



RESEARCH ARTICLE

Frequency of Extended Spectrum Beta Lactamase Producing *Escherichia coli* in Fresh and Frozen Meat

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ABSTRACT

A cross sectional study was performed to determine the frequency of *Escherichia coli* in fresh and frozen meat samples followed by antimicrobial resistance profiling and to detect different extended spectrum beta lactamases (ESBLs) genes. A total of (n=100) samples of fresh and frozen meat (n= 50 each) were collected from different butcher shops and super markets. Equal numbers of specimens were collected from chicken and mutton. Samples were processed for isolation and identification of *E. coli* by standard microbiological, biochemical and molecular characterization. The resistance pattern was detected by Kirby-Bauer disk diffusion method while presence of ESBLs was checked by double disk synergy test and PCR. The results of present study showed that among 100 meat samples, potentially pathogenic *E. coli* was isolated from 36 samples with greater contamination 20/50 (40%) in chicken samples in comparison to mutton 16/50 (32%). Similarly, the frequency of *E. coli* was more pronounced in fresh meat 30/50 (60%) rather than frozen 4/50 (8%). The highest resistance pattern (100%) was observed against ampicillin, ciprofloxacin, vancomycin and tetracycline followed by cefotaxime (91.6%) and (n=27) isolates were found multi drug resistant (MDR). The double disk synergy test found 17 (47.22%) ESBL producing isolates while *bla* CTX-M gene was identified in 5 (29.41%) isolates followed by *bla* OXA-48 in 4 (23.52%) samples and *bla* TEM gene in 1 (5.88%). This study revealed that vigilant control procedures should be implemented all over the food chain and effective surveillance should also be performed at national level to minimize the spread of MDR and ESBL producing *Escherichia coli* from raw meat.

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INTRODUCTION

Escherichia coli is considered top ranked foodborne pathogen within *Enterobacteriaceae* family. Most of *E. coli* strains are non-pathogenic but few are responsible for gastrointestinal issues including diarrhea in infants, children and adults, urinary tract infections and meningitis (Gallardo *et al.*, 2017). Poultry is recognized as main source of *E. coli* dissemination among environment, human community and animals. The pathogenic strains of *E. coli* in poultry often results in difficult to treat infections posing a direct threat to poultry industry and human health. Extraintestinal pathogenic *E. coli* (ExPEC)

causes severe losses in poultry in form of mortality and morbidity due to colibacillosis and also recognized as avian pathogenic *Escherichia coli* (APEC) (Bélangier *et al.*, 2011). These ExPEC can also cause variety of human extraintestinal diseases like neonatal meningitis, urinary tract infections and sepsis (Ranjan *et al.*, 2017). Despite of these ExPEC, poultry gut also accommodates various pathogenic intestinal *E. coli* (Lutful Kabir, 2010). Enterohemorrhagic *E. coli* (EHEC) is also a common intestinal reservoir of cattle, sheep, goat and chicken. EHEC is transmitted to human community primarily via consumption of contaminated raw milk, meat and undercooked meat products (Sethulekshmi *et al.*, 2016).

Meat and meat products are among the most important edible commodities of animal origin like cattle, sheep and poultry. Contaminated meat is a vital source of food borne illness due to the fact that it has favorable conditions and nutrients for the efficient growth of pathogenic microorganisms such as proteins, fermentable carbohydrates, minerals and growth factors (Datta *et al.*, 2012). Pathogenic strains of *Escherichia coli* are cause of foodborne illness in humans when ingested. Contamination of meat during slaughtering process can occur as a result of direct or indirect contact with feces, contaminated tools, skin, equipments, personnel and their clothings (Adzitey, 2015). The irrational and excessive usage of antibiotics for the treatment of food animals is the reason behind development of multidrug resistance in microorganisms creating a serious public health threat. This resistance is shifted to humans by consumption of meat and its products (Mouiche *et al.*, 2019).

