document, Wayne, PA pp:MI00-4.

Dillon B, Thomas L, Mohmand G, et al., 2005b. Multiplex PCR for screening of integrons in bacterial lysates. J Microbiol Methods 62:221-32.

Habeeb MA, Sarwar Y, Ali A, et al., 2013. Rapid emergence of ESBL producers in E. coli causing urinary and wound infections in Pakistan. Pakistan J Med Sci 29:540.

Hussain T, 2015. Pakistan at the verge of potential epidemics by multidrug-resistant pathogenic bacteria. Adv Life Sci 2:46-7.

Khan E, Schneider T, Zafar A, et al., 2010. Emergence of CTX-M Group I-ESBL producing Klebsiella pneumonia from a tertiary care centre in Karachi, Pakistan. J Infect Dev Countr 4:472-6.

Khatak I, Mushtaq MH, Ayaz S, et al., 2018. Incidence and Drug Resistance of Zoonotic Mycobacterium bovis Infection in Peshawar, Pakistan. Adv Exp Med Biol 1057:111-26.

Meletis G, 2016. Carbapenem resistance: overview of the problem and future perspectives. Therap Adv Infect Dis 3:15-21.

Miteva E., 2010. The role of unregulated sale and dispensing of antimicrobial agents on the development of antimicrobial resistance in developing countries. In: Antimicrobial Resistance in Developing Countries: Springer 403-11.

Mohsin M, Raza S, Schaufler K, et al., 2017. High Prevalence of CTX-M-15-Type ESBL-Producing E. coli from Migratory Avian Species in Pakistan. Front Microbiol 8.

Nordmann P, Naas T and Poirel L, 2011. Global spread of carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 17:1791.

Pakistan Go, 2016. Economic Survey of Pakistan, Government of Pakistan, Economic Advisor’s Wing, Finance Division, Islamabad, Pakistan. Accessed April 6, 2017.

Perry JD, Naqui SH, Mirza IA, et al., 2011. Prevalence of fecal carriage of Enterobacteriaceae with NDM-1 carbapenemase at military hospitals in Pakistan, and evaluation of two chromogenic media. J Antimicrob Chemother 66:2288-94.

Piet JR, van Ulsen P, ur Rahman S, et al., 2016. Meningococcocal Two-Partner Secretion Systems and Their Association with Outcome in Patients with Meningitis. Infect Immun 84:2534-40.

Poirel L, Walsh TR, Cuvelier V, et al., 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Micro Infec Dis 70:119-23.

Poirel L, Lagrutta E and Taylor P, 2010. Emergence of metallo-beta-lactamase NDM-1-producing multidrug-resistant Escherichia coli in Australia. Antimicrob Agents Chemother 54:4914-6.

Sattar AL, Raza MW, Abbasi SA, et al., 2014. Molecular epidemiology of NDM-1-producing Enterobacteriaceae and Acinetobacter baumannii isolates from Pakistan. Antimicrob Agents Chemother 58:5589-93.

Sattar H, Toleman M, Nahid F et al., 2016. Co-existence of bla NDM-1 and bla KPC-2 in clinical isolates of Klebsiella pneumoniae from Pakistan. J Chemother 28:346-9.

Tantawisat S, Tansuphasiri U, Wongwit W, et al., 2005. Development of multiplex PCR for the detection of total coliform bacteria for Escherichia coli and Clostridium perfringens in drinking water. Southeast Asian J Trop Med Public Health 36:162-9.

ur Rahman S, Ali T, Ali I, et al., 2018a. The Growing Genetic and Functional Diversity of Extended Spectrum Beta-Lactamases. BioMed Res Int 2018:14.

ur Rahman S, Ahmad S, Khan I and Pakistan P, 2018b. Incidence of ESBL-Producing-Escherichia coli in Poultry Farm Environment and Retail Poultry Meat. Pak Vet J http://dx.doi.org/10.29261/pakvet/2018.091.

ur Rahman S, Arenas J and Ozturk H, 2014. The polypeptide transport-associated (POTRA) domains of TpsB mediators determine the system specificity of two-partner secretion systems. J Biol Chem 289:19799-809.

ur Rahman S and van Ulsen P, 2013. System specificity of the TpsB transporters of coexpressed two-partner secretion systems of Neisseria meningitidis. J Bacteriol 195:788-97.

Ulsen P, Rahman S and Jong WS, 2014. Type V secretion: from biogenesis to biotechnology. Biochim Biophys Acta 1843:1592-611.

Yong D, Toleman MA, Giske CG, et al., 2009. Characterization of a new metallo-beta-lactamase gene, blαNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. Antimicrob Agents Chemother 53:5046-54.