GENOME NOTE

REVISED Draft genome sequences of three emerging beta-lactamase-producing *Escherichia coli* in the camel production system in Northern Kenya [version 2; peer review: 2 approved]

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Abstract

We report the draft genome sequences and annotation of three beta-lactamase-producing *Escherichia coli* (*E.coli*) strains isolated from fecal samples of healthy camels in Laikipia county, Kenya. This data adds to the online genome resources to support the ongoing antimicrobial resistance surveillance in the livestock-wildlife interface.

Keywords

E.coli, beta-lactamase, AMR, genome, whole genome sequencing, camel

This article is included in the Genomics and Genetics gateway.

Open Peer Review

Approval Status ✓ ✓

	1	2
version 2 (revision) 16 May 2023	✓ view	
version 1 30 Nov 2022	? view	✓ view

1. **Meenakshi S. Iyer**, Tata Institute of Fundamental Research, Bengaluru, India

2. **Janet Midega**, KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

Any reports and responses or comments on the article can be found at the end of the article.

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Author roles: Gachogo R: Data Curation, Formal Analysis, Writing – Original Draft Preparation; **Karegi I**: Methodology, Writing – Review & Editing; **Ogoti B**: Data Curation, Formal Analysis, Methodology; **Musyoki V**: Data Curation, Methodology; **Martins D**: Project Administration, Resources; **Onyambu F**: Conceptualization, Funding Acquisition, Resources, Supervision, Writing – Review & Editing; **Kamau J**: Project Administration, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

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REVISED **Amendments from Version 1**

This version highlights the rationale of sequencing *E. coli* in camels. This version contains a table showing antibiotics susceptibility and beta-lactamase genes profile of the isolates sequenced.

Any further responses from the reviewers can be found at the end of the article

Introduction

Antimicrobial resistance (AMR), especially on the readily available beta-lactam antibiotics continues to threaten effective healthcare management and global economic success in livestock farming (Fashae et al., 2021; Kiiru et al., 2012). Recently, concerted efforts have been employed to explore the AMR situation following a One Health Approach Previously, we and others have demonstrated an increased occurrence of broad-spectrum producing *Enterobacteriaceae* in livestock (Akunda et al., 2023; Nüesch-Inderbinen et al., 2020). Importantly, extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* have been implicated in the development of multi-drug resistance in both humans and animals. However, there still exists a gap in whole genome sequencing data on *Enterobacteriaceae* harboring AMR genes in camels (*Camelus dromedaries*), a key component of livestock farming in arid and semi-arid regions of Northern Kenya. We therefore aimed at sequencing the genomes of extended-spectrum beta lactamase *Enterobacteriaceae* using *Escherichia coli* (*E. coli*) as our model organism.

Methods

Here, we report three draft genome sequences of beta-lactamase-producing *E. coli* isolated from camel fecal samples in Laikipia county, Northern Kenya as previously described (Akunda et al., 2023). Fecal samples were collected from healthy camels reared under both ranching systems and pastoralism, transported in Cary Blair media (HiMedia Lab, Mumbai, India) and stored at 4°C awaiting processing.

The samples were cultured on MacConkey agar plate (HiMedia Lab, Mumbai, India) and incubated at 37°C for 18 hours. A single colony per MacConkey agar plate that displayed *E. coli* traits morphologically was identified through Gram staining and the IMViC (indol, methyl red, Voges Proskauer, and citrate) biochemical method and subjected to antimicrobial susceptibility testing (AST) against the beta-lactam antibiotic spectrum. Briefly, antimicrobial susceptibility testing (AST) was performed on Mueller Hinton agar (HiMedia Lab, Mumbai, India) using Kirby Bauer disk diffusion method. Antibiotics tested included, Ampicillin-10 µg (AMP), Chloramphenicol-30 µg (CHL), Tetracycline-30 µg (TCY), Gentamycin-10 µg (GEN), Streptomycin-10 µg (STR1), Trimethoprim-sulfamethoxazole-25 µg (SXT), Norfloxacin-10 µg (NOR), Ciprofloxacin-5 µg (CIP), Cefaclor-30 µg (CEC), Ceftriaxone-30 µg (CRO), Cefotaxime-30 µg (CTX), Cefuroxime-30 µg (CXM), Cefepime-30 µg (FEP), Amoxicillin–Clavulanate-20/10 µg (AMC), and Ceftazidime-30 µg (CAZ). The antibiotic susceptibility profile of the sequenced isolates is as shown in Table 1.

Production of beta-lactamase genes was assessed by conducting PCR to confirm genes encoding for CTX-M, TEM, CMY, SHV and OXA for the resistant isolates (Livermore et al., 2007), results are as shown in Table 1. Briefly, a 20 ul PCR reaction was prepared by adding 10 ul of the Platinum II Hot-Start PCR Master Mix (Invitrogen, Carlsbad, CA, U.S), 0.4 ul of 10 nmol of forward and reverse primers, 4 ul of Platinum GC enhancer, 0.2 ul nuclease free water and 5 ul DNA. Cycling was performed on Veriti™ Dx 96-well Thermal Cycler (Thermofisher, Waltham, Massachusetts, U.S) using

Table 1. Antibiotic sensitivity profile and beta-lactamase genes profile (+) present, (-) absent.