There are various strategies which bacteria can adopt to attain drug resistance including enzymatic degradation of drugs, active efflux and alteration in the target site of drugs. The degradation by beta lactamase enzymes is the key mechanism for acquired resistance against beta lactam antibiotics. The increasing trend and spread of extended spectrum beta lactamase (ESBL) producing bacteria are posing a serious threat because they can degenerate clinically important 3rd as well as 4th generation cephalosporins but unable to inactivate carbapenems (Rahman *et al.*, 2018).

The ESBLs are mostly produced among *Enterobacteriaceae*, predominantly in *Escherichia coli*. Moreover, *E. coli* encoding ESBLs are resistant to more than two classes of antimicrobials and referred as multidrug resistant (MDR), presenting a serious challenge in healthcare settings as very few treatment options are left for treatment (Meletis, 2016). Many studies on ESBL producing *E. coli* from clinical settings have been conducted in Pakistan during past few years but there are very few reports regarding their prevalence in meat samples from Pakistan. Due to these facts, the current research was planned to find out incidence of ESBL producing *E. coli* in fresh and frozen meat samples from Pakistan.

MATERIALS AND METHODS

Samples collection: Total (n=100) fresh and frozen meat samples (poultry and mutton) were collected from various butcher shops and super markets. Fifty samples were collected from each category and equal numbers of samples were collected from chicken and mutton. The samples were carefully packaged into polyethylene bags, labeled properly then transferred in the icebox to the laboratory for further processing.

Isolation and identification of *Escherichia coli*: A 25 g of each meat specimen was mixed aseptically in the 225 ml of sterilize peptone water and subjected to overnight enrichment followed by inoculation on MacConkey's agar or Eosin Methylene Blue (EMB) agar (Oxoid, UK) and incubated for 24-48 hours at 37°C aerobically. Pinkish colonies on MacConkey agar or distinct metallic green colonies on EMB agar indicated positive growth for *E. coli* which was further confirmed by Gram's staining and

biochemical tests (Nawaz *et al.*, 2019). Preservation of isolates was done in brain heart infusion with 30% glycerol and stored at -80°C (Rahman *et al.*, 2018).

Molecular confirmation of *Escherichia coli*: Molecular confirmation of isolates was done by polymerase chain reaction (PCR) targeting the *uidA* (b-glucuronidase) gene. The DNA was separated using commercial Kit (Thermo Scientific, USA), PCR was performed in thermal cyclers (Bio Rad, USA) and products were visualized using 1% gel by electrophoresis as described by (Maynou *et al.*, 2017).

Antimicrobial susceptibility testing: After the confirmation of *E. coli*, antibiotic susceptibility profile of all isolates was investigated against commonly used antimicrobials including ampicillin, amoxicillin/clavulanic acid, Amikacin, Cefotaxime, Ciprofloxacin, Ceftriaxone, Ceftazidime, Gantamicin, Tetracycline, Vancomycine, Imipenem, Meropenem and Dorepenem. The result analysis was done according to the guidelines of Clinical Laboratory Standards Institute as described by (Nawaz *et al.*, 2019).

Phenotypic detection of ESBL production: Modified double disc synergy test (MDDST) was used for detection of ESBL producing *E. coli* using cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), ceftriaxone (CRO, 30 µg) and amoxicillin-clavulanate (20/10 µg) discs. The strains ATCC 35218 and ATCC 25922 were used as negative and positive controls of *E. coli*, respectively. Amoxicillin-clavulanate (20/10 µg) disc was placed to center of the agar plate and other antibiotics discs were placed at 15 to 20mm distance. The ESBL production considered as positive as any increase in zone in the direction of disc of amoxicillin-clavulanate (Saleem *et al.*, 2017).

