Original Article

Molecular characterizationof Beta-Lactamase Gene Variants in *Escherichia coli* in the northern Indian region

Hamid Ahmad Shah, Dalip K. Kakru, Mohd Altaf Bhat

Abstract:

Background: With the rise of bacteria that are multidrug-resistant, antimicrobial resistance (AMR) is an extensive public health problem. *Escherichia coli*, a common bacterial pathogen, has demonstrated resistance to beta-lactam antibiotics through the formation of beta-lactamase enzymes. Understanding the genetic diversity of β-lactamase genes in *E. coli* is essential for developing effective strategies to combat AMR.

Methods: In this study, we conducted a comprehensive molecular characterization of beta-lactamase gene variants in *E. coli* isolates collected from clinical in the northern Indian region. To find and categorize beta-lactamase genes, 210 *E. coli* isolates were submitted for phenotypic and genotypic investigation. Different beta-lactamase gene variations were detected using DNA sequencing and polymerase chain reaction (PCR).

Results: Our findings revealed a diverse range of beta-lactamase gene variants in the *E. coli* isolates, including SHV, TEM, and CTX-M types. Importantly, we seen a greater existence of extended-spectrum beta-lactamase (ESBL)-making the strains of E. coli, indicating the growing concern of ESBL-mediated resistance in the region.

Conclusion: This research provides valuable awareness of the molecular epidemiology of beta-lactamase gene variants in *E. coli* in the northern Indian part, highlighting the urgent need for targeted interventions to curb the spread of antimicrobial resistance. Understanding the genetic diversity and mechanisms of resistance is crucial for the development of effective antibiotic stewardship programs and the design of novel therapeutic approaches to combat AMR in this region.

JK-Practitioner 2023;28(3-4):50-56

Introduction:

The Major public health concern globally nowadays is antibiotic resistance caused by enteric bacteria.[1] Numerous resistance mechanisms were reported in Enterobacteriaceae and non-fermentative gram-negative bacilli.[2-4] The enteric bacteria are primarily causative agents of septicemia, urinary tract, wound, and respiratory infections. The usage of beta-lactam antibiotics has increased which has resulted in an increase in Enterobacteriaceae resistance because of the formation of the β-lactamases which act on the beta-lactam ring of antibiotics. ESBL are a subset of β-lactamases generated by gram-negative bacteria, primarily Enterobacteriaceae and *Pseudomonas aueruginosa*.[5] Klebsiella & *E.coli* are the most common strains that produce ESBLs.[6,7] The emergence of ESBL-producing bacteria places restrictions on the prescribing of beta-lactam antibiotics, has led to numerous disease outbreaks around the world, and poses challenging infection control measures. The emergence of beta-lactamases *E. coli* is a serious concern in hospital settings. Only a few genes, particularly *TEM-1*, *TEM-2*, and *SHV-1* were described during early phases of resistances.[8,9] More than 450 variants of /*TEM*, *SHV*, and *CTX-M* enzymes are found which are produced by ESBL harboring bacteria.[10] The goal of the current research had been to detect the ESBL isolates from various clinical isolates. There hasn't been any research done to use molecular cloning and sequencing analysis to find distinct variations of the ESBL resistance genes. The current research focused on detecting variants like*TEM-1,TEM-2, SHV-1,and CTX-M15.*

Author Affiliations
Hamid Ahmad Shah, Dalip K. Kakru, Department of Microbiology, SMSR, Sharda University, Greater Noida, Uttar Pradesh, India, **Mohd Altaf Bhat** , Department of Veterinary Microbiology & Immunology, F.V.Sc & A.H., SKUAST-K, Shuhama, Srinagar, Jammu & Kashmir, India.

Correspondence
Hamid Ahmad Shah, Department of Microbiology, SMSR, Sharda University, Greater Noida, Uttar Pradesh, India,
Email: syedhamydh@gmail.com

Indexed
EMBASE, SCOPUS, IndMED, ESBCO, Google Scholar besides other national and International Databases

Cite This Article as
Shah HA, Kakru DK, Bhat A. Molecular characterization of Beta-Lactamase Gene Variants in *Escherichia coli* in the northern Indian region. JK Pract2023; 28(3-4), 50-56

Full length article available at **jkpractitioner.com** one month after publication

Keywords
Antimicrobial resistance, DNA sequencing, *Escherichia coli*, ESBL-mediated resistance, Molecular epidemiology.

