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Clinical validation of loop-mediated isothermal amplification for the detection of *Escherichia coli* sequence type complex 131

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ABSTRACT The dissemination of *Escherichia coli* multidrug-resistant (MDR) STc131 is related to its persistence in the human gastrointestinal tract as efficient gut colonizers. Infection and prevention measures are the cornerstones for preventing STc131 spread. Oral decolonization therapies that target ST131 are being developed. There are no rapid methods available to identify STc131 in human specimens. A loop-mediated isothermal amplification (LAMP) assay (named LAMP-ST131) was developed for the detection of STc131 on well-characterized *E. coli* isolates and then compared to culture and PCR for urines and stool swabs. With *E. coli* isolates (n = 720), LAMP-ST131 had a sensitivity (sens) of 100% [95% confidence interval (C.I.) = 98.1–100%)] and a specificity (spec) of 98.9% (95% C.I. = 97.5–99.5%). On urines (n = 550), LAMP-ST131 had a sens of 97.6% (95% C.I. = 89.68–94.33%) and a spec of 92.3% (95% C.I. = 87.68–99.88%), while on stool swabs (n = 278), LAMP-ST131 had a sens of 100% (95% C.I. = 88.7–100%) and a spec of 83.9% (95% C.I. = 78.8–87.9%). LAMP-ST131 detected 10 (urines) and 100 (stool swabs) gene copies/µL. LAMP-ST131 accurately identified STc131 within *E. coli* isolates and human specimens. The implementation of LAMP-ST131 will aid genomic surveys, enable the rapid implementation of effective infection prevention measures, and identify patients suitable for ST131 decolonization therapies. Such approaches will curb the spread of STc131 and decrease incidence rates of global MDR *E. coli* infections.

IMPORTANCE We developed an accurate non-culture-based loop-mediated isothermal amplification (LAMP) methodology for the detection of (sequence type) STc131 among *Escherichia coli* isolates and human specimens. The use of LAMP-ST131 for global genomic surveillance studies and to identify patients that are suitable for ST131 decolonization therapies will be important for decreasing multidrug-resistant *E. coli* infections across the globe.

KEYWORDS LAMP, *E. coli* ST131, specimens

The *Escherichia coli* (sequence type) ST131 is a well-established fluoroquinolone-resistant, CTX-M- and carbapenemase-producing global high-risk clone, and is responsible for millions of community-acquired urinary tract infections (UTIs) and bloodstream infections annually (1). ST131 single locus variants (SLVs) have rarely been reported and, collectively with ST131, are referred to as the sequence type complex STc131 (2). Retrospective genomic studies have shown that STc131 had largely been responsible for the increase in global multidrug-resistant (MDR) extra-intestinal *E. coli* infections during the mid to late 2000s (3). ST131 belongs to three clades (A, B, and C) and four subclades (C0, C1, C1_M27, and C2) (4). The MDR clade ST131-C with CTX-M β-lactamases dominates the global population structure of this lineage. The global spread of ST131-C has indirectly led to the increased use of carbapenems, with the subsequent increase in carbapenem resistance (5).

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Multilocus sequence typing is the gold standard to identify STc131 isolates (https://github.com/tseemann/mlst), while whole genome sequencing (WGS) phylogenetic analysis is necessary to confirm different ST131 clades (6). Several non-sequenced-based methods are available for the rapid detection of STc131 (7). These techniques are performed on bacterial isolates and include the following: several PCRs (7), multiplex PCR to identify different clades (8), PCR followed by high-resolution melting (9), matrix-assisted laser desorption ionization–time of flight mass spectrometry (10), and monoclonal antibodies (11).

We developed a loop-mediated isothermal amplification (LAMP) assay (named LAMP-ST131) for the detection of *E. coli* STc131 on bacterial isolates and human specimens. LAMP-ST131 can be utilized to identify STc131 among *E. coli* collections in genomic surveys and to detect patients colonized and infected with this lineage for decolonization therapies.

MATERIALS AND METHODS

Overview of the study design

The LAMP-ST131 primers were designed using Primer Explorer V5. These primers were first validated using *E. coli* isolates (n = 260) that had previously been characterized with WGS. The next step was to evaluate the performance of LAMP-ST131 on a Calgary clinical *E. coli* collection (n = 460) that was previously tested with a ST131 PCR.

