Author/s:
Acharya, J;Jha, R;Bhatta, RR;Shrestha, L;Sharma, BK;Chapagain, S;Gompo, TR;Rijal, N;Jha, P;Baines, SL;Judd, LM;Ioannidis, L;Howden, BP;Kansakar, P

Title:
Extended spectrum beta-lactamase producing Escherichia coli and antimicrobial resistance gene sharing at the interface of human, poultry and environment: results of ESBL tricycle surveillance in Kathmandu, Nepal

Date:
2025-04-16

Citation:
Acharya, J., Jha, R., Bhatta, R. R., Shrestha, L., Sharma, B. K., Chapagain, S., Gompo, T. R., Rijal, N., Jha, P., Baines, S. L., Judd, L. M., Ioannidis, L., Howden, B. P. & Kansakar, P. (2025). Extended spectrum beta-lactamase producing Escherichia coli and antimicrobial resistance gene sharing at the interface of human, poultry and environment: results of ESBL tricycle surveillance in Kathmandu, Nepal. One Health Outlook, 7 (1), https://doi.org/10.1186/s42522-025-00145-9.

Persistent Link:
https://hdl.handle.net/11343/360529

One Health Outlook

RESEARCH Open Access

Extended spectrum beta-lactamase producing *Escherichia coli* and antimicrobial resistance gene sharing at the interface of human, poultry and environment: results of ESBL tricycle surveillance in Kathmandu, Nepal

Jyoti Acharya[1], Runa Jha[1], Ranjan Raj Bhatta[1], Lilee Shrestha[1], Barun Kumar Sharma[2], Sharmila Chapagain[2], Tulsi Ram Gompo[2], Nisha Rijal[3], Priya Jha[3], Sarah L. Baines[4,5,6†], Louise M. Judd[4,5], Lisa Ioannidis[4,5], Benjamin P. Howden[4,5,6†] and Palpasa Kansakar[3*]

Abstract

Background The spread of antimicrobial resistant pathogens, including extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* is a global health threat and can be addressed only through a One Health approach. We aimed to characterize ESBL producing *Escherichia coli* isolates from World Health Organization Tricycle surveillance using data from whole genome sequencing (WGS) to decipher the potential dynamics of their circulation at the human, poultry and environment interface.

Methods WGS was performed on 100 non-duplicate representative ESBL *E. coli* isolates including 28 isolates from humans, 36 from poultry caeca, and 36 from water samples. Minimum Inhibitory Concentration (MIC) was determined using Vitek 2 Compact. WGS was performed on Illumina NextSeq 2000 platform and open-source bioinformatics pipelines were used to analyze WGS data for genomic characterization including phylogenetic analysis and in silico multi-locus sequence typing and, serotyping and, ESBL gene detection.

Results Most isolates were susceptible to imipenem (98%), meropenem (94%) and tigecycline (94%). Six ESBL *E. coli* isolates from poultry were resistant to colistin (MIC ≥ 4 µg/ml). WGS revealed high genetic diversity representing 56 sequence types (ST) including three novel STs. ST131 (7 isolates) was the most prevalent comprising human and environment isolates, followed by ST2179 (6 isolates, all poultry) and ST155 (5 isolates across the three sectors). All

†Sarah L. Baines and Benjamin P. Howden contributed equally to this work.

*Correspondence:
Palpasa Kansakar
pkansakar@who.int

Full list of author information is available at the end of the article

eight recognized *E. coli* phylogroups were observed, with majority (86%) of the isolates belonging to A, B1, B2 and D phylogroups. Of the100 isolates, 98 carried bla_{CTX-M} gene, with $bla_{CTX-M-15}$ the most prevalent allele (76%). AmpC type ESBL genes were found in four and OXA type β lactamases in six isolates. In our study, bla_{NDM-5} was detected in two imipenem resistant isolates from human. Coexistence of more than one β-lactamase genes was seen in 26% isolates.

Conclusion Our findings indicate high genetic diversity among ESBL *E. coli* strains from all three sectors and sharing of identical strains and resistance genes within and between sectors. ST131, the globally dominant ESBL *E. coli* clade is gaining prevalence in Nepal with bla_{CTX-M} being the most common ESBL gene across the phylogroups and all source groups. Antimicrobial stewardship should be promoted in one health approach to combat antimicrobial resistance.

Keywords ESBL *E. coli*, One health, bla_{CTX-M}, Nepal

Introduction

The global spread of antimicrobial resistant (AMR) pathogens, including extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae* is a major global health threat, affecting humans, animals, and the environment. In the AMR era, the evolving resistance caused by ESBLs is associated with higher morbidity, prolonged hospital stays, and expensive treatment options [1]. ESBL producing *Escherichia coli* (ESBL *E. coli*) are characterized by resistance to third generation cephalosporins, but are unable to hydrolyze cephamycin or carbapenems [2]. Though carbapenems are considered the drug of choice for treating infections caused by ESBL producers [3], emergence and spread of carbapenemase producing *Enterobacteriaceae* (CPE) raises serious public health concerns [4].

AMR also affects animal health, food safety and security, and environmental health. The importance of One Health approach with epidemiological surveillance is now acknowledged as a key element in the fight against AMR [5]. In this regard, WHO has developed a simplified, integrated, multisectoral surveillance protocol known as Tricycle, which uses a One Health approach to survey AMR in three major sectors: human (carriage and bloodstream infections), the food chain (poultry), and the environment (surface water, slaughterhouse effluents, and wastewater) using ESBL *E. coli* as the indicator organism [6]. Using a sympatric approach to cross-sectoral sample collection, Nepal adopted the WHO ESBL *E. coli* Tricycle protocol in 2022 to assess the prevalence of ESBL *E. coli* across these sectors. Tricycle surveillance in Kathmandu, Nepal showed a high prevalence of ESBL *E. coli* in all these three sectors [7]. The increasing application of WGS brings a level of discrimination and information on genetic relatedness and resistance mechanisms that surpass previous typing methods [8]. With the objective to gain insights on the genetic links between ESBL *E. coli* from the three sectors, predominant clones circulating and associated ESBL genes, we conducted whole-genome sequencing (WGS) of representative ESBL *E. coli* isolates from Tricycle surveillance in Kathmandu, Nepal. To the best of our knowledge, this is the first study on ESBL producing *E. coli* isolates from Nepal through One Health approach using genomics.

Materials and methods

Ethical clearance

Ethical approval for this study was obtained from the Nepal Health Research Council, Government of Nepal (NHRC Registration no. 845–2019).

Information on study samples

For human sampling five hospitals were selected for both cases (blood samples referred for culture and sensitivity from patients with bloodstream infections) and controls (stool samples from healthy pregnant females in the third trimester of pregnancy visiting the same hospital for antenatal care).

For poultry sampling, caeca samples from birds were collected from five different poultry live markets and slaughter areas that were selected based on their spatial distributions within proximity to the human sampling sites.

For the environment sampling, four sample types-namely upstream, downstream, communal and hospital effluent wastewater samples were selected. Sites for environment sampling were selected based on the proximity to human sampling locations, effluent discharging from hospitals, and adjoining areas near poultry market [7].

Information on ESBL *E. coli* isolates used in the study.