Materials and Methods

Collection of isolates and ESBL identification

From January 2021 to December 2022, the research was conducted in the Department of Veterinary Microbiology, FVSc & AH., SKUAST-K, and the Department of Microbiology, SMSR, Sharda University. A total of 210 isolates of the *E. coli* have been gathered through the various sites in the Noida, Uttar Pradesh, and Kashmir valley regions. These isolates were from people who may have had *E. coli* infections. Phenotypic identification was done by using ESBL chromogenic agar, Double disc synergy test, E-test, and Disk diffusion test. Presumptive isolates found to be positive in phenotypic tests underwent genotypic detection by multiplex PCR amplification utilizing specific primers set.

Extraction of bacterial DNA

Utilizing a DNA extraction kit that is available commercially, the DNA was isolated from the bacterial lysate (Wizard Genomic DNA purification kit, Promega). This helps us to achieve the pure DNA without any PCR inhibitors. The DNA extracted was subjected to O.D check before using them as a template in the PCR reaction. A ratio of 260/280 in the range of 1.6-1.8 was appropriate to consider.

Detection of ESBL gene variants *TEM* group, *SHV* group, and *CTX-M*15

Using the Go-green Master mix, all of the PCR experiments in this investigation were carried out in a 25µl reaction volume (Promega). DNA taken by the lysate subjected to PCR amplification for the detection of ESBL gene variants utilizing a set of primers as mentioned in Table-1 The cyclic conditions are mentioned in Table-2

. **Table-1 Details of Primer sets used in this** "**study**

S.No	PCR name	β-lactamase(s) targeted	Sequence (50 –30)	Length (bases)	Amplicon size (bp)	Primer concentration (pmol/mL)	Reference
1.	**Multiplex I** TEM	**TEM variants** including TEM-1 and TEM-2	F-CATTTCCGTGTCGCCCTTATTC	22	800	0.4	[11]
			R-CGTTCATCCATAGTTGCCTGAC	22		0.4	
2.	**Multiplex I** SHV	**SHV variants** including SHV-1	F-AGCCGCTTGAGCAAATTAAAC	21	713	0.4	[11]
			R-ATCCCGCAGATAAATCACCAC	21		0.4	
3	**Singleplex**	**CTX-M-15**	F-CACACGTGGAATTTAGGGACT	21	996	0.1	[12]
			R-GCCGTCTAAGGCGATAAACA	20		0.1"	

Table-2: Cyclic conditions for PCR amplification of ESBL gene variants

"Gene	Initial Denaturation	Denaturation	Annealing	Extension	Final Extension"
TEM Variants including TEM-1 and TEM-2	94°C for 10 min	94°C for 40s	60°C for 40s	72°C for 1 min	72°C for 7 mins
SHV variant including SHV-1	94°C for 10 min	94°C for 40s	60°C for 40s	72°C for 1 min	72°C for 10 mins
CTX-M 15	95°C for 10 min	94°C for 40s	50°C for 40s	72°C for 1 min	72°C for 10 mins

Electrophoresis and Documentation

A 500 ml Erlenmeyer flask has been utilized to heat the necessary agarose quantity (Sigma Aldrich, St. Louis, USA) with 50 ml of a 1X tris acetate EDTA (TAE) (appendix) buffer. This resulted in an agarose gel that had a weight-to-volume ratio of 1.5 percent. After the flask had been chilled to 60 degrees Celsius, ethidium bromide has been poured until the final concentration read 0.5 µg per ml. After allowing the heated agarose solution to totally set at room temperature for 30 mins, the solution was poured into a plastic holder equipped with an appropriate comb (with one mm wells). Following the removal of the comb, the gel was installed on an electrophoresis tank (manufactured by Amersham Pharmacia Biotech, UK), and the electrophoresis tank was then filled with 1X TAE buffer. On the submerged gel, individual PCR samples were placed into wells of their own. In addition, the standard molecular weight (100 bp) marker that was supplied by promega was injected into one well. A voltage ranging from 1 to 5V/cm was applied across the gel till the yellow dye of the master mix migrated to the correct distance. After removing the gel, it was recorded using ultraviolet light, and photographs were taken using the Gel Documentation System (Ultra-lumInc.Imaging System, UVP, UK).