The next phase of the study was the validation of LAMP-ST131 on patient specimens [i.e., urines (n = 550) and stool swabs (n = 278)]. This included the level of detection on patient specimens.

LAMP-ST131 primer design, specimen preparation, and assay conditions

An ST131-specific region was chosen as the amplification target for LAMP-ST131. This region consisted of a 580 bp amplicon that was previously identified for a ST131 specific PCR assay (8). The LAMP-ST131 primer set (a total of six primers) was designed using "Primer Explorer V5" (http://primerexplorer.jp/e/) and the specificity of the primers was confirmed using the Primer-BLAST website (https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The LAMP-ST131 primer sequences are shown in Table 1.

Bacterial isolates, urines, and stool swabs were processed as follows: (i) *E. coli* isolates were grown from frozen overnight on blood agar plates. Three to five colonies of each isolate were suspended in 300 µL of 10 mM Tris-EDTA buffer, heated at 95°C for 10 min, and subsequently centrifuged for 2 min at 14,000 rpm. (ii) Urine specimens were defrosted from −80°C, then briefly vortexed, and 300 µL was aliquoted to a microfuge tube, heated at 95°C for 10 min, and centrifuged for 2 min at 14,000 rpm. (iii) Stool swabs were defrosted from −80°C, then vortexed briefly in 300 µL of 10 mM Tris-EDTA buffer in a microfuge tube. Stool suspensions were heated at 95°C for 10 min and subsequently centrifuged for 2 min at 14,000 rpm.

LAMP-ST131 reactions were set up in a MicroAmp Fast Optical 96-Well plate (Applied Biosystems, USA), with each well containing 12.5 µL WarmStart 2X LAMP master mix, 0.125 µL of fluorescent dye (New England Biolabs, ON, Canada), 0.3 µM of each outer primer (F3/B3), 1.6 µM of each inner primer (FIP/BIP), 0.8 µM of each loop primer (LF/LB),

TABLE 1 LAMP-ST131 primers

Primer name	Nucleotide sequence (5′–3′)	Size (bp)
ST131-ID20_F3	GTTCGCCAGCCAGAACAT	18
ST131-ID20_B3	CGTGATACCAGCAACGAAGT	20
ST131-ID20_FIP	GCACGCCCGGTATTGGTGATGATAAAACAGTCAGCGGCAGA	41
ST131-ID20_BIP	GTGGAACAGCGGCACAACCAAGCCCAGGTTACCGTATCC	39
ST131-ID20_LF	GCCAGCCAGGCCTTTAACG	19
ST131-ID20_LB	GACAAAGCAGAGACATAACCGT	22

TABLE 2 Performance of LAMP-ST131 with *E. coli* isolates, human urines, and stool swabs[a]

	LAMP-ST131 positive	LAMP-ST131 negative
Well-characterized global *E. coli* collection (*n* = 260)		
ST1c131 (*n* = 84)[b]	84	0
Non-ST131 (*n* = 176)[c]	0	176
Calgary clinical blood *E. coli* collection (*n* = 460)		
ST131 PCR positive (*n* = 111)	111	0
ST131 PCR negative (*n* = 349)	6[d]	343
Urines (*n* = 550)		
ST131 culture positive (*n* = 42)	41	1
ST131 culture negative (*n* = 508)	39	469
Stool swabs (*n* = 278)		
ST131 culture positive (*n* = 30)	30	0
ST131 culture negative (*n* = 248)	40	208