Implementation of ESBL *E. coli* Tricycle surveillance in Kathmandu, Nepal in 2022 through sampling performed within the same temporal scale and in a limited geographical area, led to the isolation of 165 ESBL producing *E. coli* isolates (Table 1). These included 15 isolates from stool cultures of healthy pregnant women (human control), 26 from human blood stream infections (BSI, human case), 85 from poultry caeca and 39 from environmental water samples (upstream, downstream, communal rivers, and hospital effluent) [7]. Sampling and microbiological procedures for isolation and

Table 1 Source, seasonal and site distribution of ESBL *E. coli* isolates

Isolates by sample source (number)	Seasonal distribution (number of isolates)				Distribution by sampling site (number of isolates)				
	Spring (Feb-Apr)	Autumn (Aug-Oct)	Winter (Nov-Jan)	Summer/ monsoon (May-July)	Hospital A	Hospital B	Hospital C	Hospital D	Hospital E
Pregnant women ($n=10$)	2	0	8	0	4	2	2	0	2
Bloodstream infections ($n=18$)	5	4	2	7	3	4	4	7	0
Poultry caeca ($n=36$)	Spring (Feb-Apr)	Autumn (Aug-Oct)	Winter (Nov-Jan)	Summer/ monsoon (May-July)	Chabahil	Chandragiri	Jorpati	Shobha Bhagwati	Lagankhel
	4	30	23	28	19	19	19	13	15
Environment water ($n=36$)	Spring (Feb-Apr)	Autumn (Aug-Oct)	Winter (Nov-Jan)	Summer/ monsoon (May-July)	Upstream	Downstream	Communal	Hospital effluent	-
	11	6	11	11	0*	8	23	8	-

* No ESBL *E. coli* were detected in water samples from upstream rivers

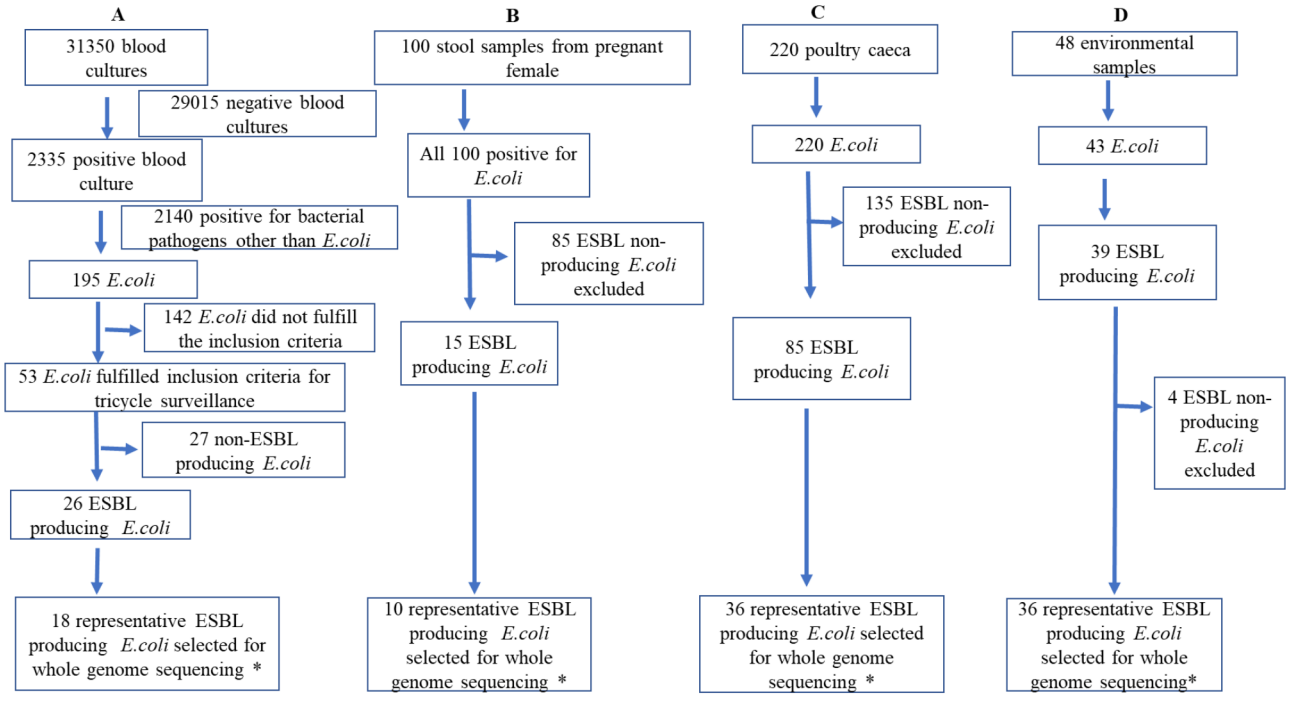

Fig. 1 Flowchart for selection of ESBL *E. coli* isolates for WGS

identification of these isolates were performed according to the WHO ESBL *E. coli* Tricycle protocol [6]. These isolates were preserved at -80° C at National Public Health Laboratory, Kathmandu, Nepal for further analysis. Among these, a collection of 100 non-duplicate ESBL *E. coli* representing from all three sectors were selected for antimicrobial susceptibility testing and WGS. These isolates were selected based on the frequency of isolation, source of isolation, sampling sites and sampling time to maximize representation in the WGS dataset. These included 28 human isolates (10 controls and 18 cases), 36 poultry isolates and 36 environment isolates. The selection of isolates for WGS is shown in Fig. 1 and isolate information in Table 1.

Antimicrobial susceptibility testing and ESBL screening

Antimicrobial susceptibility testing of the isolates selected was conducted by VITEK-2 Compact Automated ID/AST system (Biomerieux, France) using N280 AST panels for Gram negative bacteria. The panel included

amoxicillin/clavulanate (4/2–32/16 µg/mL), piperacillin/tazobactam (2/4–48/8 µg/mL), cefepime (2–32 µg/mL), imipenem (1–12 µg/mL), meropenem (0.5–12 µg/mL), amikacin (4–32 µg/mL), gentamicin (4–32 µg/mL), ciprofloxacin (0.5–4 µg/mL), tigecycline (0.75–4 µg/mL) and cotrimoxazole (1/19–16/304 µg/mL). The MIC of colistin was determined using microbroth dilution Sensititre kits (ComASP Colistin, Lot no.011323505 from Liofilchem S.R.L). The inoculum preparation, test procedure and AST result interpretation (supplemental table S1) was done using CLSI-M100, 2022 [9] except for tigecycline for which EUCAST guidelines [10]were used. Multidrug resistance was defined as non-susceptibility to three or more classes of antimicrobial agents.

DNA extraction

Bacterial DNA was extracted and purified with Qiagen Kit (QIAmp DNA Micro kit, Invitrogen by Thermo Fischer Scientific, Carlsbad, CA, United States) according to manufacturer's instructions.

The assessment of DNA quality was carried out using a Nano Drop ND-1000 spectrophotometer (Thermo Fischer Scientific, Wilmington, DE, United States) and DNA quantity was measured using a Qubit 2.0 fluorometer (Invitrogen, Life Technologies, Carlsbad, CA, United States). An optical density of 1.8–2.0 at 260/280 nm and a DNA concentration of 20 ng/µl was set as threshold for quality control.