2.5 Cloning of gene variants *TEM* group, *SHV* group, and *CTX-M*15

The representative positive samples, one from each of the SHV group, CTX-M group (CTX-M15), and 2 from the TEM group had been cloned in a pGEMT-easy cloning vector (Promega Corporation, Madison, USA) and then sent out to be sequenced so that the identity of ESBL *E. coli* could be determined. To summarise, the PCR products have been ligated into the pGEMT-easy cloning vector in accordance with the proposed procedure provided by the manufacturer. In order to convert DH5α Escherichia coli cells, a heat shock treatment was performed in a water bath while the ligation combination was present. Two types of colonies were observed on the plates white (positive) and blue (negative). 5 positive (white) colonies were selected for every transformation, placed in Luria Bertani (LB) broth (Difco), and afterward placed in a rotary shaking incubator set to 37 ° C for one night. The shaking rate of the incubator was 200 revolutions per minute (JEIO TECH, Korea).

Screening of Recombinant Colonies

Both colony PCR and restriction enzyme analysis were used to screen the recombinant colonies to determine whether or not they contained the insert in addition to the plasmid DNA. Each pellet was suspended in 500 l distilled water and heated for 10 minutes followed by rapid cooling over ice for 15 minutes. then centrifuged at 10,000rpm for 10 min the supernatant had been kept in another 1.5ml micro-centrifuge tube and has been utilized as the template for PCR. The PCR was carried out using*TEM-2*, *TEM-1*, *CTX-M 15* and *SHV-1* primers as described previously to confirm the presence of insert. The amplicons were checked by agarose gel electrophoresis.

Extraction of Recombinant plasmid and Restriction digestion

After utilizing QIAprep® Miniprep Kit (Qiagen, Hilden, Germany) to extract the plasmid DNA from chosen colonies, the concentration of the DNA was modified to be 200 ng/µl. The isolated plasmids were digested separately with restriction enzyme EcoRI for the confirmation of insert. Each of the digestion reactions consisted of ~5µg of plasmid (2 µl), 1µl of Fast Digest green buffer, 10 units of EcoRI enzyme, and remaining nuclease-free water to make up an overall volume of 10 µl. The reaction solutions has been incubated in a water bath at the temp of 37°C for the time of 20min and then 2 µl of this plasmid was loaded onto 0.8% agarose gel and subsequently visualized under a UV gel documentation system for the presence of the insert. After determining the concentration of the isolated plasmid using absorbance at λ260 (using a Biophotometer made by Eppendroff in Germany), the sample was placed in a 1.5 ml centrifuge tube and frozen before being sequenced. The plasmids were sequenced by Sanger's dideoxy chain termination method [13] in Unipath Specialty Laboratory Ltd. India. Following the completion of the sequencing, homology searches have been carried out by utilizing the BLAST aproach.(http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Occurrence of ESBL *E. coli* in clinical isolates

During the study period out of 210 clinical isolates collected only 158(75.23%) isolates were phenotypically presumptive positive ESBL *E. coli* based on the phenotypic confirmatory test (ESBL Chromogenic agar), E-test, and double disk synergy test as depicted in Fig-1a, 1b,1c. All these presumptive isolates underwent molecular detection by PCR utilizing a particular set of primers for the determination of the ESBL gene in isolatesof *E. coli*. Only 124 isolates were positive for *bla* SHV, *bla* CTX-M, and *bla* TEM as depicted in Fig-2.

