Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2018.091

RESEARCH ARTICLE

Incidence of ESBL-Producing-*Escherichia coli* in Poultry Farm Environment and Retail Poultry Meat

Sadeeq ur Rahman[1]*, Shakoor Ahmad[2] and Imad Khan[1]

[1]College of Veterinary Sciences and Animal Husbandry, Section Microbiology, Abdul Wali Khan University, Mardan-Khyber Pakhtunkhwa Pakistan; [2]Department of Animal Health, The University of Agriculture, Peshawar – Khyber Pakhtunkhwa Pakistan
*Corresponding author: sadeeq@awkum.edu.pk

ARTICLE HISTORY (18-279)

Received: July 29, 2018
Revised: August 26, 2018
Accepted: September 03, 2018
Published online: September 27, 2018

Key words:
Antimicrobial resistance
CTXM
ESBL
Mardan KP
NDM-1
Pakistan

A B S T R A C T

Antimicrobials are excessively used in Pakistan during broiler poultry production, however, status of antimicrobial resistance (AMR) is not known. We report on the frequency of extended spectrum β-lactamase (ESBL) –producing-*Escherichia coli* recovered from samples obtained from poultry farm environment (n=100) and chicken meat samples (n=100) collected at sales point of live bird market in District Peshawar, Pakistan. Phenotypically confirmed ESBL producers were further investigated for the presence of ESBL- and carbapenemase-encoding genes and types of integrons. Our results showed that 38.8% (14/36) and 47.6% (20/42) of *E. coli* recovered from farm environment and chicken meat, respectively, were found to be ESBL producers. PCR based results showed that 30% (6/20), 10% (2/20) and 20% (4/20) of these *E. coli* from chicken-meat were harboring bla_{CTXM}, $bla_{\text{CTXM-1}}$ and bla_{SHV2}, respectively, while 20% were carrying bla_{OXA-48} and bla_{NDM-1}. On the other hands, 28.5% (4/14), 28.5% (4/14) and 14.2% (2/14) of *E. coli* isolated from chicken farm-environment were harboring bla_{CTXM}, $bla_{\text{CTXM-1}}$ and bla_{SHV2}, respectively, while, 28.5% (4/14) and 14.2% (2/14) were carrying $bla_{\text{OXA-48}}$ and bla_{NDM} -1. Integron 2 was PCR amplified from 17 isolates and integron 1 from 16 isolates, while, integron 3 was absent from all. Finally, insertion sequence IS*CR1* was PCR-amplified from 15 isolates (41.6%), which was found associated with bla_{CTXM} among 6 (16.6%) isolates suggesting its role in mobilization. Overall, our results suggest a high rate of occurrence of ESBL- and carbapenemase-carrying *E. coli* in poultry farm environment and chicken meat.

To Cite This Article: Rahman SU, Ahmad S and Khan I, 2019. Incidence of ESBL-producing-*Escherichia coli* in poultry farm environment and retail poultry meat. Pak Vet J, 39(1): 116-120. http://dx.doi.org/10.29261/pakvetj/2018.091

INTRODUCTION

Antimicrobial resistance (AMR) is a global emerging threat and is one of the major health issues Pakistan is facing at the moment. In particular, resistance to *β*-lactams and carbapenems is quite serious as these drugs are proven safe and efficient, but are losing its effect due to emerging AMR. Bacteria evolve this feature against *β*-lactams through production of extended spectrum β-lactamases (ESBL) that inactivate antimicrobials including third, fourth-generation cephalosporins and monobactams, however, could not inactivate cephamycins and carbapenems (El Salabi *et al.,* 2013; Rahman *et al.,* 2018). Carbapenemases, on the other hand, are enzymes that can inactivate carbapenem drugs-the most effective and last resort of antibiotic when extended-spectrum cephalosporins fail to work. ESBL and carbapenemases are predominantly produced in *Enterobacteriaceae*, mainly in *Escherichia coli* that is utilized as crucial resistance mechanism to cephalosporins and carbapenems (Meletis, 2016). Furthermore, generally, *E. coli* encoding for ESBL and carabepenemases are resistant to more than one class of antimicrobials and hence are multidrug resistant (MDR), and thus presenting a serious challenge in healthcare settings. In addition to implications of antimicrobials for prophylaxis and as growth promoters, recurrent bacterial infections encourages excessive and constant usage of these antimicrobials thereby resulting emergence and development of resistance against these compounds (Rahman *et al.,* 2018). Emergence of resistance against carbapenem and cephalosporins are particularly more

worrisome as no option left for serious infections in humans.

