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Original Article

Molecular mechanism of antimicrobial co-resistance Colistin (*mcr*-1) and ESBLs genes among *Escherichia coli* isolates from commercial chickens in Pakistan

Mecanismo molecular de corresistência antimicrobiana de Colistina (mcr-1) e genes ESBLs entre isolados de Escherichia coli em frangos comerciais no Paquistão

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Abstract

Emergence of plasmid mediated colistin and extended spectrum β-lactamases (ESBL) resistant genes has been impacted the efficacy of colistin and β-lactams drugs like 3rd, 4th generation cephalosporin. Current study was aimed to investigate antimicrobial resistance genes (ARGs) among *Escherichia coli* isolates from meat producing commercial broilers in Pakistan. Two hundred (n=200) fecal samples were collected during January-2018 to August-2019. For isolation of *E. coli*, pink colonies on MacConkey agar were transferred to EMB agar. Metallic sheen color colonies were tested biochemically using API-20E kit. The molecular identification of *E. coli* (n=153) was targeted by amplification of *uid* gene through polymerase chain reaction (PCR) and different ARGs i.e. gentamicin, streptomycin, tetracycline, colistin, β-lactams drugs, quinolone and ampicillin followed by sequence analysis. Genotypically, followed by phenotypically of resistant ARGs of isolated PCR-confirmed *E. coli* (153) shoed resistant against gentamicin (*aac(3)-IV),* streptomycin (*aadA1*), tetracycline (*tet*A), colistine (*mcr*-1), ampicillin (bla-$_{TEM}$) and bla-$_{CTX-M}$ were 86%, 88%, 86%, 88%, 83% & 77% respectively. 33/38 (86%) of the isolate was positive for quinolone resistance. Colistine (*mcr*-1), ESBLs (bla-$_{TEM}$) and (bla-$_{CTX-M)}$ resistance genes were 88%, 83% and 77% respectively. About 33 isolated *E. coli* harbored the both *mcr*-1 and ESBLs genes. All of *E. coli* isolates were found sensitive to ceftriaxone (CTX-30) and imipenem (IMP-10). The Isolated *E. coli* showed single or multi clade decadency. The *E. coli* and ARGs sequences showed single or multi clade decadency. This is first comprehensive study from Pakistan that described the molecular evidences of ARGs and their co-existence in single isolates originated from commercial poultry. Commercial chicken (Broilers) can act as melting pot of antibiotic resistance genes for human being. It is alarming situation for surveillance of antibiotic resistance program because of more regulated prescription of antimicrobial agents in Pakistan

Keywords: antimicrobial resistance genes, food animals and *Escherichia coli* Colistin, extended spectrum β-lactamases.

Resumo

O surgimento de colistina mediada por plasmídeo e genes de resistência a β-lactamases de espectro estendido (ESBL) afetou a eficácia de medicamentos colistina e β-lactâmicos, como as cefalosporinas de 3ª e 4ª geração. O presente estudo teve como objetivo investigar genes de resistência antimicrobiana (ARGs) entre isolados de *Escherichia coli* em frangos de corte comerciais no Paquistão. Duzentas (n = 200) amostras fecais foram coletadas durante janeiro de 2018 a agosto de 2019. Para o isolamento de *E. coli*, colônias rosas em ágar MacConkey foram transferidas para ágar EMB. As colônias de cores de brilho metálico foram testadas bioquimicamente usando o kit API-20E. A identificação molecular de *E. coli* (n = 153) foi direcionada pela amplificação do gene *uid* através da reação em cadeia da polimerase (PCR) e diferentes ARGs, ou seja, gentamicina, estreptomicina, tetraciclina, colistina, medicamentos β-lactâmicos, quinolona e ampicilina, seguido de análise de sequência. Genotipicamente, seguido por fenotipicamente de ARGs resistentes de *E. coli* isoladas foram confirmadas por PCR (153) como resistente contra gentamicina (aac(3)-IV), estreptomicina (aadA1), tetraciclina (tetA), colistina (mcr-1), ampicilina (bla-TEM) e bla-CTX-M, demonstrando resultados de 86%, 88%, 86%, 88%, 83% e 77%, respectivamente. Cerca de 33/38 (86%) do isolado foi positivo para resistência às quinolonas. Os genes de resistência à colistina (mcr-1), ESBLs (bla-TEM) e (bla-CTX-M) foram 88%, 83% e 77%, respectivamente. Cerca de 33 *E. coli* isoladas continham os genes mcr-1 e ESBLs. Todos os isolados de *E. coli* foram considerados sensíveis à ceftriaxona (CTX-30) e imipenem (IMP-10). A *E. coli*