Detection of ESBL gene variants *TEM* group, *SHV* group, and *CTX-M*15

All these 124 isolates that have been positive for the *bla TEM*, *bla SHV*, and *bla CTX-M* were subjected to another round of PCR to determine the type of variants using a specific set of primers. Out of 124 ESBL *E. coli* isolates, 54(43.5%) were *TEM* variants, 14(11.29%) were *SHV* variants and 46 (37.09%) were *CTX-M15* variants. The *bla*-CTX-M15 strain was the one that was found the most frequently. Isolates that carried the *bla* TEM variant gene created an amplicon with 800 bp, those that carried the *bla* CTX-M variant displayed an amplicon with 996 bp, and the existence of the *bla* SHV gene variant had been identified by the amplification of 713 bp, as shown in Figure 3a, 3b, and 3c.

Table 3. Repartition of the Detected ESBL Genes

ESBL Genes	Types of Variant Group	No. (%)
Bla TEM	*TEM* Variants including *TEM-1 and TEM-2*	54(43.5)
	CTX-M15	46(37.09)
Bla CTX-M	*SHV*Variants including *SHV-1*	14(11.29)
Bla SHV		

Cloning of gene variants

One representative amplicon of *SHV* and *CTX-M15* and two from *TEM* group were purified and cloned separately in *E. coli* DH5α cells, using cloning vector pGEM-T. The existence of altered colonies on the LB/Ampicillin agar plates served as the basis for determining whether or not the cloning attempt was successful. The colonies with and without insert appeared white and blue, respectively as depicted in Fig-4.The white colonies were further confirmed for the presence of the respective inserts by colony touch PCR depicted in Fig-5.Restriction digestion by EcoRI also confirmed the presence of insert. Four gene amplicons from the above gene variants were sequenced commercially. BLAST analysis of nucleotide sequences of the TEM group showed 100% homology to the *E. coli* class A broad-spectrum beta-lactamase TEM-1 (blaTEM) gene, SHV group showed 99.85% of homology to beta-lactamase SHV-1 precursor gene, and CTX-M15 showed 99.90% of homology to extended-spectrum class A beta-lactamase CTX-M-15 gene. The sequences of CTX-M-15, SHV-1, and blaTEM-1 from the isolates were submitted to Genbank under accession numbers **OR350836**, **OR350837,** and **OR350838** respectively.

Discussion

The first-line drugs in infections caused by Enterobacteriaceaeare β-lactam antibiotics.[14] The present study investigates the ESBL-producing Enterobacteriaceae in two tertiary care Indian hospitals. There is variation in the existence of ESBL-producing Enterobacteriaceae around the globe. In most of the countries, where the patients are treated with antibiotics, a high prevalence rate of multidrug-resistant Enterobacteriaceae has been observed.[15] However, lower rates were found in North America and Europepreviously [16,17] and high rates in Asia. [18], South America[19], and Africa.[20] The factors responsible for the spread are encouraged by indiscriminate antimicrobial use without a prescription, use of counterfeit drugs, poor hygiene, a high prevalence of infectious diseases, and lack of diagnostic tools for detecting antibiotic resistance.[21,22] In our research, the isolation of MDR ESBL strains that expressed the CTX-M-15 gene restricted the treatment options available to clinicians for their patients. It is not enough to just focus on the strains themselves to prevent the resistant gene isolates spread; rather, continuous surveillance and the application of effective methods for infection control are essential. The findings that the majority of beta-lactam-resistant isolates had blaTEM genes emphasize the significant contribution of non-ESBL blaTEM genes to resistance. This is caused by the gene product's enzymatic capacity to hydrolyze the majority of penicillin medications, historical cephalosporins, and contemporary beta-lactams.[23,24] In our study,*Bla-CTX-M15*has been themajor represented *bla-CTX-M* type which is also confounded by previous findings from ESBL isolates from camel and domestic livestock origin in Africa[25-28] Interestingly, the isolates harboring blaCTX-M-15 and blaTEM-1 gene exhibited an MDR phenotype. This phenotypic emergence corroborated findings from prior research indicating ESBL-producing Enterobacteriaceae frequently display an MDR phenotype against nonbeta-lactam antibiotics as a result of their plasmids containing numerous resistance genes[26,27,29]

Fig-1a: Isolation of ESBL producing *E. coli* on Chromogenic agar.1b: Phenotypic confirmation of ESBLs production in *E. coli* isolates by Etest using ceftazidime/ ceftazidime + clavulanic acid Strip.1c: Phenotypic confirmation of ESBLs production in *E. coli* isolates by disk diffusion method using cefotaxime (CTX) and cefotaxime+clavulanic (CEC)acid disks.