Identification of ESBL encoding genes: The isolates of *Escherichia coli* showing positive phenotypic ESBL detection were processed for the identification of extended spectrum beta lactamase encoding genes using specific primers by PCR (Table 1). The targeted ESBL genes were *bla CTX-M*, *bla TEM* and *bla OXA₄₈* (Hosseinzadeh *et al.*, 2018; Nawaz *et al.*, 2019).

Statistical analysis: The variables were presented in form of percentages (%). The value (P<0.05) was considered as significant. All the statistical analyses were performed by Stata 11 software (Stata Corp, USA).

RESULTS

The results of present study showed that out of 100 meat samples, (n=36) were identified as *Escherichia coli* on the basis of standard microbiological and molecular methods (Fig. 1). The prevalence of *E. coli* was recorded higher 20/50 (40%) in chicken meat as compared to 16/50 (32%) in mutton. Moreover, the detection of *E. coli* was found significantly more pronounced in fresh meat samples 30/50 (60%) rather than frozen meat 4/50 (8%). Greater trend of *E. coli* was found in fresh chicken meat 44.40% than frozen chicken 11.10% (P=0.021). Similarly, significant variation was found between the fresh mutton and frozen mutton (P=0.007) as described in (Table 2).

Table 1: Name and sequences of primers used for PCR amplification

Primers Name	Sequence	Product size	References
uidA-F	ATCACCGTGGTGACGCATGTCCG	486bp	(Maynou <i>et al.</i> , 2017)
uidA-R	CACCACGATGCCATGTTTCATCTGC		
bla OXA ₄₈ F	GCGTGGTTAAGGATGAACAC	438bp	(Hosseinzadeh <i>et al.</i> , 2018)
bla OXA ₄₈ R	CATCAAGTTCAACCCAACCG		
bla TEM F	ATGAGTATTCAACATTTCCG	862bp	(Egual <i>et al.</i> , 2017)
bla TEM R	GACAGTTACCAATGCTTAATCA		
bla CTX-M F	CGTCACGCTGTTGTTAGGAA	780bp	(Bello-López <i>et al.</i> , 2017)
bla CTX-M R	ACGGCTTCTGCCTTAGTGT		

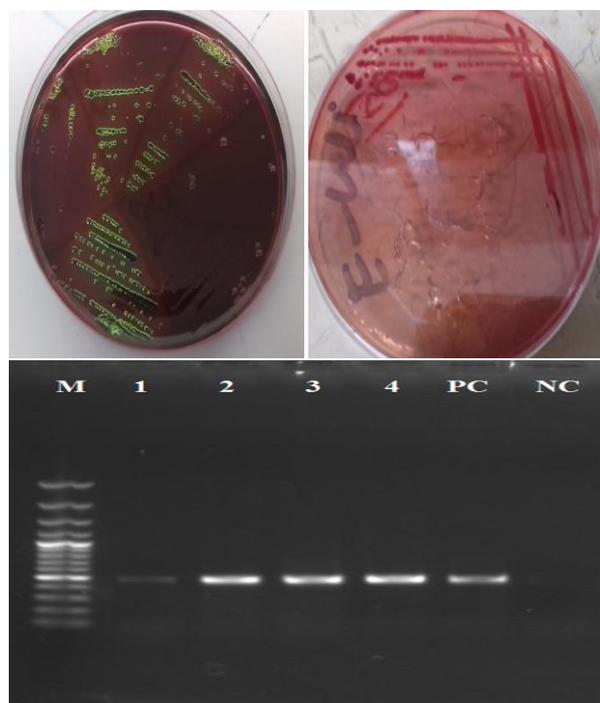


Fig. 1: Confirmation of *Escherichia coli* isolates on the basis of cultural and molecular methods. A: Brilliant green colonies with metallic sheen on Eosin Methylene Blue (EMB) agar. B: Dark pink colonies on MacConkey agar. C: Gel showing 486bp band for *uidA* gene. Lane M: 100bp Ladder, Lane 1-4: Positive samples, Lane PC: Positive control, Lane NC: Negative control.