[a]LAMP-ST131 with well-characterized isolates showed a sensitivity of 100% (95% C.I. = 95.63–100%]) a specificity of 100% (95% C.I. = 97.86–100%); with clinical blood isolates, it showed a sensitivity of 100% (95% C.I. = 96.65–100%) and a specificity of 98.28% (95% C.I. = 96.30–99.21%); with urines, it showed a sensitivity of 97.6% (95% C.I. = 89.68–94.33%) and a specificity of 92.3% (95% C.I. = 87.68–99.88%); with stools, it showed a sensitivity of 100% (95% C.I. = 88.65–100) and a specificity of 83.87% (95% C.I. = 78.78–87.93%).
[b]STc131 consisted of ST131 (*n* = 77) and ST131 SLVs (*n* = 7). The ST131 isolates belonged to the following clades: A (*n* = 10), B (*n* = 15), C0 (*n* = 5), C1-nonM27 (*n* = 20), C1-M27 (*n* = 4), and C2 (*n* = 23). The ST131 SLVs belonged to ST4171, ST5941, ST9126, ST12149, and ST12151 (one each) and two ST8671 isolates.
[c]Non-ST131 isolates consisted of the following: ST101 (*n* = 1), ST10 (*n* = 3), ST12 (*n* = 6), ST69 (*n* = 4), ST11316 (*n* = 1), ST117 (*n* = 2), ST1193 (*n* = 5), ST1196 (*n* = 1), ST127 (*n* = 3), ST1312 (*n* = 1), ST14 (*n* = 10), ST141 (*n* = 3), ST1431 (*n* = 2), ST144 (*n* = 3), ST155 (*n* = 1), ST156 (*n* = 2), ST167 (*n* = 6), ST182 (*n* = 1), ST1858 (*n* = 1), ST1876 (*n* = 3), ST2003 (*n* = 2), ST205 (*n* = 1), ST2038 (*n* = 2), ST224 (*n* = 2), ST297 (*n* = 1), ST34 (*n* = 1), ST345 (*n* = 1), ST3478 (*n* = 1), ST349 (*n* = 1), ST354 (*n* = 2), ST357 (*n* = 1), ST361 (*n* = 3), ST372 (*n* = 2), ST38 (*n* = 6), ST393 (*n* = 3), ST399 (*n* = 1), ST4 (*n* = 1), ST404 (*n* = 8), ST405 (*n* = 4), ST409 (*n* = 4), ST410 (*n* = 6), ST4167 (*n* = 1), ST428 (*n* = 1), ST429 (*n* = 2), ST43 (*n* = 1), ST44 (*n* = 2), ST443 (*n* = 1), ST448 (*n* = 3), ST453 (*n* = 3), ST457 (*n* = 2), ST46 (*n* = 2), ST491 (*n* = 1), ST5021 (*n* = 1), ST538 (*n* = 1), ST540 (*n* = 1), ST550 (*n* = 3), ST568 (*n* = 4), ST569 (*n* = 4), ST58 (*n* = 1), ST582 (*n* = 1), ST617 (*n* = 2), ST624 (*n* = 1), ST648 (*n* = 4), ST69 (*n* = 3), ST73 (*n* = 5), ST88 (*n* = 3), and ST95 (*n* = 12).
[d]The isolates belonged to ST1193 (*n* = 1), ST73 (*n* = 3), and ST95 (*n* = 2).

and 4 µL of each (bacterial isolate, urine, or stool swab) lysate. Nuclease-free water was added up to a total volume of 25 µL. LAMP-ST131 was carried out on a QuantStudio Dx Real-Time PCR instrument (Applied Biosystems, USA) at 64°C for 60 min (120 min—capture sign every 30 s).

LAMP-ST131 performance using bacterial isolates

A total of 260 well-characterized global *E. coli* isolates were included in the initial validation of LAMP-ST131 (Table 2). The *E. coli* isolates were randomly selected and obtained from North America (i.e., Canada and USA), Latin America (i.e., Mexico, Guatemala, Colombia, Brazil, and Argentina), Europe (i.e., Spain, France, Belgium, Italy, Greece, Netherlands, Romania, and Serbia), Africa (i.e., Egypt, Morocco, Tunisia, and South Africa), Middle East (i.e., UAE, Lebanon, and Jordan), Asia (i.e., India, Vietnam, Thailand, Philippines, and Japan), and Oceania (i.e., New Zealand). This *E. coli* global collection previously underwent Illumina NextSeq500 short-read WGS (Illumina, San Diego, CA, USA) and was obtained from previous molecular epidemiology studies (12–17). The STc131 isolates consisted of ST131 (*n* = 77) and ST131 SLVs (*n* = 7) (one each of ST4171, ST5941, ST9126, ST12149, and ST12151 and two each of ST8671 isolates). The ST131 isolates belonged to the following clades: A (*n* = 10), B (*n* = 15), C0 (*n* = 5), C1-nonM27 (*n* = 20), C1-M27 (*n* = 4), and C2 (*n* = 23). The non-STc131 *E. coli* isolates (*n* = 176) consisted of 67 different sequence types and are listed in Table 2.

After the initial validation process, the performance of LAMP-ST131 was verified using a Calgary *E. coli* clinical blood collection (*n* = 460) (18) (Table 2). This collection consisted of sequential, non-repeat *E. coli* isolates obtained in 2010 and was previously tested with a ST131 specific PCR (8).