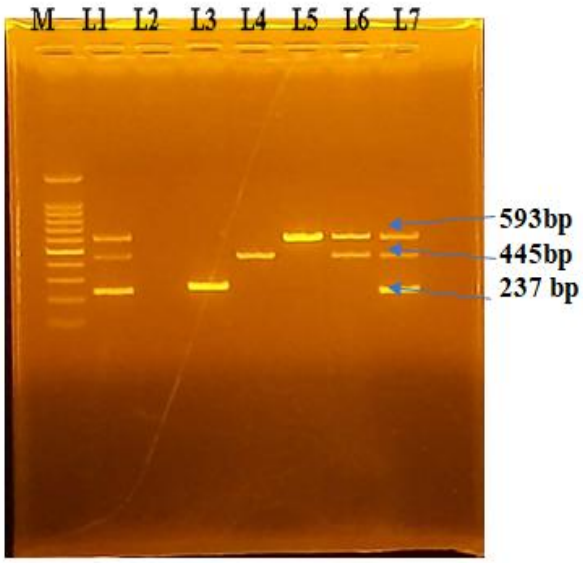

Fig-2: Representative *bla*$_{SHV}$, *bla*$_{TEM}$ and *bla*$_{CTX-M}$genes profile of phenotypically positive isolates using multiplex polymerase chain reaction (m-PCR):- Lane M: 100 bp DNA Ladder; Lane 1: Positive control for *bla*$_{SHV}$, *bla*$_{TEM}$ and *bla*$_{CTX-M}$genes; Lane 2: Negativecontrol Lane 3: *bla*$_{SHV}$positive; Lane 4: *bla*$_{TEM}$ positive; Lane 5: *bla*$_{CTX-M}$positive; Lane 6:*bla*$_{TEM}$ and *bla*$_{CTX-M}$positive and Lane 7:*bla*$_{SHV}$, *bla*$_{TEM}$ and *bla*$_{CTX-M}$positive

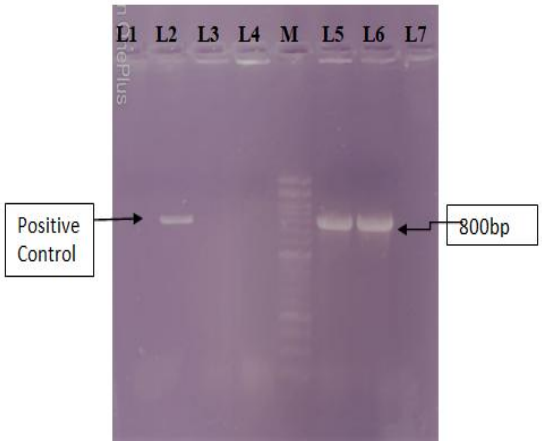

Fig-3a: PCR detection of *bla*$_{TEM}$group:- Lane M: 50 bp DNA Ladder; Lane 2: Positive control for *bla*$_{TEM}$group; Lane 1: Negativecontrol; Lane 5 and 6: *bla*$_{TEM}$grouppositive;

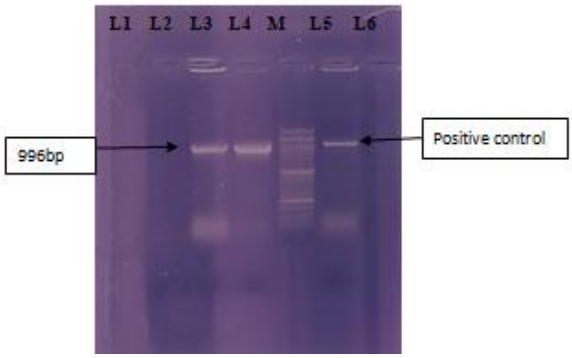

Fig-3b: PCR detection of *bla*$_{CTX-M15}$group:- Lane M: 50 bp DNA Ladder; Lane 1: Negativecontrol; Lane 3 and 4: *bla*$_{CTXM-15}$grouppositive, Lane 5: Positive control for *bla*$_{CTXM-15}$group