There are three main types, *bla*$_{CTXM}$, *bla*$_{SHV}$ and *bla*$_{TEM}$, of ESBL-encoding genes with further subtypes such as *bla*$_{CTXM}$ has five subgroups (*bla*$_{CTXM-1}$, *bla*$_{CTXM-2}$, *bla*$_{CTXM-8}$, *bla*$_{CTXM-9}$, *bla*$_{CTXM-25}$). The list of subtypes of each group is growing day by day by introducing new types with more than 160 subtypes for *bla*$_{CTXM}$ has been described so far (http://www.lahey.org/studies). ESBL and particularly *bla*$_{CTXM}$ has even frequently been isolated from food-producing-animals (Ali *et al.,* 2016; Ali *et al.,* 2017) as well as from community (Abrar *et al.,* 2017) and has been predominant in Asia mobilized by integrons and insertion sequence common region 1 (IS*CR1*) (Ali *et al.,* 2016).

Antibiotics are excessively used during poultry production in Pakistan for treatment, prevention of diseases, and as growth promoters. This provides selective pressure for the emergence of novel bacterial resistance in poultry microbiota. These resistant microbes are then excreted in the feces in large amount that can persist in the environment and thus serve as pool of resistance gene for other microbes. We thus investigated occurrence of *E. coli* recovered from poultry farm environment and chicken meat carrying ESBL- and carbapenemase- encoding genes. In the absence of nation-wide structural surveillance program on the impact and extent of AMR, such random reports become highly important for policy makers, clinicians and public.

MATERIALS AND METHODS

Ethics: The project was approved from the ethical committee of the University of Agriculture Peshawar and Abdul Wali Khan University Mardan KP. All work described here is carried out in accordance with local institutional and national guidelines and legislations.

Study area and samples: A total of 100 samples from broiler shed environment (drinking water, floor and feeding utensils), while another 100 from retail poultry meat (liver, leg piece and heart) (Table 1) in district Peshawar KP.

Isolation of *Escherichia coli,* and ESBL confirmation: Meat (25g) samples were minced properly (by sterile scissors in peptone water) and subjected to overnight pre-enrichment in tryptic soy broth containing cefotaxime (2mg/L) followed by streaking onto MacConkey agar supplemented with 2 mg/L of cefotaxime or 0.5 mg/L of meropenum and incubated for 24-48 h at 37°C. Samples from shed floor (10 g in peptone water), swab from drinking and water equipment (transported in 1 ml of peptone water) and drinking water (1 ml) (Mesa *et al.,* 2006) were streaked onto MacConkey agar containing 2mg/L cefotaxime for screening of ESBL and/or 0.5 mg/L meropenum for screening of carbapenemase enzymes as per guidelines of Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2014). Pink color *E. coli* colonies on MacConkey agar were confirmed by gram staining, colonial morphology, biochemical test and specie specific PCR as described earlier (Tantawiwat *et al.,* 2005). Confirmed ESBL-producing *E. coli* isolates were stored in brain heart infusion broth containing 30% glycerol at -80°C.

Presumptive ESBL-producing *E. coli* were confirmed by double-disc synergy test in accordance with recommendations of CLSI. For positive control, *Klebsiella pneumoniae* ATCC 700603 (ESBLs-positive strain) was used.

ESBL and carbapenemase encoding genes identification: Conventional PCR was performed to identify ESBL and carbapenemase encoding genes using specific primers already published (Supplementary Table 1). Total DNA was extracted by using standard boiling method. The polymerase chain reaction was performed in thermo cycler (Bio Rad thermo cycler T100). PCR amplification products obtained were evaluated by 1% agarose gel electrophoresis as explained (Al Agamy *et al.* 2009). Reference DNA for *bla*$_{NDM-1}$ and *bla*$_{OXA-48}$ was used from our previously confirmed isolates (manuscript under review).