LAMP-ST131 validation using patient samples

De-identified, sequential, non-repeat clinical specimens [i.e., urines (*n* = 550) and stool Amies swabs (*n* = 278)] were frozen at −80°C and used to validate LAMP-ST131. Clinical specimens were defrosted, then cultured on Thermo Scientific Oxoid MacConkey Agar, and lactose fermenting colonies underwent matrix-assisted laser desorption ionization–time of flight mass spectrometry (bioMérieux Vitek Systems Inc., Hazelwood, MO). Isolates that were identified as *E. coli* were then screened with a ST131 PCR (8). Five colonies per specimen were tested.

LAMP-ST131 limit of detection

Limit of detection (LOD) of LAMP-ST131 was done on urine and stool swab specimens. Specimens were initially resuspended in 1 mL of Tris-EDTA buffer. LOD was done using 10-fold serial dilutions of ST131 culture-positive (non-contrived) specimens in Tris-EDTA buffer. A ST131 digital droplet PCR (ddPCR) (BioRad, Hercules, USA) assay was used to quantify the number of gene copies in each dilution. The design of the ST131 ddPCR assay and calculation of ST131 gene copies are shown in Supplementary Materials.

Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of LAMP-ST131 were calculated for bacterial isolates, urines, and stool swab specimens. Optimized cut-off cycle threshold (Ct) values were estimated for each assay type by finding the highest Youden index (sensitivity + specificity − 100) in the data set.

For *E. coli* isolates, WGS (population 1) and ST131-PCR (population 2) were considered as the gold standard for comparisons against LAMP-ST131 (Table 2). For urines and stool swabs, bacterial culture followed by ST131 PCR on cultured isolates (8) was considered as the gold standard for comparisons against LAMP-ST131 (performed directly on urines and stool swabs) (Table 2).

To determine the discrimination value of our analysis, receiver operational characteristic (ROC) curves were generated for bacterial isolates, urines, and stool swabs, along with the area under the curve (AUC). All the statistical analysis, box plots, and ROC curves were generated in GraphPad Prism (version 9.5.1).

RESULTS AND DISCUSSION

LAMP-ST131 performance with bacterial isolates

LAMP assays are isothermal, rapid, detectable to the naked eye, room temperature stable, and do not require expensive/time-consuming extraction/ purification steps (19). LAMP methods had previously been utilized to detect *E. coli* isolates (20), including antimicrobial resistance determinants among *E. coli* isolates (21). LAMP is also suited for testing on human specimens because this technology is less susceptible to inhibitors (19) and has been used to detect *E. coli* in stools (22) as well as urines (23).

The initial validation of LAMP-ST131 used a global well-characterized *E. coli* population (Table 2): STc131 (*n* = 84) *vs* known non-ST131 *E. coli* isolates (*n* = 176) (that had previously been characterized by WGS). LAMP-ST131 showed a perfect AUC of 100% with an optimal cut-off value of 36.79 Ct (Fig. S1), displaying a sensitivity of 100% (95% C.I. = 95.63–100%) and a specificity of 100% (95% C.I. = 97.86%–100%) (Table S1). The PPV and NPV were equivalent to 100% (95% C.I. = 95.63–100%) and 100% (95% C.I. = 97.86–100%), respectively.

LAMP-ST131 performance was then verified using a Calgary clinical *E. coli* collection (Table 2) consisting of PCR-positive ST131 (*n* = 111) *vs* unknown sequence type isolates (*n* = 349) (ST131 PCR negative). LAMP-ST131 showed an AUC of 98.70% with an optimal cut-off value of 35.77 Ct (Fig. S2), displaying a sensitivity of 100% (95% C.I. = 96.65–100%) and a specificity of 98.28% (95% C.I. = 96.30–99.21%). The ST131 prevalence in this collection was 24% resulting in a PPV of 94.84% (95% C.I. = 89.26–97.60%) and an NPV of 100% (95% C.I. = 98.93–100%) (Youden index = 0.9946) (Table S2).

LAMP-ST131 can be utilized to identify STc131 among *E. coli* collections for genomic surveys. Due to the potential of user-friendly and cost-effective LAMP instrumentation, LAMP-ST131 has the potential for future utilization in resource-limited countries to identify ST131 in genomic surveys (24). We are currently in the process of converting LAMP-ST131 into a cost-effective point-of-care and near-patient testing versions. Such methodologies can easily be incorporated into global MDR molecular surveillance networks including lower- and middle-income countries (LMICs) (25).