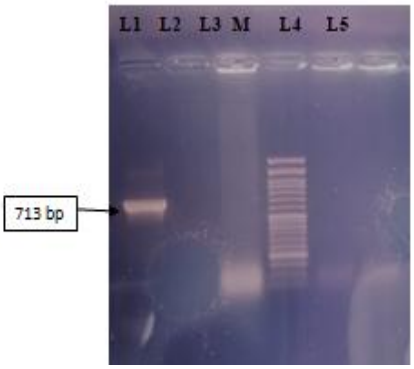

Fig-3c: PCR detection of *bla*$_{SHV}$group:- Lane M: 50 bp DNA Ladder; Lane 2: Negativecontrol; Lane 1: *bla*$_{SHV}$group positive

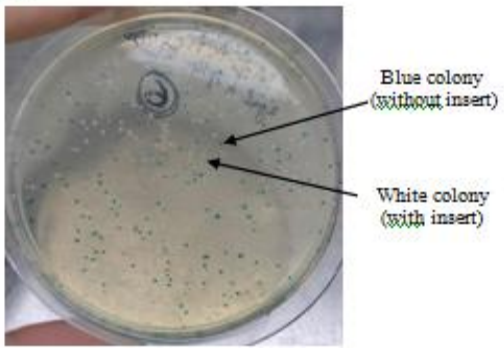

Fig-4: Blue/white screening for recombinant DH5α (LB/Amp. Agar) carrying *bla*gene in pGEM-T vector on Luria Bertaini agar.

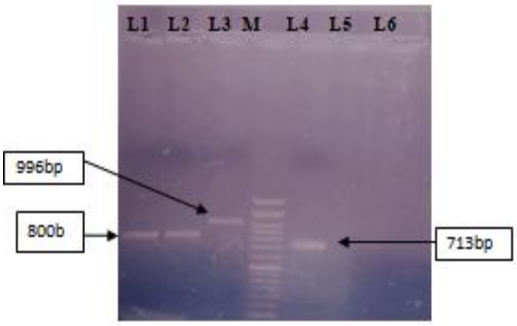

Fig-5: Colony touch PCR confirming the presence of *bla*$_{TEM}$, *bla*$_{CTXM-15}$ and *bla*$_{SHV}$ inserts in *E. coli* DH5 - α clone using pGEM-T vector

Lane M: 50 bp DNA Ladder

Lane 1-2: DH5-α clones on LB/Amp Agar showing amplification of approx. 800bp amplicons, upon colony touch PCR, confirming the presence of *bla*$_{TEM}$insert.

Lane-3: DH5-α clones on LB/Amp Agar showing amplification of approx. 996bp amplicons, upon colony touch PCR, confirming the presence of *bla*$_{CTX-M15}$insert.

Lane-4:DH5-α clones on LB/Amp Agar showing amplification of approx. 800bp amplicons, upon colony touch PCR, confirming the presence of *bla*$_{TEM}$insert.

Lane 5: Negativecontrol

Conclusion

In this study, we conclude the isolates as phenotypically presumptive positive ESBL *E. coli* based on the phenotypic confirmatory test (ESBL Chromogenic agar), E-test, and double disk synergy test. On molecular evaluation, 124 isolates were positive for *bla* TEM, *bla* SHV, and *bla* CTX-M genes mainly expressed by MDR ESBL isolates. Thus the indiscriminate use of antibiotics in the food industry is one reason for AMR development and in order to prevent the spread of ARGs among humans while also preserving the cultural practices of the pastoral communities, efforts should be taken to develop medical practices and husbandry practices to widen up the antibiotic options availability while fighting any medical health problem.