Table 2: Frequency of *Escherichia coli* from fresh and frozen meat samples

Samples Type	No. of samples collected	Positive <i>E. coli</i> samples	Positive <i>E. coli</i> samples percentage	P Value
Fresh chicken meat	25	16	44.40%	
Frozen chicken meat	25	04	11.10%	0.021*
Fresh mutton	25	14	38.90%	
Frozen mutton	25	02	5.50%	0.007*
Total	100	36	36.00%	

*Statistically significant at (P<0.05).

Table 3: Percentage resistance pattern of *Escherichia coli* against different antibiotics

Antibiotics Used	Resistance (%age)	Intermediate resistant	Sensitive (%age)
Amoxicillin (AMP)	24 (66.67%)	10 (27.78%)	2 (5.55%)
Vancomycin (VA)	36 (100%)	0 (0%)	0 (0%)
Gentamicin (GN)	24 (66.67%)	3 (8.33%)	9 (25%)
Ampicillin/Clavulanate (AMC)	36 (100%)	0 (0%)	0 (0%)
Cefotaxime (CTX)	33 (91.67%)	2 (5.5%)	1 (2.78%)
Tetracycline (TET)	36 (100%)	0 (0%)	0 (0%)
Ceftriaxone (CRO)	22 (61.12%)	12 (33.33%)	2 (5.55%)
Ciprofloxacin (CIP)	36 (100%)	0 (0%)	0 (0%)
Ceftazidime (CAZ)	24 (66.67%)	8 (22.22%)	4 (11.11%)
Amikacin (AK)	23 (63.89%)	1 (2.78%)	12 (33.33%)
Imipenem (IMP)	5 (13.89%)	7 (19.44%)	24 (66.67%)
Doripenem (DOR)	14 (38.89%)	9 (25%)	13 (36.11%)
Meropenem (MEM)	9 (25%)	12 (33.33%)	15 (41.66%)

Antimicrobial susceptibility profile of *Escherichia coli*:

Different isolates of *E. coli* showed variable pattern of antibiotic susceptibility against various antimicrobial agents. The resistance to not less than 3 different class of antibiotics was observed in (n=27) 75% of the *E. coli* isolates and these isolates were therefore referred as MDR. All the *E. coli* isolates (100%) were found resistant to ampicillin, ciprofloxacin, vancomycin and tetracycline while (91.6%) showed resistance against cefotaxime. Furthermore, 66.66% isolates were detected resistant to three drugs, ceftazidime, gentamicin and amoxicillin/clavulanic acid. The highest sensitivity was observed to imipenem (66.66%) as shown in (Table 3).

Frequency of ESBL producing *Escherichia coli*:

The double disk synergy test showed that phenotypically 17 out of 36 (47.22%) isolates of *E. coli* were ESBL producers. Among these ESBL positive (n=17) isolates, *bla* CTX-M gene was identified from 5 (29.41%) isolates followed by *bla* OXA₄₈ from 4 (23.52%) and *bla* TEM gene was detected from 1 (5.88%) isolate.

DISCUSSION

Over the last decade, ESBL producing *E. coli* has been frequently contaminating the meat of food animals and posing a global threat to public health and food security. Mostly these isolates are MDR in nature, which increase the challenge for their treatment and eradication. The multi drug resistance is getting more serious in Pakistan where antibiotic usage is not strictly regulated (Rahman *et al.*, 2018).