LAMP-ST131 validation using clinical samples

The rapid dissemination of ST131 is related to its persistence in the human gastrointestinal tract (GIT) as efficient gut colonizers (26). Prolonged gut colonization increases the ability of ST131 to spread among hosts and cause extraintestinal infections (e.g., UTIs) (26, 27).

ST131 is often the most common and most MDR clone among *E. coli* bloodstream infections (28). The elimination of ST131 will lead to significant overall decreases in incidence and MDR rates among *E. coli* isolates causing bloodstream infections (12, 29) (e.g., the removal of ST131 will decrease the overall incidence rate of Calgary *E. coli* bloodstream infections from 48.8 to 38.4/100,000 patient years). This was especially evident among Calgary long-term-care facility residents where the removal of ST131 will decrease the overall incidence rates of *E. coli* bloodstream infections from 857 to 544/100,000 patient years (12, 29).

Oral decolonization therapies (e.g., phages) targeting ST131 are being developed (30–32). Animal studies have shown that ST131-specific phages can decolonize the GIT of mice previously colonized with ST131 (30–32). Such therapies have the potential to decrease ST131 numbers and perhaps eliminate this lineage from the human GIT. The implementation of effective IP&C measures and the potential elimination of ST131 will decrease MDR *E. coli* infections and aid in curbing the MDR pandemic (33).

Culture-based approaches followed by ST131 PCR of *E. coli* isolates are the only methodologies available to identify ST131 from patient specimens (33). These methodologies are not sensitive, not standardized, labor-intensive, and time-consuming. There are no rapid specimen methods available for the detection of patients colonized or infected with ST131.

The best cut-off Ct value of LAMP-ST131 for urine specimens was 60.05 min, with a Youden index of 89.94. A sensitivity of 97.6% (95% C.I. = 89.68–94.33%) and a specificity of 92.3% (95% C.I. = 87.68–99.88%) were recorded with this cut-off. The AUC of the ROC was equivalent to 97.38, suggesting that this cut-off value is sufficient as a proxy for discrimination (Fig. S3). The ST131 prevalence among urines was 8% resulting in a PPV of 51.3% (95% C.I. = 44.90–60.01%) and an NPV of 99.8% (95% C.I. = 98.47–99.97%) (Table S3).

The best cut-off Ct value of LAMP-ST131 for stool swab specimens was 57.94 min with a Youden index of 83.87. A sensitivity of 100 (95% C.I. = 88.65–100%) and a specificity of 83.87% (95% C.I. = 78.78–87.93%) were recorded with this cut-off. The AUC of the ROC was equivalent to 93.76, suggesting that this cut-off value is sufficient as a proxy for discrimination (Fig. S4). The ST131 prevalence among stool swabs was 11% resulting in a PPV of 42.9% (95% C.I = 36.59–50.44%) and an NPV of 100% (95% C.I. = 98.24–100%) (Table S4).

One of the limitations of this study is that we used non-sensitive culture-based methodologies as the gold standard to evaluate the performance of LAMP-ST131 for the detection of STc131 on human specimens: 39/508 (7.6%) culture-negative urines and 40/248 (16%) culture negative stool swabs were false positive with LAMP-ST131. Is it likely that some of these specimens were actually positive for ST131 due to the inaccuracies of culture-based methodologies (34). It is also possible that LAMP-ST131 caused false negative results.

LAMP-ST131 will be able to identify patients colonized or infected with ST131 resulting in the rapid implementation of effective IP&C measures (33). LAMP-ST131 will

TABLE 3 LAMP-ST131 limit of detection for ST131-positive urines and ST131-positive stool swab specimens[a]

ddPCR, gene copies/µL[b]	Urines (Ct cut-off, 60.05)	Stool swabs (Ct cut-off, 57.94)
10^6	–[c]	–
10^5	8/8	–
10^4	8/8	8/8
10^3	8/8	8/8
10^2	8/8	8/8
10	8/8	3/8
1	7/8	1/8
10^{-1}	4/8	0/8
10^{-2}	0/8	0/8
10^{-3}	–	0/8

[a]Gene copies were calculated with ddPCR. Ct, cut-off cycle threshold.
[b]Order of magnitude, ddPCR corrected.
[c]Concentration not tested or out of reach.

also detect patients suitable for ST131 decolonization therapies. The potential elimination of ST131 from the GIT of patients will aid in decreasing the overall incidence rates of *E. coli* bloodstream infections, especially among LTCF residents and will also help to curb the spread of this MDR lineage (12, 29).