Antibiotic susceptibility testing: Mueller-Hinton agar (Difco^{TM}) was used to perform antibiotic susceptibility of ESBL using antibiotic discs (Becton Dickison, Sparks, MD USA) following standard Kirby-Bauer disk and interpreted as in CLSI (CLSI, 2014). *E. coli* ATCC 25922 (ESBL-negative) and *Klebsiella pneumoniae* ATCC 700603 (ESBL-positive) were used as quality control strains (CLSI, 2014).

Determination of Phylogroups of *E. coli:* ESBL- *E. coli* isolates were placed in one of the four phylogenetic groups: phylo-group A, group B1, group B2 and group D based on the triplex PCR as described earlier (Clermont, 2000).

Detection of integrons: Integrons (Integron 1-3) were detected by conventional PCR using integron specific primers as described previously (Dillon *et al.,* 2005).

IS*CR1* and association: IS*CR1* was first detected by PCR as optimized by us and published earlier (Ali *et al.,* 2016). Association of IS*CR1* with that of ESBL gene was established through PCR using a combination primer approach as we described earlier (Ali *et al.,* 2016).

RESULTS

***Escherichia coli* isolates and ESBL phenotypes:** A total of 36 *E. coli* were recovered from environmental samples while 42 were recovered from chicken meat. Of 36 environmental *E. coli*, 14 (38.8%) were confirmed as ESBL producers. Of 42 chicken *E. coli*, 20 (47.6%) were confirmed as ESBL producers as determined by double disc synergy test (Table 1). Overall, 34 ESBL isolates (43.5%) were confirmed from 78 *E. coli* isolates. Frequency of ESBL producers from different environmental samples varied such as 3, 4 and 7 ESBL producers were confirmed from water, ground floor and feeding and drinking equipments, respectively (Table 1).

Frequency and diversity of ESBL-and carbapenemase-encoding genes: The ESBL- producing isolates were

further assessed for the types of ESBL-encoding genes and results are presented in Table 2. Our results showed that *bla$_{CTXM}$* was a bit predominant carried by 29.1% (10/34) followed by *bla$_{OXA-48}$* (23.5% 8/34). No *bla$_{TEM}$* was PCR amplified from a single isolate. Interestingly, PCR based ESBL and carbapenemase genes amplification results showed that *E. coli* recovered from both sources (poultry farm and poultry retail meat) were carrying similar types of genes. Also, none of the tested gene was found distinctly associated with either chicken meat or environment. Almost all tested genes were found equally distributed among the isolates recovered from both sources suggesting widespread occurrence of ESBL- and carbapenemase-carrying *E. coli* in chicken rearing environment.

Antimicrobial susceptibility patterns: All these 34 isolates were further tested against various antibiotics and results are described in Table 3. Results indicated all isolates under study were resistant to first generation cephalosporins (Cephalexin), while resistance to third generation cephalosporins, cefotaxime and ceftazidime was noted to be 69.76 and 74.18%, respectively. Of note, majority of isolates were found susceptible to meropenum (78.8%) followed by norfloxacin (48.1%) and aztreonam (41.8%).

Table 1: Isolation of *E. coli* and ESBL phenotypic detection

Source	Numb of sample collected	*E. coli* isolation n(%)	ESBL producers n(%)
Environmental samples			
Water (drinking water from drinkers)	30	8(26.6)	3(37.5)
Ground (farm-floor)	40	21(52.5)	5(23.8)
Feeding and drinking equipments (cotton swabs)	30	7(23.3)	6(58.7)
Subtotal	100	36(36)	14(38.8)
Retail chicken			
Liver	43	21(48.8)	11(52.3)
kidney	22	04(18.1)	00(0.0)
leg piece	25	12(48.0)	5(41.6)
Skin	10	05(50.0)	4(80.0)
Subtotal	100	42(42.0)	20(47.6)
Grand total	200	78(39.0)	34/78(43.5)

Table 2: Prevalence of extended-spectrum β-lactamase encoding genes

Name of gene	Chicken	Environment	Total
CTX-M	6/20(30%)	4/14(28.57%)	10/34 (29.1)
CTX-M -1	2/20(10%)	4/14(28.57%)	6/34(17.6)
SHV-2	4/20(20%)	2/14(14.28%)	6/34(17.6)
OXA-48	4/20(20%)	4/14(28.57%)	8/34(23.5)
NDM-1	4/20(20%)	2/14(14.28%)	06/34(17.6)
TEM	0/20(0.00%)	0/0(0.00%)	0/0(0.00%)