Whole genome sequencing

DNA extracts were shipped to the Centre for Pathogen Genomics at the University of Melbourne, Australia, where data was generated using an established wet-lab workflow. Briefly, the concentration of the genomic DNA provided for sequencing was re-assessed using a Quantus fluorometer. Dual-indexed DNA sequence libraries were prepared for all samples of sufficient DNA concentration threshold for sequencing using the Illumina DNA prep kit according to the manufacturer's instructions (Illumina; protocol - https://shorturl.at/aBD08). Libraries were sequenced on an Illumina NextSeq 2000 platform with 2 × 151 bp paired-end chemistry.

All sequence reads have been uploaded to the European Nucleotide Archive under project accession PRJEB85701. Individual sample and data accessions have been included in the Supplementary Materials (supplemental table S2).

Bioinformatics analysis

The BOHRA bioinformatics pipeline (https://github.com/kristyhoran/bohra), that adheres to ISO standards, was used to: (1) assess sequence data quality, including read length, read quality, GC content, total sequence yield, and estimated genome coverage, and (2) perform a range of analyses, including de novo assembly (SPAdes v3.13.2) [11] multi-locus sequence typing (mlst v2.23.0, https://github.com/tseemann/mlst) using the global PubMLST typing schemes (https://pubmlst.org/), antimicrobial resistance gene detection (abriTAMR v1.0.14) [12] using NCBI's AMRfinderPlus database [13], plasmid replicon detection (MOB-suite v3.1.6) [14], mapping and variant calling (snippy v4.6.0, https://github.com/tseemann/snippy), and maximum likelihood phylogenetic recons truction (IQ-TREE v2.2.3) performed on a core genome alignment, with 1000 bootstrap replicates [15]. The prototypic *E. coli* strain K-12 (NCBI Reference Sequence: NC_000913.3) was used as the reference. This analysis was supplemented with in detection of phylogroups using ClermonTyping (v20.03) [16], performed to classify isolates into the globally recognized *E. coli* phylogroups, and virulence gene detection (ABRicate v1.0.1, https://github.com/tseemann/abricate) using the virulence factor database (vfdb) [17].

Results

Antibiotic susceptibility profile of the ESBL *E. coli* isolates

The majority of the isolates tested were susceptible to imipenem (98%), ertapenem (94%), meropenem (94%), tigecycline (94%) and amikacin (93%), followed by β-lactam/β lactamase inhibitor (BL-BLI) combinations-piperacillin/tazobactam (90%). Only 47%, 30% and 8% of the isolates were susceptible to co-trimoxazole, cefepime and ciprofloxacin respectively (Table 2). Four ESBL *E. coli* isolates (three from human case samples, and one from an environment sample) were resistant to meropenem, of these only two (both from human case samples) were resistant to imipenem. Six isolates (all from poultry) were resistant to colistin (MIC ≥ 4 µg/ml) and harbored *mcr-1* gene.

Variations in AST was seen among sectors. Isolates from human case were less susceptible to antibiotics tested (except for cefepime and aminoglycosides) as compared to isolates from human controls, poultry and environmental samples. Of the 100 ESBL positive isolates, 23% were MDR and highest proportion of MDR (12/36, 33.3%) was seen in poultry isolates, followed by human (7/28, 25%) and environment (4/36, 11%).

Phylogenetic analysis

Phylogenetic analysis of the 100 ESBL-positive *E. coli* demonstrated significant genetic diversity among isolates. Isolates representing samples from all sectors were broadly distributed across the phylogenetic tree with only a small number of clades dominated by a single sector (Fig. 2).

Given the extensive genetic diversity observed in the dataset, phylo grouping as defined with ClermonTyping was undertaken which allowed the isolates to be clustered

Table 2 Antibiogram of ESBL producing *E. coli* isolates from different sources

Number of isolates susceptible (% susceptibility)					
Source	**Human case (*n* = 18)**	**Human control (*n* = 10)**	**Poultry (*n* = 36)**	**Environment (*n* = 36)**	**Total (*n* = 100)**
Antibiotics tested					
Amoxicillin/Clavulanate	10 (56%)	10 (100%)	28 (78%)	33 (92%)	81 (81%)
Piperacillin/Tazobactam	13(72)	10 (100%)	35 (97%)	32 (89%)	90 (90%)
Cefepime	8 (44%)	3(30%)	13 (36%)	6 (17%)	30 (30%)
Imipenem	16 (89%)	10 (100%)	36 (100%)	36 (100%)	98 (98%)
Meropenem	15 (83%)	10 (100%)	34 (94%)	35 (97%)	94 (94%)
Amikacin	17 (94%)	10 (100%)	32(89%)	34 (94%)	93 (93%)
Gentamicin	13 (72%)	10 (100%)	24 (67%)	33(92%)	80 (80%)
Ciprofloxacin	1 (6%)	3(30%)	2(6%)	2(6%)	8 (8%)
Tigecycline	17 (94%)	10 (100%)	31 (96%)	36 (100%)	94(94%)
Cotrimoxazole	3(17%)	7 (70%)	14 (39%)	23(64%)	47 (47%)

into broader groups. All eight known phylogroups were detected, with most isolates (86%) belonging to the four phylogroups- A, B1, B2, and D. Further, the phylogroups were strongly correlated with the phylogenetic tree, providing confidence in the population structure presented in Fig. 2.

Exploration of the diversity of the dataset in the context of the isolate source groups identified several findings. First, there was representation of all source groups (animal, human, and environment) in the two largest phylogroups, A and B1 (Fig. 3). Most of the environment (28/36, 78%) and animal (25/36, 68%) isolates were assigned to these two phylogroups. There was an over-representation of human isolates (14/17, 82%) in phylogroup B2 with 64% (9/14) isolates from cases and 36% (5/14) isolates from controls. Half of the human isolates (14/28, 50%) were assigned to this phylogroup. There was a dominance of animal isolates in the lesser observed phylogroups F and G.

Sequence types (STs)

The 100 ESBL *E. coli* isolates were assigned to 56 unique MLST profiles, including three novel sequence types; two were single-locus variants of ST58/ST155 and ST46, with the third profile representing a novel combination of known alleles (allele details are available in the supplemental table S2). Singleton STs (profiles observed in only a single isolate) comprised 38% of the total isolates. The most prevalent ST was ST131, seen in seven isolates (five from human and two from environment), followed by ST2179 seen in six poultry isolates, and ST155 seen in five isolates (from human case, poultry and environment) (Fig. 3).

Twenty-eight human isolates were assigned to 16 STs, with ST131 being the most predominant. Poultry isolates were assigned to 25 STs with ST2179 being the most predominant. Environment isolates were assigned to 28 STs with ST155 being the most predominant. Only ST155 was seen in all three sectors, five sequence types (ST38, ST117, ST156, ST224, ST2179) were shared between poultry and environment, whereas four sequence types (ST131, ST1193, ST3580 and ST12452) were shared between human and environment (Fig. 3). Seven STs were assigned to the extra-intestinal, virulent-associated, B2 phylogenetic group. ST131 were detected only in human and environmental isolates. Human isolates in phylogroup B2 included four common and clinically important STs (ST131, ST1193, ST73, and ST95).