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isolada apresentou decadência de um ou vários clados. As sequências de *E. coli* e ARGs apresentaram decadência de um ou vários clados. Este é o primeiro estudo abrangente do Paquistão que descreveu as evidências moleculares de ARGs e sua coexistência em isolados únicos originados de aves comerciais. Dessa forma, é possível concluir que o Frango comercial (Broilers) pode atuar como caldeirão de genes de resistência a antibióticos para o ser humano. É uma situação alarmante para a vigilância do programa de resistência a antibióticos devido à prescrição mais regulamentada de agentes antimicrobianos no Paquistão

Palavras-chave: genes de resistência antimicrobiana, animais para alimentação e *Escherichia coli* Colistina, β-lactamases de espectro estendido.

1. Introduction

The global emergence of *Escherichia coli* harboring multiple antimicrobial resistant genes (ARGs), among different food producing animals, has evoked the attention of public health microbiologists. Most of ARGs of *E. coli* are plasmid encoded and have the ability to be transferred horizontally among different members of family Enterobacteriaceae. The important ARGs includes mobilized colistin resistance genes (*mcr*-1 to *mcr*-9), extended spectrum beta lactamases (ESBLs which further includes *bla*-$_{CTX-M}$, *bla*-$_{SHV}$ and *bla*-$_{TEM}$) and tetracycline resistance genes (*tet*-A and *tet*-B) etc (Azad et al., 2019). Colistin resistance genes and ESBLs genes have attractive global attention because Colistin and β-lactam drugs considered as last resort of antibiotic agents in both human and animals (Azad et al., 2019; Blattner et al., 1997). β- D-glucuronosidase (*uid*A) is present in *E. coli*. *Uid*A gene is enzyme code which target the β-D-glucuronoside for the genetic confirmation of *E. coli.* 1.87 kb is gene size of *Uid*A. β-D-glucuronosidase enzyme has molecular weight 68200 which is very stable and easily sensitively assayed by using specific substrate (Hayashi et al., 2006). First time, whole genomic sequence (WGS) of *E. coli* was published in 1997. It comprises 4,600,000 base pairs and 4,000 genes (Blattner et al., 1997).

Among the food producing animals i.e. chicken meat is considered as potential source of avian pathogenic *E. coli* (APEC) that can cause minor to severe infection in humans i.e. epidemic diarrhea, urinary tract infections, endocarditis, meningitis, cellulitis, and septicemia along with the ability to spread antimicrobial resistance among community (Gross, 1994).

At present, the emerged resistance against multiple antimicrobials is termed as "superbug" (Amer et al., 2018). The plasmid/ chromosomal mediated resistance is developed through genetic alterations among different bacterial species via the process of conjugation, transduction or transformation. The evolution of resistance spread is a complex mechanism that also involves the mobile genetic elements i.e. transposons or transposable elements, integrons or plasmids. Hence, the phenotypic expression of resistance is determined by the genotype (Amer et al., 2018; Bennett, 2008). The accurate detection of this sort of resistance is targeted by amplification of different resistance genes (Awad et al., 2016; Ibrahim et al., 2019). Several of the factors are involved in spread of ARGs in the community through chicken meat and other products i.e. a) off-label over the counter use of antimicrobials particularly among the developing regions of the world b) use of antimicrobials as growth promoters and c) over dosage to attain quick response (Jakobsen et al., 2010; Adelowo et al., 2014). Ultimately, the risk of severity and zoonotic potential increases which results in increased health care cost as well as the morbidity and mortality (Adelowo et al., 2014). Extensive use of different antibiotics i.e. penicillin, cephalosporins, gentamicin, streptomycin, sulphonamides, tetracycline, aminoglycosides and colistin is widely practiced across the globe particularly for the treatment of *E. coli* infection in commercial chickens (Ibrahim et al., 2019; Zakeri & Kashefi, 2012).