Table 3: Antibiotic sensitivity profile

Drug	Abb.	Concentration (µg)	Resistant %	Intermediates %	Susceptible %
Cephalexin	CLR	30	85.00	03.0	10.00
Ampicillin	AM	10	80.75	12.2	07.05
Cefotaxime	CTX	30	69.76	15.2	15.04
Ceftazidime	CAZ	30	74.18	9.10	16.80
Azetreonem	AZT	30	27.90	30.3	41.80
Gentamicin	GM	10	25.58	30.3	44.20
Tetracycline	TE	20	51.16	15.2	33.70
Meropenem	MPN	10	06.00	15.2	78.80
Norfloxacin	NOR	10	18.60	33.3	48.10

Phylogenetic analysis: Based on a triplex PCR for phylogenetic grouping, ESBL-producing *E. coli* were grouped into three phylogroups as shown in Table 4. Our results showed that the commensal phylogroup A was found to be the most prevalent (50%), followed by virulent extra-intestinal group D (32%) and phylogroup B2 (16.6%), respectively.

Integrons, variable region and insertion sequence IS*CR1*: All ESBL-producing isolates were further characterized for the type of integrons, and insertion sequence common region 1 (IS*CR1*) and results are presented in Table 5. Overall, our results showed that integron 2 was present in a total of 17 isolates (47.2) followed by integron 1, which was found in 16 (44.44%) isolates. Notably, integron 3 could not be amplified from any of the isolates. Interestingly, a combination of integron 1 and 2 was also observed in a total of 2 isolates both were from the environmental samples. Finally, insertion sequence IS*CR1* was also PCR-amplified from a total of 15 isolates (41.6%). Finally, association of the ESBL genes with the IS*CR1* was assessed by primer combination targeting IS*CR1* and an ESBL gene, indicating a total of 6 (16.6%) isolates produced an amplicon of the expected size showing association with the insertion sequence and more likely role in dissemination of these resistance encoding genes. Overall, our results showed ESBL producing multidrug resistant *E. coli* carrying diverse ESBL and carbapenemase encoding genes with *bla$_{CTXM}$*in dominance followed by *bla$_{OXA-48}$*.

DISCUSSION

Over the last couple of years, ESBL- and carbapenemase-producing *E. coli* have been frequently reported from food-producing animals presenting a global challenge for public health and food security (Seiffert *et al.*, 2013; Ali *et al.*, 2016; Ali *et al.*, 2017). Generally, these isolates also exhibit MDR phenotypes further increasing challenge to eradicate them (Nordmann and Poirel 2002; Adnan *et al.*, 2017; Rahman *et al.*, 2018). Antimicrobial resistance (AMR) is even more serious in developing countries like Pakistan where usage of antimicrobials are not strictly regulated, particularly in animals. Furthermore, lack of surveillance and monitoring data regarding usage of antimicrobials and emergence of drug resistance further complicates scenario. Poultry industry in Pakistan is one of the biggest and dynamic industry with over 200 billion Pak. Rs. investment, and this sector provides a lion share of 28% of total meat produced in the country (Pakistan, 2016). However, unfortunately, use of antibiotics in poultry industry is not strictly regulated (Mitema, 2010) raising concern of emergence of antimicrobial resistant microorganisms. For the very first time in Pakistan and particularly in the Northern Province-the Khyber Pakhtunkhwa- we designed this project to identify *E. coli* recovered from retail poultry meat and poultry farm environment carrying ESBL and carbapenemase encoding genes. Our study show that *E. coli* isolates from poultry meat and poultry farm environment carrying similar types of ESBL and carbapenemase genes suggesting its widespread dissemination.