Serotypes

In silico serotyping identified 68 unique profiles. The most prevalent serotype was 09:H9, with eight isolates observed with this profile (5 from poultry and 3 from environment), followed by O25:H4 (6 isolates-2 isolates from human control and 1 each from human case and environment), O75:H5 (4 isolates-2 isolates from human control and 1 each from human case and environment). Singleton serotypes represented the majority of the of the isolates (52%). All ST131 isolates from the current study were of serotype O25:H4 except for one environment isolate which belonged to serotype O16:H5. No serotype specific to a particular sector was observed. (supplemental table S2)

Antibiotic resistance genes

All ESBL isolates except one were concordant between phenotypic and genotypic detection of AMR with all confirmed to carry at least one gene conferring an ESBL phenotype. Of the 100 isolates, 95 contained a $bla_{\text{CTX-M}}$ type gene that encodes a class A ESBL. Among these, $bla_{\text{CTX-M-15}}$ was the most common (76 isolates) of which 46 were carried on the chromosome and 30 on plasmids. $Bla_{\text{CTX-M-55}}$ was detected in 10 isolates (9 from poultry isolates and 1 from environment), all carried on a plasmid, $bla_{\text{CTX-M-65}}$ in 8 isolates (2 carried in plasmids, 6 in the chromosome), $bla_{\text{CTX-M-27}}$ in 3 isolates (2 carried in plasmid and 1 in the chromosome), and $bla_{\text{CTX-M}}$-14 in 1 isolate carried on a plasmid. Four AmpC type ESBLs

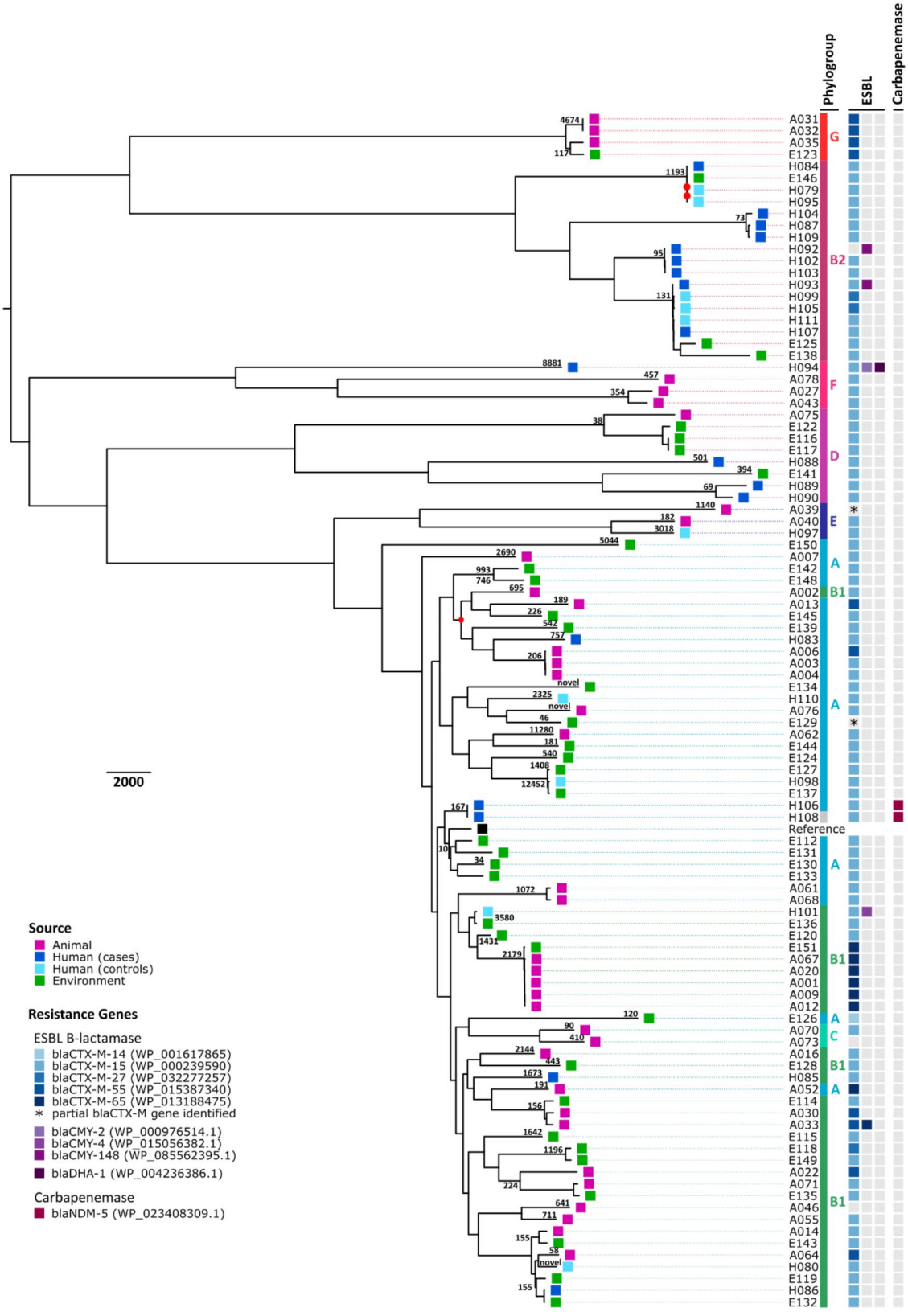

Fig. 2 Population structure of the study dataset. A midpoint rooted maximum likelihood phylogenetic tree of the 100 ESBL *E. coli* isolates. Branches with bootstrap support < 70% are indicated with a red circle. Tree annotations include isolate name (tip labels), isolate source (tip shape, colored by source), and MLST (denoted on branches/clades). Aligned to the phylogenetic tree is information on phylogroups (as defined by ClermonTyping) and the presence of key β-lactamase resistance genes