Colistine induce the derangements of cell membrane. Bacterial cell death occurs due to leakage of inner contents of cell following the increase permeability of cell membrane. Colistin is also anti endotoxin activity which bind with toxin molecules of LPS and neutralize them properly (Gupta et al., 2009)

Beta lactam drugs which contain lactam beta ring. Third generation cephalosporin, monobactam, carbapenam and penicillin are major antibiotics group which belong to beta lactam drugs. Enzyme beta lactamase breakdowns the ring of beta lactam easily which interpret the function of antibiotic and make them untreatable (Greenwood, 2007). However, transmission of *mcr*-1 mediated colistin resistance genes between human and animals poses high thread to public health (Yang et al., 2017). Colistin and beta lactam drugs are used as feed additive in United States, Asia, European Union, Australia and other countries for the prevention of gastro intestinal tract infections caused by *E. coli, Salmonella* and other enterobacteria in food producing animals (Moreno et al., 2019).

In developing countries, antibiotics resistant bacteria become major concern for public health to cure the infectious diseases. Consumption of antibiotics without prescription and their overuse lead the high incidence of antibiotics resistance. Rate of antibiotic resistance among microorganisms is high in developing countries. Waste of money and loses of costly lives of human being & animals occurred in the result of antimicrobial resistant genes of microorganisms (Hayashi et al., 2006).

Altogether, the antimicrobial resistance (AMR) is a serious threat for the public health among developed as well as developing countries (Dabo et al., 2019; Younis et al., 2017). Hence, in the current study, we targeted the molecular identification and characterization of multiple ARGs from *E. coli* isolated from commercial meat producing chickens from Faisalabad-Pakistan.

2. Material and Methods

2.1. Samples collection and isolation of E. coli

This cross sectional study was conducted from January-2018 to August-2019. Fecal samples (n=200) were collected from meat producing chickens from Three

(n=01) commercial farms in Faisalabad-Pakistan mention in Table 1. All of the fecal samples were directly streaked on MacConkey agar (Oxoid, UK) followed by re-streaking of the suspected pink colonies on Eosin Methylene Blue agar (Oxoid, UK). Metallic Sheen on EMB agar (cultured plates) were incubated overnight at 37ºC (Jamil et al., 2007). Single, well isolated and suspected bacterial colony from each sample was subjected to initial confirmation for *E. coli* using commercially available API-20E kit (BIOMÉRIEUX, France).

2.2. Molecular identification of E. coli isolates

Putative colonies from EMB agar were inoculated in Brain Heart Infusion (Oxoid, UK) and incubated overnight followed by extraction of DNA using commercially available DNA purification kit (GeneJET, Genomic DNA Purification kit, Thermo Scientific ®-UK) according to manufacturer's instructions. All of the extracted DNA was subjected to PCR amplification by targeting *uid*- gene ((Jamil et al., 2007). The sequence of the forward and reverse primers was described in Table 2. The PCR was performed in final volume of 25 µL with initial denaturation at 95 ºC and 55 ºC followed by followed by 1.5% agarose gel electrophoresis.

Table 1. Distribution of isolated *E. coli* from different farms.

Name of farms	Total no of samples	Isolated *E. coli*
Farm-1	67	51
Farm-2	67	59
Farm-3	66	43
Total	200	153

Source: Chicken broiler; Farm-1= Rana Commercial Chicken; Farm-2=Tofail Commercial Chicken; Farm-3=Tariq Commercial Chicken.

2.3. Phenotypic identification of antimicrobial resistance

All of the genotypically confirmed *E. coli* isolates were subjected to determine the antimicrobial resistance patterns using technique of antimicrobial susceptibility test against different antibiotic disks of Oxoid® UK which include tetracycline, streptomycin, gentamicin, sulfamethoxazole, quinolones, colistin, amoxicillin, ceftriaxone, cefotaxime, ceftazidime and imipenem according to the guidelines of Clinical and Laboratory Standards Institute (Liu et al., 2017).

2.4. Genotypic identification of antimicrobial resistance

The DNA of confirmed *E. coli* isolates was subjected to PCR by targeting the specific genes for identification of different ARGs, the detailed sequences and target genes were described in Table 2. The amplification was performed as described above followed by 1.5% agarose gel electrophoresis.

2.5. Gene sequencing and alignments of fragments

All the identified and purified genes were dispatched for sequencing on the basis of Sanger di-deoxy method (Macrogen-Korea). The nucleotide sequences were compared with existing NCBI GenBank nucleotide databases and the phylogenetic position of the genes was constructed

Table 2. List of primers for *E. coli* confirmation and determination of antimicrobial resistance.