The present study was designed to isolate and identify *E. coli* from fresh and frozen meat samples followed by estimation of their antimicrobial susceptibility and presence of ESBL genes. In this study, the prevalence of *E. coli* was found 36% (36/100) and similar findings with 35.40 % prevalence were reported in Nepal (Saud *et al.*, 2019) and 20.40% in chicken meat in Iran (Safarpordkhordi *et al.*, 2014). Conversely, a much higher prevalence (75%) of *E. coli* was reported in 2015 (El-Tawab *et al.*, 2015) and 49% in raw chicken meat in Bangladesh (Rahman *et al.*, 2017). The variation in the results recorded by different scientist was due to contamination of collection sites and type of meat samples. It was found that *E. coli* was more prevalent in fresh chicken and mutton meat samples than in frozen meat. The poor hygienic handling and processing is mainly responsible for the *E. coli* contamination in meat. Same type of findings was also recorded in Saudi Arab (Hemeg, 2018) as the growth of *Escherichia coli* cells is impaired at temperatures below 21°C and stops at 7.5°C.

The antimicrobial susceptibility profiling was done for (n=36) *E. coli* isolates against 14 different antibiotics. The results showed highest resistance pattern (100%) against ampicillin, ciprofloxacin, vancomycin and tetracycline followed by cefotaxime (91.6%). The results finding of (Adzitey, 2015) and (Dehkordi *et al.*, 2014) showed higher resistance to ciprofloxacin and ampicillin, while the results of (Nahar *et al.*, 2018) showed 100% resistance to ampicillin and cefotaxime. *E. coli* isolates 75% (n=27) were categorized as multi drug resistant *Escherichia coli* (MDR-EC). The increased trend of multi drug resistant *E. coli* was also reported by (Ahmed *et al.*, 2009) in Japan, (Adzitey, 2015) in Ghana, (Altalhi *et al.*, 2010) in Saudi Arabia, (Dehkordi *et al.*, 2014) in Iran and (Abdel-Rahman *et al.*, 2015) in Egypt. This difference in results of antibiotic susceptibility profile was due to excessive use of antibiotics in commercial poultry and veterinary practice for both prevention and treatment. Among the carbapenems, highest resistance was found against doripenem (38.89%) and meropenem (25%) which is comparable to the findings of (Batool *et al.*, 2016) in Pakistan and (Ahmed *et al.*, 2009) in Japan while these results are in contrast with the findings of (Zhao *et al.*, 2018).

The results of present study showed 47.22% (n=17) isolates were ESBL producers phenotypically using double disk synergy test which is very close to the findings of (Hussain *et al.*, 2017) with 46% from India and (Rahman *et al.*, 2018) with 47.6% in Pakistan. A much-elevated level (70%) of ESBL producing *E. coli* was detected by (Saleem *et al.*, 2017) in Pakistan and (Nahar *et al.*, 2018) from Japan. This elevated level of ESBL producing *E. coli* in the meat of food animals is posing a potential threat to humans. It was also observed that among the phenotypically ESBL positive (n=17) *E. coli* isolates; *bla* CTX-M gene was detected in 5 (29.41%) isolates which is also supported by (Chishimba *et al.*, 2016) in Zambia with 13% presence of *bla* CTX-M, 58% in South Africa by (Montso *et al.*, 2019) and 75% in Ghana by (Eibach *et al.*, 2018). Furthermore, *bla* OXA-48 was detected in 4 (23.52%) and *bla* TEM gene was found in only 1 (5.88%) isolate. The results of (Falgenhauer *et al.*, 2019) also supported the fact that *bla* CTX-M is the most prominent type of ESBL present in meat samples followed by *bla* TEM.

Conclusions: The results of current study concluded that poultry and mutton meat samples were contaminated with MDR-*Escherichia coli* and this contamination was more pronounced in fresh meat samples. Chicken meat is highly contaminated with *E. coli* than mutton and increased occurrence of ESBL-EC in meat robustly suggest for involvement of efforts to drop this emerging challenge of drug resistance.

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Authors contribution: ZN and ABS conceived and designed the study; MM and MUQ performed the experiments; BA and RA compiled the data and results;

MAZ, SA and AR wrote and critically reviewed the manuscript.