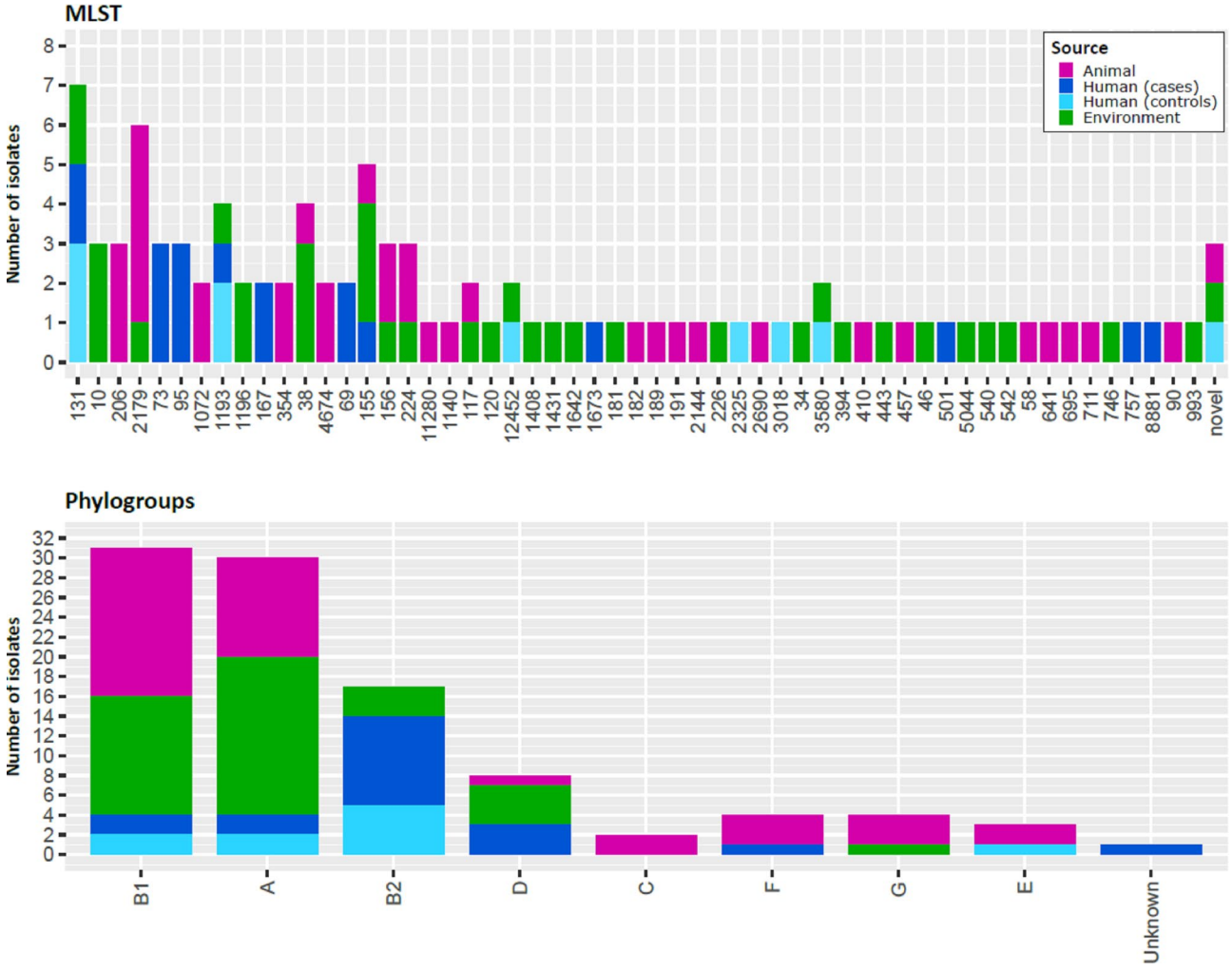

Fig. 3 Phylogroup and MLST distribution of isolates sequenced by source

genes ($bla_{CMY-148}$, bla_{CMY-2}, bla_{CMY-4} and bla_{DHA-1}) were present in five isolates, all carried plasmids. One of these isolates carried two different *Amp*C genes, bla_{CMY-2} and bla_{DHA-1}. OXA type β lactamases were detected in three isolates. Four isolates contained two or more ESBL genes whereas 26 isolates contained more than one β lactamase genes. List of β lactamase genes detected is shown in Table 3. Genes encoding intrinsic β-lactamases (i.e., bla_{EC}, also known as bla_{AmpC}) is not included in Table 3 as it is extremely rare for these genes to mediate a cephalosporin resistance phenotype in their native repressed state.

All except two isolates carried either class A and/or AmpC type ESBL genes. The other two isolates carried bla_{TEM-1} and $bla_{TEM-135}$. The carriage of bla_{CTX-M} was distributed across the phylogenetic tree, with only a small cluster (representing the animal-associated lineage ST2179) carrying $bla_{CTX-M-65}$ (Fig. 2). Further investigation of ESBL genes in the context of the phylogenetic tree revealed that $bla_{CTX-M-15}$ (the most common ESBL gene in the dataset) was carried by isolates representing seven of the eight phylogroups. Phylogroup G was the exception and exclusively carried $bla_{CTX-M-55}$. Gene encoding metallo β-lactamase bla_{NDM-5} were βdetected in two isolates (both from human blood stream infections) which were resistant to both imipenem and meropenem. No carbapenem resistance genes were detected in isolates that were resistant to meropenem but imipenem susceptible. This study has not looked for other mechanisms of resistance, such as mutation.

Discussion

Tricycle surveillance project implementation in Nepal detected a high ESBL*E. coli* prevalence in human BSI, healthy pregnant women, poultry and the environment samples [7]. Besides poultry, some studies in Nepal have also highlighted the prevalence of ESBL *E. coli* in cattle such as cow/buffalo (47.7%) and goats (51%) [18]. We characterized 100 representative ESBL producing *E. coli* isolates from Tricycle surveillance in Nepal using WGS

Table 3 β-lactamase and carbapenemase genes distribution by source

(Class-A) Extended spectrum β-lactamases	Total Number	Human control (10)	BSI (18)	Poultry (36)	Environment (36)
$bla_{\text{CTX-M-15}}$	76	8	17	19	32
$bla_{\text{CTX-M-55}}$	10	0	0	9	1
$bla_{\text{CTX-M-65}}$	8	0	0	7	1
$bla_{\text{CTX-M-27}}$	3	2	0	0	1
$bla_{\text{CTX-M-14}}$	1	0	0	0	1
Total	98 genes				
(Class-A) Narrow spectrum β-lactamases					
$bla_{\text{TEM-1}}$	30	0	5	17	8
$bla_{\text{TEM-135}}$	1	0	0	1	0
$bla_{\text{TEM-176}}$	1	0	0	1	0
$bla_{\text{TEM-190}}$	1	0	0	0	1
Total	33 genes				
(Class C) Extended spectrum β-lactamases-AmpC type					
$bla_{\text{CMY-148}}$	2	0	2	0	0
$bla_{\text{CMY-2}}$	1	0	1	0	0
$bla_{\text{CMY-4}}$	1	1	0	0	0
$bla_{\text{DHA-1}}$	1	0	1	0	0
Total	5 genes				
(Class D) OXA-type β-lactamases					
$bla_{\text{OXA-1}}$	5	0	4	1	0
$bla_{\text{OXA-9}}$	1	0	0	1	0
Total	6 genes				
Carbapenemase					
$bla_{\text{NDM-5}}$	2	0	2	0	0
Total	2 genes				

and compared the genomic diversity and resistance determinants of the strains from the three sectors. To our knowledge, the present study is the first to address the circulation of antibiotic resistance from a One Health perspective in Nepal which carried out genomic analysis of the results of the implementation of the WHO Tricycle protocol.

ESBL producing *E. coli* isolates were found to be genetically diverse and were assigned to 56 MLST profiles. High genomic diversity is not unexpected for *E. coli* given the diverse sampling represented in the dataset. ST131 was the most prevalent and comprised mainly human isolates providing important insight to the epidemiology and increasing spread of this globally successful pathogenic clone. Although detected at a low proportion, our results indicate that ST131 is a predominant ST (7%) among sequence types observed and that the $bla_{\text{CTX-M-15}}$ gene is commonly carried (97%) in ESBL *E. coli* in Nepal. This is consistent with global trends that indicate that the drug-resistant *E. coli* ST131, associated with the $bla_{\text{CTX-M-15}}$ gene, has been increasing in prevalence [19, 20]. Sharing of the same STs and resistance genes among isolates from different sources was observed. Overall, five STs were common among isolates from poultry and environment, four among environment and human, and one ST (ST155) shared by all three sectors. ST155 is considered to have zoonotic potential, responsible for the transmission of ESBL *E. coli* to human [21]. This suggests a higher possibility of the exchange of resistant strains and genes through human-environment interactions. such as transmission from human wastewater to the environment.