No.	Description	Target Gene	Sequence 5`-3`	Annealing Temperature	Product size	References
1	*E. coli*	*uid*	ATCACCGTGGTGACGCATGTCGC CACCACGATGCCATGTTCATC TGC	55	486bp	(Jamil et al., 2007)
2	Gentamicin	*aac(3)-IV*	CTTCAGGATGGCAAGTTGGT TCATCTCGTTCTCCGCTCAT	55	286bp	Momtaz et al. (2012)
3	Streptomycin	*aadA1*	TATCCAGCTAAGCGCGAACT ATTTGCCGACTACCTTGGTC	55	447bp	Momtaz et al. (2012)
4	Colistin	*mcr*-1	ATGATGCAGCATACTTCTGTGTGGT TCAGCGGATGAATGCGGTGCAATC	58	1626bp	Zhang et al. (2018)
5	Quinolones	*qnrA*	GGGTATGGATATTATTGATAAAG CTAATCCGGCAGCACTATTTA	52	860bp	Momtaz et al. (2012)
6	Tetracycline	*tet*-A	GTGAAACCCAACATACCCC GAAGGCAAGCAGGATGTAG	51	888bp	Zhang et al. (2018)
7	Ampicillin	bla-$_{TEM}$	ATGAGTATTCAACATTTCCG TTAATCAGTGAGGCACCTAT	58	867bp	Cullik et al. (2010)
8	Ceftrixone	bla-$_{CTX-M}$	SCSATGTGCAGYACCAGT ACCAGAAYVAGCGGBGC	52	585bp	Tofteland et al. (2007)

between the isolates and with existing databases using bioinformatics tools i.e. Logiciels BioEdit and MEGA-X (Vuthy et al., 2017; Kumar et al., 2018)

3. Results

A total of 181 samples produced pink colonies on MacConkey agar which were transferred to EMB agar for further purification. The 173 isolates produced typical metallic sheen color on EMB agar. A total of 153 were confirmed on the basis of API-20 E profiles, hence, these 153 isolates were finally included in the current study. All of the 153 isolates were positive for *uid*- gene which resulted in 485 bp DNA amplification. Phenotypically, antibiotic resistant *E. coli* (Isolated) against gentamicin (CN-10µg), streptomycin (STR-10µg), tetracycline (TET-30µg), quinolones (CIP-5µg), colistin (ST-10µg), amoxicillin (AM-30µg), cefotaxime (CTX-30µg), ceftazidime (CAZ-30µg), were found 83/153 (54%), 96/153(62%), 119/153 (77%), 38/153 (24%), 76/153 (49%), 100/153 (65%), 81/153 (52%) & 83/153 (54%) respectively. No Zone of Inhibition were found against Ceftriaxone (CRO-30µg) & imipenem (IPM-20µg) which were described in Table 3.

The sequence analysis showed that all of the isolated *E. coli* belonged to single clade as shown in Figure 1A.

The phenotypic resistance patterns revealed the existence of resistance among *E. coli* isolates against different antimicrobials. A total 115 isolates also showed resistance to more than two antimicrobials and termed as existence of co-resistance in single isolate. This co-resistance was also confirmed on the basis of PCR as these 33 isolates contained more than two antimicrobial resistant genes. The phenotypic and genotypic resistance is described in Table 3. All of *E. coli* isolates were found sensitive to ceftriaxone (CTX-30) and imipenem (IMP-10).

The sequence analysis of each of the ARGs was compared with each other and with existing data bases. The resulting phylogenetic position of each gene was shown in Figure 1B to 1D. on the basis of sequence analysis, antimicrobial resistant genes among *E. coli* from these farms were found 99% similarity. No distinct results were found among this isolated *E. coli.*

Genotypically, Gentamicin (*aac(3),* Streptomycin (*aadA1),*Tetracycline (*tet*-A), Quinolones (*qnrA),* Colistin (*mcr*-1), Ampicillin $_{(bla\text{-TEM})}$ & ESBLs gene $_{bla\text{-CTX-M}}$ resistant genes among isolated *E. coli* were found 72/83 (86%), 85/96 (86%), 103/119 (86%), 33/38 (86%), 67/76 (88%), 83/100 (83%), 57/81 (70%) respectively which were described in Table 3. The complete framework was showed in Figure 2. Statistical graph was presented for each antimicrobial resistant genes (AGRs) against different antimicrobial agents which showed results in percentage (%) genotypically in Figure 3.