REFERENCES

- Abdel-Rahman SH, Khalifa SM, El Galil KHA, *et al.*, 2015. Prevalence of toxins and antimicrobial resistance among *E. coli* isolated from meat. *Adv Microbiol* 5:737-8.
- Adzitey F, 2015. Antibiotic resistance of *Escherichia coli* isolated from beef and its related samples in Techiman municipality of Ghana. *Asian J Animal Sci* 9:233-40.
- Ahmed AM, Shimabukuro H and Shimamoto T, 2009. Isolation and Molecular Characterization of Multidrug-Resistant Strains of *Escherichia coli* and *Salmonella* from Retail Chicken Meat in Japan. *J Food Sci* 74:405-10.
- Altalhi AD, Gherbawy YA and Hassan SA, 2010. Antibiotic resistance in *Escherichia coli* isolated from retail raw chicken meat in Taif, Saudi Arabia. *Foodborne Pathog Dis* 7:281-5.
- Batool A, Baig H and Qamar MU, 2016. Extended spectrum--lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* causing urinary tract infection. *Afr J Microbiol Res* 10:1775-8.
- Bélanger L, Garenoux A, Harel J, *et al.*, 2011. *Escherichia coli* from animal reservoirs as a potential source of human extraintestinal pathogenic *E. coli*. *FEMS Immunol Med Microbiol* 62:1-10.
- Bello-López JM and Rojo-Medina J, 2017. Detection of antibiotic resistance genes β -lactamases in bacterial strains isolated from Umbilical Cord Blood Units for transplant. *Rev Méd Hosp Gen México* 80:31-6.
- Chishimba K, Hang'Ombe BM, Muzandu K, *et al.*, 2016. Detection of extended-spectrum beta-lactamase-producing *Escherichia coli* in market-ready chickens in Zambia. *Int J Microbiol* 16:1-5.
- Datta S, Akter A, Shah IG, *et al.*, 2012. Microbiological quality assessment of raw meat and meat products and antibiotic susceptibility of isolated *Staphylococcus aureus*. *Agric Food Anal Bacteriol* 2:187-94.
- Dehkordi FS, Yazdani F and Mozafari J, 2014. Virulence factors, serogroups and antimicrobial resistance properties of *Escherichia coli* strains in fermented dairy products. *BMC Res Notes* 7:217.
- Egualé T, Birungi J, Asrat D, *et al.*, 2017. Genetic markers associated with resistance to beta-lactam and quinolone antimicrobials in non-typhoidal *Salmonella* isolates from humans and animals in central Ethiopia. *Antimicrob Resist Infect Control* 6:13.
- Eibach D, Dekker D, Boahen K, *et al.*, 2018. Extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in local and imported poultry meat in Ghana. *Vet Microbiol* 217:7-12.
- El Tawab AAA, El-Hofy FI, Nada SM, *et al.*, 2015. Detection of virulence genes of enterohaemorrhagic *E. coli* isolated from some meat products by polymerase chain reaction. *Benha Vet Med J* 29:45-52.
- Falgenhauer J, Imirzalioglu C, Falgenhauer L, *et al.*, 2019. Whole-Genome Sequences of clinical *Enterobacter bugandensis* isolates from Germany. *Microbiol Resour Announc* 8:e00465.
- Gallardo P, Izquierdo M, Vidal RM, *et al.*, 2017. Distinctive gut microbiota is associated with diarrheagenic *Escherichia coli* infections in children. *Front Cell Infect Microbiol* 7:424.
- Hemeg HA, 2018. Molecular characterization of antibiotic resistant *Escherichia coli* isolates recovered from food samples and outpatient Clinics, KSA. *Saudi J Biol Sci* 25:928-31.
- Hosseinzadeh Z, Saraie HSE, Sarvari J, *et al.*, 2018. Emerge of *bla*_{NDM-1} and *bla*_{OXA-48}-like harboring carbapenem-resistant *Klebsiella pneumoniae* isolates from hospitalized patients in southwestern Iran. *J Chin Med Assoc* 81:536-40.
- Hussain A, Shaik S, Ranjan A, *et al.*, 2017. Risk of transmission of antimicrobial resistant *Escherichia coli* from commercial broiler and free-range retail chicken in India. *Front Microbiol* 8:2120.
- Lutful Kabir SM, 2010. Avian colibacillosis and salmonellosis: a closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. *Int J Environ Res Public Health* 7:89-114.
- Maynou G, Bach A and Terré M, 2017. Feeding of waste milk to Holstein calves affects antimicrobial resistance of *Escherichia coli* and *Pasteurella multocida* isolated from fecal and nasal swabs. *J Dairy Sci* 100:2682-94.
- Meletis G, 2016. Carbapenem resistance: overview of the problem and future perspectives. *Therap Adv Infect Dis* 3:15-21.
- Montso KP, Dlamini SB, Kumar A, *et al.*, 2019. Antimicrobial resistance factors of Extended-Spectrum Beta-Lactamases producing *Escherichia coli* and *Klebsiella pneumoniae* isolated from cattle farms