Table 4: Molecular characterization of ESBL producers*

Sr. No	ID		P.G	ESBL genotype				Carbapenemase genotype		Integron typing			ISCR1	ISR1+ ESBL	R/I phenotypes
				CTXM	CTXM1	SHV	TEM	Oxa-48	NDM-1	Int.1	Int.2	Int.3			
1.	HC-2	Chicken	D	+	-	+	-	-	-	+	-	-	-	-	CLR, TE, AM
2.	HC-4		A	-	-	-	-	-	-	-	-	-	-	-	-
3.	HC11		A	-	-	-	-	-	-	-	-	-	-	-	CLR
4.	HC14		A	+	-	-	-	+	-	-	-	-	-	-	CLR, GM, C,
5.	HC19		A	+	-	-	-	-	-	-	+	-	+	-	CLR, AM
6.	HC42		A	+	-	-	-	-	+	+	-	-	+	-	CLR, AM, GM,TE, C
7.	HC52		A	-	-	-	-	+	-	-	+	-	-	-	CLR, AM
8.	HC55		B2	-	-	-	-	-	-	+	-	-	+	-	CLR, AM
9.	E6		B2	+	-	-	-	+	-	+	+	-	+	+	CLR, GM, TE
10.	E7	Environment	A	-	-	-	-	-	-	-	-	-	+	-	CLR
11.	E11		D	-	-	-	-	-	-	-	+	-	-	-	CLR
12.	E31		B2	+	-	-	-	+	+	+	-	-	-	-	CLR, TE, NOR, C
13.	E40		A	+	+	+	-	-	-	-	-	-	-	-	CLR, TE, C, AM
14.	E69		A	-	-	-	-	-	-	-	+	-	-	-	-
15.	E70		A	-	+	-	-	-	-	+	+	-	+	-	CLR, AM

*To avoid clutter, only a few representative isolates characteristics are described + present, - absent, Int.1 integron class 1, Int. 2 integron class 2, Int.3 Integron class 3, P.G phylogenetic groups, CLR Cephalexin, C Chloramphenicol, GM Gentamicin, TE Tetracycline.

Supplementary Table 1: Primers, Targeted Genes and Amplicon size

Primer	Sequence (5′ to3′)	Target gene	Annealing Temperature	Amplicon Size	References
ESBL producing genes					
CTX-M –F	CGCTTTGCGATGTGCAG	*bla*$_{CTXM}$	55°C	~550	(Villegas *et al.,* 2004)
CTX-M –R	ACCGCGATATCGTTGGT				
CTXM1-F	GCT GTT GTT AGG AAG TGT GC	*bla*$_{CTXM-1}$	55°C	~490	(Shibata *et al.,* 2006)
CTXM1-R	CCA TTG CCC GAG GTG AAG				
SHV –F	GGG TTA TTC TTA TTT GTC GC	*bla*$_{SHV}$	55°C	~567	(Chang *et al.,* 2001; Yao *et al.,* 2007)
SHV -R	TTAGCGTTGCCAAGTGCTC				
TEM-F	ATA AAA TTC TTG AAG ACG AAA	*Bla*$_{TEM-1,\ -52,\ -71,\ -104\ -105}$	54°C	~1086	(Yao *et al.,* 2007)
TEM-R	GAC AGT TAC CAA TGC TTA ATC				
Carbapenemase encoding genes					
OXA-48-F	GCGTGGTTAAGGATGAACAC	blaOXA-48	55°C	438	(Poirel *et al.,* 2011)
OXA-48-R	CATCAAGTTCAACCCAACCG				
NDM-F	GGTTTGGCGATCTGGTTTTC	blaNDM	55°C	621	(Poirel *et al.,* 2011)
NDM-R	CGGAATGGCTCATCACGATC				
Integrons					
intI1-F	CCT CCC GCA CGA TGA TC	intI1	54°C	280-bp	(Dillon *et al.,* 2005)
intI1-R	TCC ACG CAT CGT CAG GC				
intI2-F	AAA TCT TTA ACC CGC AAA CGC	intI2	54°C	439-bp	(Dillon *et al.,* 2005)
intI2-R	ATG TCT AAC AGT CCA TTT TTA AAT TCT A				
intI3-F	AGT GGG TGG CGA ATG AGT G	intI3	54°C	599-bp	(Dillon *et al.,* 2005)
intI3-R	TGT TCT TGT ATC GGC AGG TG				
E. coli-Specific					
UAL	TGG TAA TTA CCG ACG AAA ACG GC	uidA	62°C	147-bp	(Tantawiwat *et al.,* 2005)
UAR	ACG CGT GGT TAC AGT CTT GCG				
PHYLO-Group					
ChuA-F	GAC GAA CCA ACG GTC AGG AT	ChuA	55°C	279-bp	(Clermont, 2000)
ChuA-R	TGC CGC CAG TAC CAA AGA CA				
YjaA-F	TGA AGT GTC AGG AGA CGC TG	YjaA	55oC	211-bp	(Clermont, 2000)
YjaA-R	ATG GAG AAT GCG TTC CTC AAC				
TspE4C2-F	GAG TAA TGT CGG GGC ATT CA	TspE4C2	55°C	152-bp	(Clermont 2000)
TspE4C2-R	CGC GCC AAC AAA GTA TTA CG				
IS*CR1*					
ISCR1-F	CGC CCA CTC AAA CAA ACG	ISCR1	55°C	469-bp	(Ali *et al.,* 2016)
ISCR1-R	GAG GCT TTG GTG TAA CCG				