4. Discussion

In general, the antimicrobial resistance has increased globally and high prevalence of resistant *E. coli* strains is emerged among different food producing animals/ birds that could be potential threat for public health. The un-due usage of antimicrobials is also rendering meat/ eggs for human consumption as well as transfer the resistance to humans (Dube and Mbanga, 2018). Principles of Hazard Analysis and Critical Control Point (HACCP) make sure about consumption of chicken meat under the strict rule of surveillance and adherence to avoid the antimicrobial resistant contaminated strains through food chain (Mensah et al., 2022). Raw chicken meat is ideal substrate for contamination of infected strain due to poor hygienic condition, lack of knowledge about antimicrobial destruction while consuming the meat (Odwar et al., 2014)

Therefore, in this cross sectional study, we investigated the phenotypic and genotypic basis of antimicrobial resistance among *E. coli* isolates from meat producing commercial chickens from different commercial farms located in Faisalabad-Pakistan. Initially, we found a total of 153 *E. coli* isolates which were confirmed on the basis of *uid*- gene amplification, as described in one of the previous

Table 3. *E. coli* isolates resistant to different antibiotics along with positive antimicrobial resistance genes.

Sr. No	Antimicrobials (Conc.)	Phenotypic Resistance *E. coli* (%)	Genotypic Resistance
1	Gentamicin (CN-10µg)	83/153 (54%)	72/83 (85%)
2	Streptomycin (STR-10µg)	96/153 (62%)	85/96 (86%)
3	Tetracycline (TET-30µg)	119/153 (77%)	103/119 (86%)
4	Quinolones (CIP-5µg)	38/153 (24%)	33/38 (86%)
5	Colistin (ST-10µg)	76/153 (49%)	67/76 (88%)
6	Amoxicillin (AM-30µg) ($bla\text{-}_{TEM}$)	100/153 (65%)	83/100 (83%)
7	Ceftriaxone (CRO-30µg)	No Zone of Inhibition	-
8	Cefotaxime (CTX-30µg) ($bla\text{-}_{CTX\text{-}M}$)	81/153 (52%)	57/81 (70%)
9	Ceftazidime (CAZ-30µg)	83/153 (54%)	57/81 (70%)
10	Imipenem (IPM-20µg)	No Zone of Inhibition	-