	E. coli **(IPR_LC 17)**	***E. coli*** **(IPR_LC19)**	***E. coli*** **(IPR_LC20)**
Livestock production System	Intensive	Extensive	Intensive
Antibiotic sensitivity profile	AMC, CAZ, CTX, CXM, CEC, CHL, TCY	AMC, CAZ, CTX, CRO, CXM, FEP, CEC, SXT	AMC, CAZ, CTX, CRO, CXM, FEP, CEC, TCY
CTX-M	+	+	+
TEM	+	+	+
SHV	-	-	-
OXA	-	-	-

Table 2. Primers used for detection of blaTEM, blaCTX-M, blaCMY, blaOXA, and blaSHV gene(s) minutes (min), Seconds (sec), base pairs (bp).

Primers	Oligonucleotide Sequence (5′-3′)	Annealing Temperature	PCR conditions	Product Size (bp)
TEM	F-ATGAGTATTCAACATTTCCG R-CTGACAGTTACCAATGCTTA	55°C	94°C: 2 min 94°C: 15 Sec X35 Annealing Temperature: 15 Sec X35 68°C:15 Sec X35 4°C:10 min	840
SHV	F-GGTTATGCGTTATATTCGCC R-TTAGCGTTGCCAGTGCTC	50°C		854
CTX-M	F-ATGTGCAGYACCAGTAARGT R-TGGGTRAARTARGTSACCAGA	60°C		593
CMY	F-ATGATGAAAAAATCGTTATGC R-TTGCAGCTTTTCAAGAATGCGC	55°C		1200
OXA	F-TCAACTTTCAAGATCGCA R-GTGTGTTTAGAATGGTGA	62°C		820

primers and PCR conditions as in Table 2. The respective gene fragment sizes were confirmed in 1% agarose gel (Invitrogen, Carlsbad, CA, U.S). ESBL producing *E. coli* isolates were phenotypically confirmed using double-disc synergy test (DDST) (Drieux *et al.*, 2008).

Genomic DNA of the confirmed ESBL producing *E. coli* isolates was extracted using Isolate II Genomic DNA Kit (Bioline, UK) and sequencing performed using Oxford Nanopore Technologies (ONT, Oxford, UK). Briefly, the manufacturer's sample barcodes (Native Barcoding Expansion 1-12) (EXP-NBD104) and sequencing adapters (Ligation Sequencing kit) (SQK-LSK109)) were added following kit instructions. Sequencing was performed on MinION device using R9.4.1 flow cell to generate 186, 38153, and 31152 raw reads for the *E. coli* strains IPR_LC17, IPR_LC19 and IPR_LC20, respectively. Basecalling and demultiplexing of raw reads were performed using Guppy v3.6.1 (https://nanoporetech.com). Adaptors were trimmed using PORECHOP v0.2.4 (Wick et al., 2017) and poor quality reads, less than 500 bp and with an average quality score of below 10 were removed using NANOFLIT v2.8.0 (De Coster et al., 2018).

E. coli PR_LC17 genome was assembled using Canu v2.2 (Koren et al., 2017) while *E. coli* (IPR_LC19) and *E. coli* (IPR_LC20) were assembled using Flye v2.9.1 (Kolmogorov et al., 2019). The taxonomy of the organism was confirmed using public databases for molecular typing and microbial genome diversity (PubMLST) (Jolley et al., 2018). Quality of the assembled genomes was assessed using QUAST v5.1.0 (Gurevich et al., 2013) and annotated using NCBI's PGAP (Tatusova et al., 2016). The assembly metrics and annotation summaries are as shown in Table 3.

Table 3. PGAP genome annotation of *E. coli* (IPR_LC17, IPR_LC19 & IPR_LC20 strains).

Feature	*E. coli* (IPR_LC17)	*E. coli* (IPR_LC19)	*E. coli* (IPR_LC20)
Genome size (Mbp)	5.08	4.70	5.66
GC content (%)	50.76	51.00	50.52
Sequencing coverage (X)	33	18	10
No. of contigs	2	2	13
Genome fraction (%)	74.07	72.62	78.97
N_{50} (bp)	4,984,001	4,606,403	4, 015,521
Genes (total)	5398	4849	6491
CDSs (total)	5277	4730	6367
Genes (coding)	1409	1604	2115
Pseudo Genes	3868	3126	4252
rRNAs	22	22	22
tRNAs	89	85	92
ncRNAs	10	12	10
CRISPR sequences	0	0	1

Ethical considerations
The project was approved by Institute of Primate Research (IPR) review committee (ISERC/10/2020).