Our results showed an occurrence of 38.8% and 47.6% of *E. coli* carrying ESBL producing genes recovered from chicken rearing environment and chicken body parts, respectively. Apparently, there seems not a big difference of ESBL producers between environmental and chicken *E. coli*, the results however do suggest that environmental *E. coli* are carrying and expressing ESBL enzymes thereby, increasing the chances of fast dissemination of these genes among similar strains. Inversely, this also suggests that cephalosporin residues might still be present in the environment as contaminant which provide passive pressure for *E. coli* to maintain and express ESBL genes. This phenomenon of development of resistance is similar to production of secretory toxins in the presence of signaling molecules in bacterial surroundings (Rahman *et al*., 2014; van Ulsen *et al*., 2014). We will soon be in future analyzing the residues of drugs on floor, water and other resources.

Of note, our observed incidence rate of ESBL producing-*E. coli* recovered from poultry remained higher as compared to previously published report of 30% in poultry in Bangladesh (Hasan *et al.*, 2012) and in 10.7% samples from healthy chicken in France (Girlich *et al.*, 2007). The higher incidence rate of ESBL-producing *E. coli* is possibly due to over use or consistent usage of antibiotics during poultry production. ESBL-producing *E. coli* has been widely reported from- human patients that were hospitalized in Pakistan (Abrar *et al.*, 2017; Abrar *et al*., 2018) community and environment, (Ullah *et al.*, 2009) and even wild migratory birds (Mohsin *et al*., 2017;

Raza *et al*., 2017). Though, results of our study cannot be generalized as we analyzed limited number of samples from a single district Peshawar of Khyber Pakhtunkhwa province, we assume that the actual incidence rate might be even higher. Our study indicates that bla_{CTXM} remained a predominant genotype among ESBL-producing *E. coli*. These results are in agreement with other contemporary findings from Pakistan (Khan *et al.,* 2010; Abrar *et al.,* 2017), China (Ali *et al.,* 2016, 2017) and India (Upadhyay *et al.,* 2015), *etc*. This strongly suggests that bla_{CTXM} is the most dominant genotype of ESBL in Asia. This goes along with higher occurrence of SHV genotype in our study and as reported by others (Habeeb *et al.,* 2013). More worrisome was the combination of these two ESBL genes with carbapenemase encoding genes such bla_{OXA-48} and bla_{NDM-1}. Presence of ESBL and carbapenemase encoding genes and its association with integron and IS*CR1* are quite worrisome as these elements would speed up dissemination of these resistance elements. This study emphasize on extension of this work to other parts of the province to identify an overall provincial level extent of ESBL occurrence and factors helping its fast dissemination.

Conclusions: Taken together, the current high occurrence of ESBL-and carbapenemase-producing genes in *E. coli* recovered from poultry meat and its rearing environment with clinical class 1 integrons strongly suggest for intervention and efforts to mitigate the challenge of AMR.

Acknowledgements: A partial funding was provided by National Natural Science Foundation of China (No. 31550110200).

Authors contribution: SUR designed and perceive the experiments and got the funding. SA and IK helped in sample collection analysis. All authors have approved the final version of the manuscript.

REFERENCES

Abrar S, Vajeeha A, Ul-Ain N, *et al*., 2017. Distribution of CTX-M group I and group III beta-lactamases produced by *Escherichia coli* and *klebsiella pneumoniae* in Lahore, Pakistan. Microb Pathog, 103:8-12.

Abrar S, Hussain S, Khan RA, *et al*., 2018. Prevalence of extended-spectrum-β-lactamase-producing *Enterobacteriaceae*: first systematic meta-analysis report from Pakistan. Antimicrob Resist Infect Control 7:26.

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, Rahman SU, 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. Oncotarget 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 integrons with CTX-M linked to ISCR1. Front Microbiol 7:1931.