- and raw beef in North-West province, South Africa. *BioMed Res Int* 2019:4318306.
- Mouiche MMM, Moffo F, Akoachere JTK, et al., 2019. Antimicrobial resistance from a one health perspective in Cameroon: A systematic review and meta-analysis. *BMC Public Health* 19:1135.
- Nahar A, Awasthi SP, Hatanaka N, et al., 2018. Prevalence and characteristics of extended-spectrum β -lactamase-producing *Escherichia coli* in domestic and imported chicken meats in Japan. *J Vet Med Sci* 80:510-7.
- Nawaz Z, Zahoor MK, Siddique AB, et al., 2019. Molecular identification of *bla*CTX-M and *bla*TEM genes among multidrug resistant Enteropathogenic *Escherichia coli* isolated from children. *Pak J Pharm Sci* 32:1215-8.
- Rahman MA, Rahman AKMA, Islam MA, et al., 2017. Antimicrobial resistance of *Escherichia coli* isolated from milk, beef and chicken meat in Bangladesh. *Bangl J Vet Med* 15:141-6.
- Rahman SU, Ali T, Ali I, et al., 2018. The growing genetic and functional diversity of extended spectrum Beta-Lactamases. *BioMed Res Int* pp:9519718.
- Rahman SU, Ahmad S and Khan I, 2018. Incidence of ESBL-producing-*Escherichia coli* in poultry farm environment and retail poultry meat. *Pak Vet J* 39:116-20.
- Ranjan A, Shaik S, Nandanwar N, et al., 2017. Comparative genomics of *Escherichia coli* isolated from skin and soft tissue and other extraintestinal infections. *MBio* 8:e01070-17.
- Safarpordehkordi F, Yahaghi E and Khodaverdi DE, 2014. Prevalence of antibiotic resistance in *Escherichia coli* isolated from poultry meat supply in Isfahan. *Iran J Med Microbiol* 8: 41-7.
- Saleem R, Ejaz H, Zafar A, et al., 2017. Phenotypic characterization of extended-spectrum-beta-lactamase producing *E. coli* from healthy individuals, patients, sewage sludge, cattle, chickens and raw meat. *Pak J Med Sci* 33:886-90.
- Saud B, Paudel G, Khichaju S, et al., 2019. Multidrug-Resistant bacteria from raw meat of buffalo and chicken, Nepal. *Vet Med Int* 2019:1-7.
- Sethulekshmi C, Latha C and Sunil B, 2016. Occurrence of Enterohaemorrhagic *E. coli* in raw meat samples in Kerala. *Int J Adv Res Bio Sci* 3:220-2.
- Zhao M, Huang D, Zhang X, et al., 2018. Metabolic engineering of *Escherichia coli* for producing adipic acid through the reverse adipate-degradation pathway. *Metab Eng* 47:254-62.

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