Conflict of Interest

All authors declare there is not any conflict of interest or any affiliation or involvement in any organization whether it is academic, commercial, financial, personal and professionally.

Authors' Contribution

HAS, DKK, MAB conceptualized the work and conducted research. HAS also wrote the manuscript.

References

1. World Health Organization. Antimicrobial resistance global report on surveillance.World Health Organization; 2014. 256 p.
2. Raji MA, Jamal W, Ojemhen O, Rotimi VO. Point-surveillance of antibioticresistance in Enterobacteriaceae isolates from patients in a LagosTeaching Hospital, Nigeria. *J Infect Public Health*. 2013;6(6):431–7. doi:10.1016/j.jiph.2013.05.002. [PubMed: 23999335].
3. Leski TA, Taitt CR, Bangura U, Stockelman MG, Ansumana R, CooperWH 3rd, et al. High prevalence of multidrug resistant Enterobacteriaceaeisolated from outpatient urine samples but not the hospitalenvironment in Bo, Sierra Leone. *BMC Infect Dis*. 2016;16:167.doi: 10.1186/s12879-016-1495-1. [PubMed: 27090787]. [PubMed Central:PMC4836052].
4. Ruppe E, Armand-Lefevre L, Estellat C, Consigny PH, El Mniai A, BoussadiaY, et al. High rate of acquisition but short duration of carriageof multidrug-resistant enterobacteriaceae after travel to the tropics.*Clin Infect Dis.* 2015;61(4):593–600. doi: 10.1093/cid/civ333. [PubMed:25904368].
5. Chaudhary U, Aggarwal R. Extended spectrum beta-lactamases (ESBL) - An emerging threat to clinical therapeutics. *Indian J Med Microbiol.* 2004;22(2):75–80.
6. Jacoby GA, Medeiros AA. More extended-spectrum beta-lactamases.*Antimicrob Agents Chemother*. 1991;35(9):1697–704.
7. Tzelepi E, Giakkoupi P, Sofianou D, Loukova V. Kemeroglou a,Tsakris a. Detection of extended spectrum beta lactamases in clinical isolates of Enterobacter cloacae and Enterobacter aerogenes. *J. ClinMicrobiol*. 2000;38(2):542-6.
8. Abdallah HM, Reuland EA, Wintermans BB, Al Naiemi N, Koek A,Abdelwahab AM, et al. Extended-spectrum beta-lactamases and/orcarbapenemases-producing enterobacteriaceae isolated from retailchicken meat in Zagazig, Egypt. *PLoS One*. 2015;10(8). e0136052. doi:10.1371/journal.pone.0136052. [PubMed: 26284654]. [PubMed Central:PMC4540287].
9. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases:A clinical update. *ClinMicrobiol Rev*. 2005;18(4):657–86. doi:10.1128/CMR.18.4.657-686.2005. [PubMed: 16223952]. [PubMed Central:PMC1265908].
10. Smet A, Van Nieuwerburgh F, Vandekerckhove TT, Martel A, Deforce D, Butaye P, et al. Complete nucleotide sequence of CTX-M-15-plasmids from clinical Escherichia coli isolates: insertional events of transposons and insertion sequences. *PLoS One*. 2010; 5(6):e11202. https://doi.org/10.1371/journal.pone.0011202 PMID: 20585456.
11. ÇakirErdoğan, D, Cömert, F, Aktaş, E, Köktürk, F&Külah, C. Fecal carriage of extended-spectrum beta-lactamase-producingEscherichia coli and Klebsiella spp. in a Turkish community. *Turk. J. Med. Sci.*2017; 47(1), 172–179.
12. Matsumura Y. et al. Rapid identification of different Escherichia coli sequence type 131 clades. *Antimicrob. Agents Chemother.* https://doi.org/10.1128/AAC.00179 -17 (2017).