Clermont, 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:M100-S124.

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

El Salabi A, Walsh TR and Chouchani C, 2013. Extended spectrum beta-lactamases, carbapenemases and mobile genetic elements responsible for antibiotics resistance in Gram-negative bacteria. Crit Rev Microbiol 39:113-22.

Girlich D, Poirel L, Carattoli A, *et al*., 2007. Extended-spectrum beta-lactamase CTX-M-1 in *Escherichia coli* isolates from healthy poultry in France. Appl Environ Microbiol 73:4681-5.

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

Hasan B, Sandegren L, Melhus Å, *et al*., 2012. Antimicrobial drug-resistant *Escherichia coli* in wild birds and free-range poultry, Bangladesh. Emerg Infect Dis 18:2055-8.

Khan E, Schneiders T, Zafar A, *et al*., 2010. Emergence of CTX-M Group 1-ESBL producing *Klebsiella pneumonia* from a tertiary care centre in Karachi, Pakistan. J Infect Dev Ctries 4:472-6.

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

Mesa RJ, Blanc V, Blanch AR, *et al*., 2006. Extended-spectrum β-lactamase-producing Enterobacteriaceae in different environments (humans, food, animal farms and sewage). J Antim Chemoth 58:211-5.

Mitema ES, 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 pp: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 8:2476.

Nordmann P and Poirel L, 2002. Emerging carbapenemases in Gram negative aerobes. Clin Microbiol Infec 8:321-31.

Pakistan Govt, 2016. Economic Survey of Pakistan, Government of Pakistan, Economic Advisor's Wing, Finance Division, Islamabad, Pakistan.

Seiffert SN, Hilty M, Perreten V, *et al*., 2013. Extended-spectrum cephalosporin-resistant Gram-negative organisms in livestock: an emerging problem for human health? Drug Resist Updat 16:22-45.

Tantawiwat 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.

Ullah F, Malik SA and Ahmed J, 2009. Antimicrobial susceptibility pattern and ESBL prevalence in Klebsiella pneumoniae from urinary tract infections in the North-West of Pakistan. Afric J Microbiol Res 3:676-80.

Upadhyay S, Hussain A, Mishra S, *et al*., 2015. Genetic environment of plasmid mediated CTX-M-15 extended spectrum beta-lactamases from clinical and food borne bacteria in North-Eastern India. PloS one 10:e0138056.

Rahman SU, Ali T, Ali I, *et al*., 2018. The growing genetic and functional diversity of extended spectrum Beta-Lactamases. BioMed Res Int:9519718.

Chang FY, Siu LK, Fung CP, *et al*., 2001. Diversity of SHV and TEM beta-lactamases in Klebsiella pneumoniae: gene evolution in Northern Taiwan and two novel beta-lactamases, SHV-25 and SHV-26. Antimicrob Agents Chemother 45:2407-13.

Poirel L, Walsh TR, Cuvillier V, *et al*., 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70:119-23.

Raza S, Mohsin M, Madni WA, *et al*., 2017. First Report of bla CTX-M-15-Type ESBL-Producing Klebsiella pneumoniae in wild migratory birds in Pakistan. EcoHealth 14:182-6.

Shibata N, Kurokawa H, Doi Y, *et al*., 2006. PCR classification of CTX-M-type β-lactamase genes identified in clinically isolated gram-negative bacilli in Japan. Antimicrob Agents Chemoth 50:791-5.

Rahman SU, Arenas J, Ozturk H, *et al*., 2014. The polypeptide transport-associated (POTRA) domains of TpsB transporters determine the system specificity of two-partner secretion systems. J Biol Chem 289:19799-809.

van Ulsen P, Rahman SU, Jong WS, *et al*., 2014. Type V secretion: from biogenesis to biotechnology. Biochim. Biophys. Acta 1843:1592-611.

Villegas MV, Correa A, Perez F, *et al*., 2004. CTX-M-12 β-lactamase in a *Klebsiella pneumoniae* clinical isolate in Colombia. Antimicrob Agents Chemother 48:629-31.

Yao F, Qian Y, Chen S, *et al*., 2007. Incidence of extended-spectrum beta-lactamases and characterization of integrons in extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolated in Shantou, China. Acta Biochim Biophys Sin (Shanghai) 39:527-32.