All eight recognized phylogroups (A, B1, B2, C, D, E, F, and G) were observed in this dataset, a finding strongly supporting that the study dataset is both genetically and ecologically diverse. There was representation of all source groups (animal, human, and environment) in the two largest phylogroups, A and B1. This is consistent with these phylogroups representing the 'generalist' *E. coli* lineages [22]. Most of the environment (28/36, 78%) and animal (25/36, 68%) isolates were assigned to these two phylogroups, a finding which agrees with the previous reports in which these lineages are frequently recovered from animals and secondary habitats [22]. There was an over-representation of human isolates in phylogroup B2 (14/17, 82%) – the predominant Extraintestinal pathogenic *E. coli* (ExPEC) lineage with 64% (9/14) isolates from cases and 36% (5/14) isolates from control samples. Half of the human isolates (14/28, 50%) were assigned to this phylogroup and represent four common and clinically important STs: ST131, ST1193, ST73, and ST95 [23]. ST131 has become the dominant ExPEC lineage causing infections in humans worldwide [24–26]. ExPEC of ST131 is a globally dispersed clonal lineage often

associated with multidrug resistance (MDR), conferring resistance to ESBLs and fluoroquinolones [24], but the prevalence of this lineage in Nepal remains unknown. The small dataset in this work estimates ST131 to represent 7% of ESBL *E. coli* and a dominance of animal isolates in the lesser observed phylogroups F and G. This correlation may change with increased sampling.

ESBL have been frequently reported in the Asian subcontinent since the late 1990s. In Nepal, ESBL have been reported for more than a decade [27]. Most ESBL in the world belong to the group A ESBL, which includes several types of sulfhydryl reagent variable (SHV) β-lactamases, Temoniera (TEM) β-lactamases, and cefotaxime-M (CTX-M β-lactamases). More recent outbreaks involving ESBLs have been mediated by CTX-M [28]. Our results also showed that in 98% isolates, the ESBL phenotype was due to a $bla_{\text{CTX-M}}$ gene, and $bla_{\text{CTX-M-15}}$ was the most frequent in each sector, which is consistent with the global ESBL epidemiology [29–31]. The globally dominant ESBL $bla_{\text{CTX-M-15}}$ was first reported in India in the mid-1990s and is still a dominant ESBL type in India, Bangladesh, and Pakistan [28]. Here we identified the $bla_{\text{CTX-M-15}}$ gene across a diverse set of lineages. This finding is similar to other studies from Tanzania and Malawi, where the $bla_{\text{CTX-M-15}}$ gene was found across numerous STs [32, 33]. We were unable to confirm a plasmid location for 53 of the identified $bla_{\text{CTX-M}}$ genes. Of note, 49/53 $bla_{\text{CTX-M}}$ genes were located on contigs identified as putative chromosomal in origin. However, long read sequencing is required to confirm this finding. High rates of $bla_{\text{CTX-M}}$ chromosomal integration are being increasingly reported, reaching, for example, 64% in a Japanese study [34]. Our results indicate that ST131 is more prevalent (7%) among sequence types observed and $bla_{\text{CTX-M-15}}$ gene is occurring at a higher frequency (98%) among ESBL genes observed. This is consistent with global trends that indicate that the highly drug-resistant *E. coli* ST131, associated with the $bla_{\text{CTX-M-15}}$ gene, has been increasing in prevalence [32]. In our study $bla_{\text{CTX-M-55}}$ and $bla_{\text{CTX-M-65}}$ were observed in animal-associated lineages.

In one isolate, the ESBL phenotype was linked to AmpC type ESBL, whereas in remaining two isolates, no ESBL genes were detected, but they contained $bla_{\text{TEM-1}}$ and $bla_{\text{TEM-135}}$. About 90% of *E. coli* harboring TEM-1 can confer resistance to ampicillin, penicillin, and first-generation cephalosporins but not to oxyimino cephalosporin [28]. During the 1980s, evolution of TEM-1 from non-ESBL to ESBL in *K. pneumoniae* and *E. coli* strains, respectively, via specific amino acid substitutions, made them more capable of hydrolyzing oxyimino-cephalosporins [28]. Other possible explanations for the discrepancy between phenotype and genotype in the two isolates include loss of a plasmid harboring an ESBL gene during laboratory processing or the presence of a combination of bla_{TEM} with other mechanisms like efflux and decreased membrane permeability. Co-existence of different β-lactamase genes were observed in 26% isolates and similar findings have been reported earlier among *E. coli* isolates from Nepal [35].

Carbapenems are regarded as primary treatment option for infection caused by ESBL-producing *Enterobacteriaceae*, however, increasing carbapenem-resistance has not only limited the treatment options but also created additional challenges for healthcare facilities worldwide regarding the implementation of effective infection control measures to limit nosocomial spread [36]. In our study, four isolates were resistant to meropenem. Carbapenem resistance gene $bla_{\text{NDM-5}}$, encoding New Delhi metallo-β-lactamase (NDM) was detected in two of these isolates which were also resistant to imipenem (both from human BSI cases). NDM can inactivate carbapenem and other β-lactam antibiotics except aztreonam and therapeutic options are more limited as a result of the evolution of new variants of NDM [37]. NDM-type-metallo-β-lactamase-producing *E. coli* clinical isolates have been reported earlier from Nepal [38–40].

Recently, a global priority list of antibiotic resistant bacteria was published by the World Health Organization to increase and encourage research into new treatments for such pathogens [41]. The list included extended-spectrum β-lactamase producing and carbapenem resistant *Enterobacteriaceae* which calls for concern both in healthcare and community settings.

Six poultry isolates in our study were resistant to colistin (with MIC > 4 μg/mL) and harbored *mcr-1* gene. The first report of colistin resistant *E. coli* carrying *mcr*-1-gene was from China in 2015 [20], but there are increasing report of colistin resistance among *E. coli* isolates harboring mcr-1 gene from Nepal as well [42–44]. Studies from Kathmandu valley shows that 47% of the farms use colistin alone or in conjunction with other antibiotics in poultry as feed supplements [45]. In our study, colistin resistance was not seen among human isolates, however, detection of colistin-resistant isolates in poultry is a serious concern, as it could potentially lead to colistin resistance in human pathogens through horizontal transfer of resistant genes from poultry to humans.

Through One Health Tricycle surveillance implementation in Nepal, we evidenced the circulation of both ESBL *E. coli* strains and ESBL genes, between and among the three sectors, despite specific sectoral population structures. Although our results showed sharing of the same sequence types of ESBL *E. coli* and resistance genes between sectors, the dynamics of *E. coli* movement between sectors remains undetermined. Of note, isolates carrying carbapenem resistant genes were from human

clinical samples only, while isolates resistant to colistin were identified in poultry samples only.

Conclusion

Through genomic analysis, we revealed extensive genetic diversity among the ESBL *E. coli* isolates, with ST131, the globally dominant *E. coli* strain being the most prevalent ST in Nepal also. The One Health sampling design of this study revealed the sharing of identical strains and resistance genes within and between sectors highlights the need for coordinated monitoring and intervention strategies across sectors. A genetic mechanism to explain the ESBL phenotype of almost all isolates was identified, with $bla_{\text{CTX-M}}$ being the most common and were broadly represented across most phylogroups and all source groups. This dataset will serve as a good baseline on the genetic diversity of ESBL *E. coli*, identification of region-specific AMR high-risk clones and ESBL determinants to restrict their dissemination nationally and internationally. Awareness programs, including implementation and regulation of strict polices on antimicrobial use in human and veterinary sector, infection prevention and control in healthcare facilities as well as farm biosecurity measures, and waste disposal are essential to contain AMR.