Data availability
Underlying data
IPR_LC20

Genbank: Escherichia coli strain IPR_LC20, whole genome shotgun sequencing project, Accession number JAOZEZ000000000: https://www.ncbi.nlm.nih.gov/nuccore/JAOZEZ000000000.1/

Sequence Read Archive (SRA): Oxford Nanopore Reads of Escherichia coli in camel production systems in Northern Kenya, Accession number SRR21998714: https://www.ncbi.nlm.nih.gov/sra/SRR21998714

IPR_LC19

Genbank: Escherichia coli strain IPR_LC19, whole genome shotgun sequencing project, Accession number JAOZFA000000000: https://www.ncbi.nlm.nih.gov/nuccore/JAOZFA000000000.1/

Sequence Read Archive (SRA): Oxford Nanopore Reads of Escherichia coli in camel production systems in Northern Kenya, Accession number SRR21998715: https://www.ncbi.nlm.nih.gov/sra/SRR21998715

IPR_LC17

Genbank: Escherichia coli strain IPR_LC17, whole genome shotgun sequencing project, Accession number JAOZFB000000000: https://www.ncbi.nlm.nih.gov/nuccore/JAOZFB000000000.1/

Sequence Read Archive (SRA): Oxford Nanopore Reads of Escherichia coli in camel production systems in Northern Kenya, Accession number SRR21998716: https://www.ncbi.nlm.nih.gov/sra/SRR21998716

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PubMed Abstract | Publisher Full Text

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Open Peer Review

Current Peer Review Status: ✔ ✔

Version 2

Reviewer Report 29 August 2023

https://doi.org/10.5256/f1000research.147722.r173957

✔ **Meenakshi S. Iyer**
National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru, India

I have gone through the revised version of the manuscript. Most of the suggested changes have been addressed. I approve this version of the manuscript.

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genomics, Transcriptomics, Anti-microbial resistance, Protein sequence-structure-funcion relationships, Database and algorithm development, Big-data analysis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Reviewer Report 02 March 2023

https://doi.org/10.5256/f1000research.140538.r162944

✔ **Janet Midega**
KEMRI-Wellcome Trust Research Programme, Kilifi, Kenya

The authors provide details of a study in which beta lactamase producing Ecoli from fecal samples are sequenced from Camels in Northern Kenya. Whilst this is an important study given the close

interactions between human and camels in this part of Kenya, hence the likelihood of Animal to human transmission of beta lactamase producing E coli, It might help if the authors can write down a justification for why the study was conducted.

Why was there a focus on beta-lactamase producing E coli only? For example is it the most important isolate in this (human and camel) population?

In total how many camels are represented in the study or were these random fecal samples?

Is it possible to be more precise about the location, for example provide GPS co-ordinates of the sampling locations - A county is a very wide area.

Are the rationale for sequencing the genome and the species significance clearly described?
Partly

Are the protocols appropriate and is the work technically sound?
Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?
Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?
Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genomic epidemiology, Microbiology

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Reviewer Report 04 January 2023

https://doi.org/10.5256/f1000research.140538.r157281

?

Meenakshi S. Iyer
National Centre for Biological Sciences, Tata Institute of Fundamental Research, Bengaluru, India

In this manuscript the authors present a genome sequences of three *E. coli* strains isolated from camels in Northern Kenya. The importance of studying drug-resistant bacteria from livestock is

obvious and these sequences are of course a very useful resource for the community. However I have a request for minor revisions. I found that the paper needs to address a few more points:

1. The authors need to provide more background on the need for sequencing *E. coli* samples from camel populations. There are some recent studies on beta-lactamase producing isolates from camle and other populations from Kenya. These studies (and more) can be referenced: Karegi I et al., 2022 DOI:https://doi.org/10.1016/j.ijid.2021.12.018; Anyanwu MU et al., 2012 https://doi.org/10.1155/2021/6630379; and Nueesch-Inderbinen M et al., 2020 https://doi.org/10.1016/j.sciaf.2020.e00274.

2. The results can be recorded in a separate section. Table 2 on genome annotation statistics can be moved to this section.

3. The results for the antibiotic susceptibility testing for the three isolates can be provided as a separate table.

4. The results of the PCR reactions for the 3 isolates describing which plasmids were detected from the samples need to be provided. These results can be corroborated with existing literature on the beta-lactamase encoding plasmid prevalence in Africa.

5. In Table 2, the statistics for partial genes detected in each strain can be listed. Overall, the authors can comment on the draft nature of the genome in the results section (% completeness, number of contigs etc.).

References

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3. Nüesch-Inderbinen M, Kindle P, Baschera M, Liljander A, et al.: Antimicrobial resistant and extended-spectrum ß-lactamase (ESBL) producing Escherichia coli isolated from fecal samples of African dromedary camels.*Sci Afr*. 2020; **7**: e00274 PubMed Abstract | Publisher Full Text

Are the rationale for sequencing the genome and the species significance clearly described?

Partly

Are the protocols appropriate and is the work technically sound?

Yes

Are sufficient details of the sequencing and extraction, software used, and materials provided to allow replication by others?

Yes

Are the datasets clearly presented in a usable and accessible format, and the assembly and annotation available in an appropriate subject-specific repository?

Yes

Competing Interests: No competing interests were disclosed.

Reviewer Expertise: Genomics, Transcriptomics, Anti-microbial resistance, Protein sequence-structure-funcion relationships, Database and algorithm development, Big-data analysis

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.