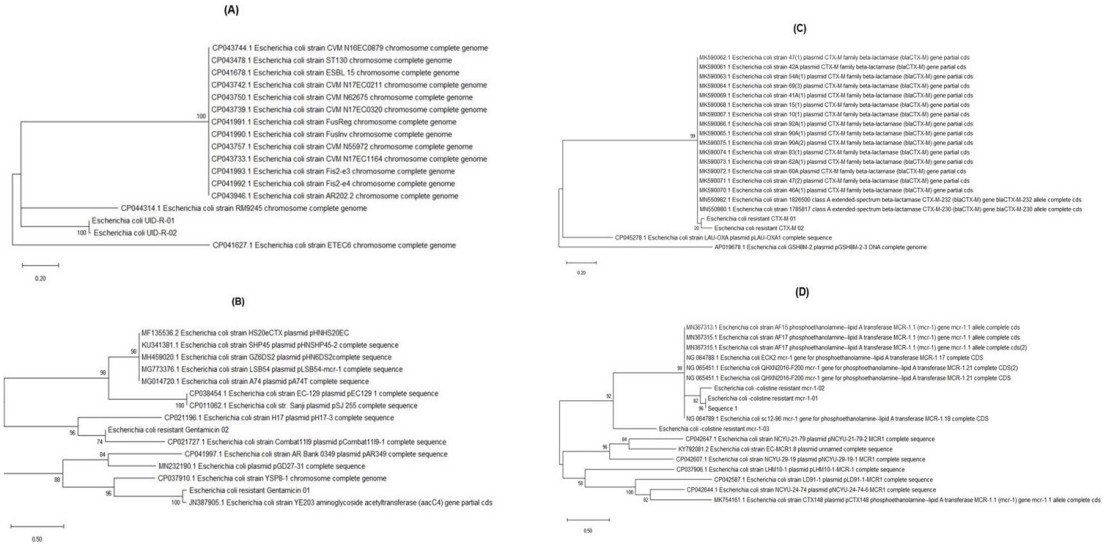

Figure 1. (A) Phylogenetic position of *E. coli* in comparison to selected sequences (on the basis of top similarlty) from NCBI data base (Mohsin et al., 2017). In currently study, our isolated *E. coli* were found in single clade decadency which were related to Fore-*E. coli* of Multi-clade decadency. In fourth clade, both *E. coli* were found 99% similarity almost with each other; (**B)** Similarity index of *aac(3)*-IV gene (gentamycin resistance) within the isoaltes and with existing sequences from NCBI GenBank database (Ahmed et al., 2004). *Escherichia coli* resistant Gentamicin (01) & (02) were found to different clade decadency that related to Multi clade decadency; (**C)** Similarity index of ESBLs (bla-$_{CTX-M}$) gene within the isoaltes and with existing sequences from NCBI GenBank database. Sequences CTX-M (01) & (02) resistant genes of isolated *E. coli* were found similar to Each other almost 99% in analysis which were linked each other and related to different reported sequence of CTX-M of *E. coli*; (**D):** Similarity index of *mcr*-1 gene (colistin resistance) within the isoaltes and with existing sequences from NCBI GenBank database. Three sequences of *mcr*-1 (01), (02) and (sequence -1) were found similar to each other about 99% and also seen to multi clade decadency which show that all were similar up to 95% which showed isolated strains were sister's strains in first cluster.

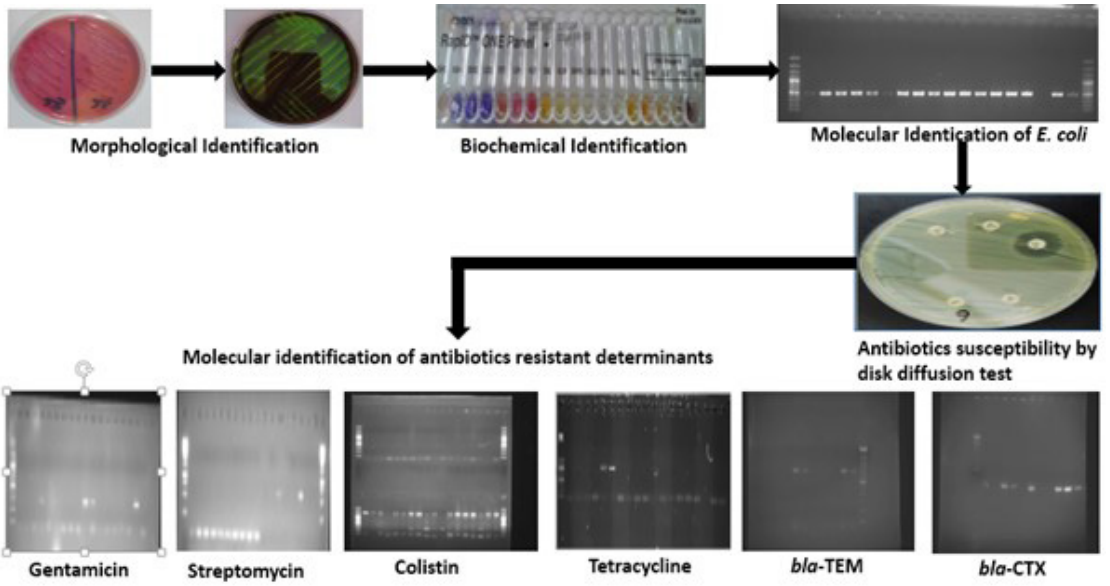

Figure 2. Frame Work of Current Study (January-2018 to August-2019) in which from left upper start of morphological picture of *E. coli* colony on selected agar, then API-20 kit were used to perform different biochemical tests to confirm *E. coli*. Then molecular confirmation of *E. coli* using *uid* gene, following performed Antibiotic sensitivity test against different antibiotics phenotypically. Last, genotypically performed PCR to confirm resistant genes mention in figure.

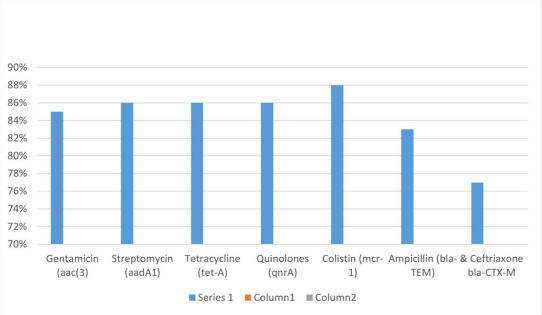

Figure 3. Statistical view of genotype AGRs among isolated *E. coli* which were found resistance to antibiotics phenotypically.

studies (Jamil et al., 2007). The sequence analysis of the isolates showed that isolated *E. coli* belonged to single clade with existing nucleotide database (Younis et al., 2017; Jamil et al., 2007).