Abbreviations

AMR	Antimicrobial Resistance
AST	Antibiotic Susceptibility Testing
BSI	Bloodstream Infection
CPE	Carbapenemase Producing Enterobacteriaceae
ESBL	Extended Spectrum B Lactamases
ExPEC	Extraintestinal Pathogenic E. coli
MDR	Multidrug Resistant
MIC	Minimum Inhibitory Concentration
MLST	Multi-locus Sequence Types
ST	Sequence Types
WGS	Whole genome Sequencing
WHO	World Health Organization

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42522-025-00145-9.

Supplementary Material 1

Supplementary Material 2

Acknowledgements

The authors would like to acknowledge Dr. Rajesh Sambhajirao Pandav (WHO Representative for Nepal), Dr. Allison Gocotano, Team Lead, WHO Health Emergencies Programme and Dr. Arunkumar Govindakarnavar, Technical Officer, Public Health Laboratories, from WHO Country office, Nepal for providing support throughout the WHO-AMR program. We greatly acknowledge, all laboratory staff from National Public Health Laboratory, Central Veterinary Laboratory and Tricycle surveillance sentinel hospital laboratories who contributed to sample collection, transportation, and provision of the isolates.

Author contributions

J.A: Project conceptualization, sample selection, isolate biorepository and genome extraction, R.R.B, R.J, L.S: Project conceptualization and monitoring, and project implementation for human health sector, B.K.S, S.C: Project conceptualization, sample selection and project implementation in veterinary sector, T.R.G: laboratory testing and isolate biorepository in veterinary sector, S.L.B, L.M.J, L.I, B.P.H: Whole genome sequencing of isolates and bioinformatics analysis and have contributed equally, N.R: isolate reconfirmation and genome extraction, P.J: isolate reconfirmation and antibiotic susceptibility testing, P.K: Project implementation, data analysis, manuscript conceptualization and drafting. All authors reviewed the manuscript.

Funding

The study was supported by the Fleming fund grant received by WHO Country Office, Nepal.

Data availability

The data on antibiotic susceptibility testing, beta lactamase genes and phylogenetic analysis that support the findings of this study are available in the manuscript itself. All sequence reads have been uploaded to the European Nucelotide Arrhive with the project accession PRJEB85701. Individual sample and data accessions have been included in the Supplementary file (Supplementary Table 2).

Declarations

Ethics approval and consent to participate

Ethical approval for this study was obtained from the Nepal Health Research Council, Government of Nepal (NHRC Registration no. 845–2019).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Disclaimer

The views and opinions expressed in this work are solely those of the author and do not necessarily reflect the official policy or position of the organization for whom the authors are employed. Any content or information presented in this work should be considered the author's personal viewpoint and should not be attributed to the organization as a whole. The organization is not responsible for any use that may be made of the information contained in this work.

Author details

[1]National Public Health Laboratory, Teku, Kathmandu, Nepal
[2]Central Veterinary Laboratory, Tripureshwor, Kathmandu, Nepal
[3]WHO Country Office, Nepal, UN House, Kupondole, Pulchowk, Lalitpur, Kathmandu, Nepal
[4]Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia
[5]Centre for Pathogen Genomics, The University of Melbourne, Melbourne, Australia
[6]WHO Collaborating Centre for Antimicrobial Resistance, The Peter Doherty Institute for Infection and Immunity, Melbourne, Australia

Received: 13 November 2024 / Accepted: 28 February 2025
Published online: 16 April 2025

References

1. Pana ZD, Zaoutis T. Treatment of extended-spectrum β-lactamase-producing enterobacteriaceae (ESBLS) infections: what have we learned until now? F1000Research. 2018;7(0):1–9.
2. Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. JAC-Antimicrobial Resist. 2021;3(3).
3. Vardakas KZ, Tansarli GS, Rafailidis PI, Falagas ME. Carbapenems versus alternative antibiotics for the treatment of bacteraemia due to enterobacteriaceae producing extended-spectrum β-lactamases: a systematic review and meta-analysis. J Antimicrob Chemother. 2012;67(12):2793–803.