13. Murphy G and Kavanagh T. Speeding-up the sequencing of double-stranded DNA. *Nucleic Acids Research*, 1988; *16*(11), 5198.
14. Denton M. 2007. Corrigendum to "Enterobacteriaceae"[Int. J. Antimicrob. Agents 29 (2007) S9–S22]. *International Journal of Antimicrobial Agents*, *6*(30), p.568.
15. Oberoi L,Singh N, Sharma P, Aggarwal A. ESBL, MBL and AMPC β lactamases producing superbugs—Havoc in the intensive care units of Punjab India. *J. Clin. Diagn. Res.* 2013; *7*, 70–73
16. Cantón R, Bryan, J. Global antimicrobial resistance: From surveillance to stewardship. Part 1: Surveillance and risk factors for resistance. *Expert Rev. Anti. Infect. Ther.* 2012; *10*, 1269–127
17. Peirano G, Pitout JDD. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: Update on molecular epidemiology and treatment options. *Drugs*, 2019; *79*, 1529–1541.
18. Jean SS, Hsueh, PR. High burden of antimicrobial resistance in Asia. *Int. J. Antimicrob. Agents.* 2011;37(4):291-5
19. Bonelli RR, Moreira, BM, Picão RC. Antimicrobial resistance among *Enterobacteriaceae* in South America: History, current dissemination status and associated socioeconomic factors. *Drug Resist. Updat.* 2014; *17*, 24–36.
20. Leopold SJ, van Leth F, Tarekegn H, Schultsz C. Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: A systematic review. *J. Antimicrob. Chemother.* 2014; *69*, 2337–2353.
21. Sonda T, Kumburu H, van Zwetselaar M, Alifrangis M, Lund O, Kibiki G, Aarestrup FM. Meta-analysis of proportion estimates of Extended-Spectrum-Beta-Lactamase-producing Enterobacteriaceae in East Africa hospitals. *Antimicrob. Resist. Infect. Control*, 2016; *5*, 1–9.
22. Chong Y, Shimoda S, Shimono N. Current epidemiology, genetic evolution and clinical impact of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella* pneumoniae. *Infect. Genet. Evol.* 2018; *61*, 185–188.
23. Bush K and Jacoby GA. Updated functional classification of beta-lactamases. *Antimicrobial Agents and Chemotherapy*, 2010; 54(3), 969–976.
24. Nagshetty K, Shilpa B, Patil S, Shivannavar C, &Manjula N. An overview of extended spectrum beta lactamases and metallo beta lactamases. *Advances in Microbiology*, 2021; 11(1), 37–62.
25. Alonso CA, Zarazaga M, Sallem RB, Jouini A, Slama KB & Torres C. Antibiotic resistance in Escherichia coli in husbandry animals: The African perspective. *Letters in Applied Microbiology*,2016; 64(5), 318–334.
26. Carvalho I, Tejedor-Junco MT, González-Martín M, Corbera JA, Silva V, Igrejas G, Torres C, &Poeta P. Escherichia coli producing extended-spectrum β-lactamases (ESBL) from domestic camels in the Canary Islands: A One Health approach. *Animals*, 2020; 10(8), 1295.
27. Saidani M, Messadi L, Mefteh J, Chaouechi A, Soudani A, Selmi R, Dâaloul-Jedidi M, Ben Chehida F, Mamlouk A, Jemli MH, Madec JY, &Haenni M. Various Inc-type plasmids and lineages of Escherichia coli and Klebsiella pneumoniae spreading blaCTX-M-15, blaCTX-M-1 and mcr-1 genes in camels in Tunisia. *Journal of Global Antimicrobial Resistance*, 2019; 19, 280–283.
28. Nüesch-Inderbinen M, Kindle P, Baschera M, Liljander A, Jores J, Corman VM, & Stephan R. Antimicrobial resistant and extended-spectrum ß-lactamase (ESBL) producing Escherichia coli isolated from fecal samples of African dromedary camels. *Scientific African*, 2020; 7, e00274.
29. Musila L, Kyany'a C, Maybank R, Stam J, Oundo V, & Sang, W. Detection of diverse carbapenem and multidrug resistance genes and high-risk strain types among carbapenem non-susceptible clinical isolates of target Gram-negative bacteria in Kenya. *PLoS ONE*, 2021; 16(2).