In the current study, phenotypic resistance was observed against gentamicin (54%), streptomycin (62%), tetracycline (77%), quinolones (24%), colistin (49%) and beta lactam drugs (70%). Recent studies also showed almost similar findings and described that resistance to tetracycline (70%), gentamicin (32%) and beta lactam drugs (40%) (Dikid et al., 2013; Hussain et al., 2017). One of previous studies showed that the isolated *E. coli* strains were highly sensitive to quinolones (Azad et al., 2019). However, all of the 153 *E. coli* isolates were found positive for at least one or more than one antimicrobial resistance genes (ARGs). Therefore, the findings of the current study were quite alarming that the co-resistance is increasing among food producing animals. The detection of ESBL genes (bla-$_{TEM}$ 83% & bla-$_{CTX\text{-}M(}$ 68-77%) among isolated *E. coli* is an emerging global issue because of extensive usage of β-lactam antimicrobials in veterinary medicine as describe (Beninati et al., 2015)

Further, in the current study, the distribution of antibiotic resistance genes *E. coli* isolates were characterized i.e. prevalence of tetracycline resistance gene *tet*-A was found (86%), similar results were reported recently in Pakistan (Amir et al., 2019). However, that study only described the *tet*-A gene and no co-resistance was studied. This is also interesting that the prevalence and diversity of ARGs from *E. coli* isolates of fecal origin of broilers was significantly higher as compared to rural or backyard poultry, because of the fact that rural/ backyard chickens usually provided with the organic feed with low usage of the antimicrobials (Guo et al., 2018). This is also observed that commercial broilers have significantly diverse *E. coli* harboring ARGs as compared to the control birds (Waseem et al., 2019).

The collective genotypic findings of current study were significantly different for different ARGs i.e. 85% gentamicin resistance (*aac(3)-IV*), 86% streptomycin (*aad*A1), 86% tetracycline (*tet*-A), 88% colistin (*mcr*-1), 83% (bla-$_{TEM}$) and 68-77% (bla-$_{CTX}$). All the 153 *E. coli* isolates were resistant against more than two isolates, phenotypically as well as genotypically. All of the current findings were in line with one of the recent studies described in Bangladesh that showed isolation of *E. coli* from broilers from different commercial farms. These isolates were reported to contain antibiotics resistance genes including streptomycin (88%), tetracycline (95%), trimethoprim (65%), erythromycin (84%) and ampicillin (91%) (Azad et al., 2019). Similarly, the ARGs were also reported from Egypt, Nigeria and columbia. The interesting fact is that these all countries are included in developing regions of the world (Adelowo et al., 2014; Dabo et al., 2019; Vuthy et al., 2017).

In a similar study, about 50% isolated *E. coli* harbored the antimicrobial resistance genes against more than one antimicrobials from the samples collected from chickens and turkey birds (Davis et al., 2018). In another study related to detection of *mcr*-1 genes among *E. coli* from poultry and bovine. 08% colistine resistance genes were reported from Portugal while detection of *mcr*-2 was not reported. The detection of *mcr*-1 gene (88%) in present study indicated the risk of spread of ARGs to public health, further, it was noted that *mcr*-1 was clustered in two different clades. Recently, this sort of resistance is described as critical because beta lactam drugs and colistin are last choice for the treatment of different infectious in human and veterinary (Clemente et al., 2019; Momtaz et al., 2012).

Variabilities among different strains of antimicrobial resistance genes among isolated *E. coli* present genotypic difference from phenotype due to lack of diagnostic, therapeutic methods. Regional microbial characteristic also play important rule during genotypic investigations of phenotypic study. In current study, difference among genotypic antimicrobial resistance genes found from phenotypic antimicrobial resistance genes among isolated *E. coli* according to study of (Davari Abad et al., 2019).

5. Conclusion

The food producing animals could be responsible for the transmission of different antimicrobial resistance genes to human, environment and other animals. This spread and transmission is also triggered by extensive usage of antimicrobials among food producing animals, hence a critical evaluation of existing antimicrobial policies has to upgraded and reviewed.

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