4. Vaux S, Carbonne A, Thiolet JM, Jarlier V, Coignard B. Emergence of carbapenemase-producing enterobacteriaceae in France, 2004 to 2011. Eurosurveillance. 2011;16(22):1–7.
5. Kahn LH. Antimicrobial resistance: a one health perspective. Trans R Soc Trop Med Hyg. 2017;111(6):255–60.
6. World Health Organization. WHO integrated global surveillance on ESBL-producing E. coli using a. One Health approach: implementation and opportunities. Licence: CC BY-NC-SA 3.0 IGO. 2021. 76 p. Available from: https://www.who.int/publications/i/item/9789240021402%0Ahttps://www.who.int/publications/i/item/who-integrated-global-surveillance-on-esbl-producing-e.-coli-using-a-one-health-approach
7. Acharya J, Jha R, Gompo TR, Chapagain S, Shrestha L, Rijal N et al. Prevalence of Extended-Spectrum Beta-Lactamase (ESBL) – Producing *Escherichia coli* in Humans, Food, and Environment in Kathmandu, Nepal: Findings From ESBL *E. coli* Tricycle Project. 2024;2024.
8. Simar SR, Hanson BM, Arias CA. Techniques in bacterial strain typing: past, present, and future. Curr Opin Infect Dis. 2021;34(4):339–45.
9. Weinstein M. M100 performance standards for antimicrobial susceptibility testing. J Serv Mark. 2021;8:18–260.
10. Antimicrobial susceptibility testing clinical breakpoints.Breakpoint tables for interpretation of MICs and zone diameters,version 12.0,2022.. 12th ed. 2012. Available from: http://www.eucast.org/clinical_breakpoints
11. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol. 2012;19(5):455–77.
12. Sherry NL, Horan KA, Ballard SA, Gonçalves da Silva A, Gorrie CL, Schultz MB et al. An ISO-certified genomics workflow for identification and surveillance of antimicrobial resistance. Nat Commun. 2023;14(1).
13. Feldgarden M, Brover V, Gonzalez-Escalona N, Frye JG, Haendiges J, Haft DH et al. AMRFinderPlus and the reference gene catalog facilitate examination of the genomic links among antimicrobial resistance, stress response, and virulence. Sci Rep. 2021;11(1):1–9. Available from: https://doi.org/10.1038/s41598-021-91456-0
14. Robertson J, Nash JHE. MOB-suite: software tools for clustering, reconstruction and typing of plasmids from draft assemblies. Microb Genomics. 2018;4(8).
15. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, Von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. Mol Biol Evol. 2020;37(5):1530–4.
16. Beghain J, Bridier-Nahmias A, Nagard H, Le, Denamur E, Clermont O. ClermonTyping: an easy-to-use and accurate in silico method for *Escherichia* genus strain phylotyping. Microb Genomics. 2018;4(7):1–8.
17. Liu B, Zheng D, Zhou S, Chen L, Yang J. VFDB 2022: a general classification scheme for bacterial virulence factors. Nucleic Acids Res. 2022;50D1:D912–7.
18. Subramanya SH, Bairy I, Metok Y, Baral BP. Detection and characterization of ESBL – producing *Enterobacteriaceae* from the gut of subsistence farmers, their livestock, and the surrounding environment in rural Nepal. Sci Rep. 2021;1–13. Available from: https://doi.org/10.1038/s41598-021-81315-3
19. Milenkov M, Proux C, Lalaina Rasolofoarison T, Angelot Rakotomalala F, Rasoanandrasana S, Vonintsoa Rahajamanana L et al. Tricycle surveillance in Antananarivo, Madagascar: circulation of both extended-spectrum beta-lactamase producing *Escherichia coli* strains and plasmids among humans, chickens and the environment. medRxiv. 2023;2023.01.16.23284583. https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2021.786146/full
20. Runcharoen C, Raven KE, Reuter S, Kallonen T, Paksanont S, Thammachote J, et al. Whole genome sequencing of ESBL-producing *Escherichia coli* isolated from patients, farm waste and canals in Thailand. Genome Med. 2017;9(1):1–11.
21. Aworh MK, Kwaga J, Okolocha E, Harden L, Hull D, Hendriksen RS et al. Escherichia coli among humans, chickens and poultry environments in Abuja, Nigeria. One Heal. 2020;2(8).
22. Touchon M, Perrin A, De Sousa JAM, Vangchhia B, Burn S, O'Brien CL, et al. Phylogenetic background and habitat drive the genetic diversification of *Escherichia coli*. PLoS Genet. 2020;16:1–43.
23. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global extraintestinal pathogenic *Escherichia coli* (Expec) lineages. Clin Microbiol Rev. 2019;32(3):1–25.
24. Nicolas-Chanoine MH, Bertrand X, Madec JY. *Escherichia coli* st131, an intriguing clonal group. Clin Microbiol Rev. 2014;27(3):543–74.
25. Banerjee R, Johnson JR. A new clone sweeps clean: the enigmatic emergence of *Escherichia coli* sequence type 131. Antimicrob Agents Chemother. 2014;58(9):4997–5004.
26. Mathers AJ, Peirano G, Pitout JDD. The role of epidemic resistance plasmids and international high- risk clones in the spread of multidrug-resistant Enterobacteriaceae. Clin Microbiol Rev. 2015;28(3):565–91.
27. Shakya G, Upadhyaya B, Sc M, Rijal N, Sc M, Mahato MK, et al. Beta Lactam / Beta lactamase inhibitor combination in the treatment of urinary tract infections caused by extended spectrum Beta lactamase producing *Escherichia coli*. (J Infect Dis Antimicrob Agents 2014. 2014;31(2):1–9.
28. Ozsolak F, Milos PM. RNA sequencing: advances, challenges and opportunities. Nat Rev Genet. 2011 Feb;12(2):87-98. doi: 10.1038/nrg2934. Epub 2010 Dec 30. PMID: 21191423; PMCID: PMC3031867. https://pubmed.ncbi.nlm.nih.gov/21191423/
29. Mazumder R, Abdullah A, Ahmed D, Hussain A. High prevalence of blactx-m-15 gene among extended-spectrum β-lactamase-producing *Escherichia coli* isolates causing extraintestinal infections in Bangladesh. Antibiotics. 2020;9(11):1–11.
30. Mohsin M, Raza S, Schaufler K, Roschanski N, Sarwar F, Semmler T et al. High prevalence of CTX-M-15-type ESBL-producing *E. coli* from migratory avian species in Pakistan. Front Microbiol. 2017;8(DEC).
31. Tacão M, Laço J, Teixeira P, Henriques I. CTX-M-producing bacteria isolated from a highly polluted river system in Portugal. Int J Environ Res Public Health. 2022;19:19.
32. Tegha G, Ciccone EJ, Krysiak R, Kaphatika J, Chikaonda T, Ndhlovu I, et al. Genomic epidemiology of *Escherichia coli* isolates from a tertiary referral center in Lilongwe, Malawi. Microb Genomics. 2021;7(1):1–12.
33. Minja CA, Shirima G, Mshana SE. Conjugative plasmids disseminating ctx-m-15 among human, animals and the environment in Mwanza Tanzania: a need to intensify one health approach. Antibiotics. 2021;10(7).
34. Gomi R, Yamamoto M, Tanaka M, Matsumura Y. Chromosomal integration of blaCTX-M genes in diverse *Escherichia coli* isolates recovered from river water in Japan. Curr Res Microb Sci. 2022;3(June):100144. Available from: https://doi.org/10.1016/j.crmicr.2022.100144
35. Pokhrel RH, Thapa B, Kafle R, Shah PK, Tribuddharat C. Co-existence of beta-lactamases in clinical isolates of *Escherichia coli* from Kathmandu, Nepal. BMC Res Notes. 2014;7(1):1–5.
36. Lee Nyao, Lee C chi, Huang W. han, Tsui K chung, Hsueh P ren, Ko W chien. Carbapenem therapy for bacteremia due to extended-spectrum- ␣ -lactamase-producing *Escherichia coli* or *Klebsiella pneumoniae*: implications of ertapenem susceptibility. 2012;56(6):2888–93.
37. Nepal S, Koirala S, Thakur S, Bhattarai S, Dhungana S, Giri S, et al. Detection of extended spectrum β-lactamases (ESBL) producing bacteria in sepsis suspected neonates. Nepal J Heal Sci. 2021;1(2):7–14.
38. Tada T, Miyoshi-Akiyama T, Dahal RK, Sah MK, Ohara H, Kirikae T, et al. NDM-8 metallo-β lactamase in a multidrug-resistant *Escherichia coli* strain isolated in Nepal. Antimicrob Agents Chemother. 2013;57(5):2394–6.
39. Shrestha B, Tada T, Shimada K, Shrestha S, Ohara H, Pokhrel BM, et al. Emergence of various NDM-type- metallo-β lactamase producing *Escherichia coli* clinical isolates in Nepal. Antimicrob Agents Chemother. 2017;61(12):1–6.
40. Thapa A, Upreti MK, Bimali NK, Shrestha B, Sah AK, Nepal K, et al. Detection of NDM variants (blaNDM-1, blaNDM-2, blaNDM-3) from carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae*: first report from Nepal. Infect Drug Resist. 2022;15(August):4419–34.
41. OMS. WHO bacterial priority pathogens list. 2024. Bacterial pathogens of public health importance to guide research, development and strategies to prevent and control antimicrobial resistance. 2024. 72 p. Available from: https://www.who.int/publications/i/item/9789240093461
42. Muktan B, Thapa Shrestha U, Dhungel B, Mishra BC, Shrestha N, Adhikari N et al. Plasmid mediated colistin resistant mcr-1 and co-existence of OXA-48 among *Escherichia coli* from clinical and poultry isolates: first report from Nepal. Gut Pathog. 2020;12(1):1–9. Available from: https://doi.org/10.1186/s13099-020-00382-5
43. Karki D, Dhungel B, Bhandari S, Kunwar A, Joshi PR, Shrestha B et al. Antibiotic resistance and detection of plasmid mediated colistin resistance mcr-1 gene among *Escherichia coli* and *Klebsiella pneumoniae* isolated from clinical samples. Gut Pathog. 2021;13(1):1–16. Available from: https://doi.org/10.1186/s13099-021-00441-5
44. Bista S, Shrestha UT, Dhungel B, Koirala P, Gompo TR, Shrestha N, et al. Detection of plasmid-mediated colistin resistant mcr-1 gene in *Escherichia coli* isolated from infected chicken livers in Nepal. Animals. 2020;10(11):1–13.

45. Koirala A, Bhandari P, Shewade HD, Tao W, Thapa B, Terry R et al. Antibiotic use in broiler poultry farms in Kathmandu Valley of Nepal: which antibiotics and why? Trop Med Infect Dis. 2021;6(2).

Publisher's note