Limit of detection

The LOD for LAMP-ST131 was determined for urine and stool swab specimens. A 10-fold serially diluted sample from each sample type was tested with LAMP-ST131 and quantified with ddPCR. The LOD for urines and stool swabs were confirmed at 10 and 100 gene copies/µL, respectively (Table 3).

Conclusion

We developed an accurate non-culture-based LAMP methodology for the detection of STc131 among *E. coli* isolates and human specimens. Youden index analysis showed similar Ct cut-offs for bacterial populations when compared to Ct cut-offs for clinical specimens. This is likely due to the lower number of gene copies in clinical specimens. Cost-effective LAMP methodologies that contain LAMP-ST131 can easily be incorporated into LMIC MDR molecular surveillance networks. The use of LAMP-ST131 for global genomic surveillance studies and to identify patients that are suitable for ST131 decolonization therapies will be important for decreasing MDR *E. coli* infections across the globe.

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AUTHOR CONTRIBUTIONS

Gisele Peirano, Conceptualization, Data curation, Formal analysis, Methodology, Validation | L. Ricardo Castellanos, Conceptualization, Data curation, Methodology, Project administration, Writing – review and editing | Ryan Chaffee, Formal analysis, Methodology, Writing – review and editing | Daniel Castañeda-Mogollón, Data curation, Formal analysis, Methodology, Writing – review and editing | Johann D. D. Pitout, Conceptualization, Data curation, Funding acquisition, Methodology, Resources, Validation, Writing – original draft.

ETHICS APPROVAL

Ethics approval for this study was obtained through the University of Calgary Conjoint Health Research Ethics Board (REB17-0277).

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Supplemental material (JCM01687-23-s0001.docx). Supplemental text, tables, and figures.

REFERENCES

1. Pitout JD, Peirano G, DeVinney R. 2023. The contributions of multidrug resistant clones to the success of pandemic extra-intestinal pathogenic *Escherichia coli*. Expert Rev Anti Infect Ther 21:343–353. https://doi.org/10.1080/14787210.2023.2184348
2. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. 2019. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. Clin Microbiol Rev 32:e00135-18. https://doi.org/10.1128/CMR.00135-18
3. Pitout JDD, DeVinney R. 2017. *Escherichia coli* ST131: a multidrug-resistant clone primed for global domination. F1000Res 6:F1000 Faculty Rev-195. https://doi.org/10.12688/f1000research.10609.1
4. Pitout J.D.D, Finn TJ. 2020. The evolutionary puzzle of *Escherichia coli* ST131. Infect Genet Evol 81:104265. https://doi.org/10.1016/j.meegid.2020.104265
5. Pitout JDD, Peirano G, Chen L, DeVinney R, Matsumura Y. 2022. *Escherichia coli* ST1193: following in the footsteps of *E. coli* ST131. Antimicrob Agents Chemother 66:e0051122. https://doi.org/10.1128/aac.00511-22
6. Petty NK, Ben Zakour NL, Stanton-Cook M, Skippington E, Totsika M, Forde BM, Phan M-D, Gomes Moriel D, Peters KM, Davies M, Rogers BA, Dougan G, Rodriguez-Baño J, Pascual A, Pitout JDD, Upton M, Paterson DL, Walsh TR, Schembri MA, Beatson SA. 2014. Global dissemination of a multidrug resistant *Escherichia coli* clone. Proc Natl Acad Sci U S A 111:5694–5699. https://doi.org/10.1073/pnas.1322678111
7. Mathers AJ, Peirano G, Pitout JDD. 2015. *Escherichia coli* ST131: the quintessential example of an international multiresistant high-risk clone. Adv Appl Microbiol 90:109–154. https://doi.org/10.1016/bs.aambs.2014.09.002
8. Matsumura Y, Pitout JDD, Peirano G, DeVinney R, Noguchi T, Yamamoto M, Gomi R, Matsuda T, Nakano S, Nagao M, Tanaka M, Ichiyama S. 2017. Rapid identification of different *Escherichia coli* sequence type 131 clades. Antimicrob Agents Chemother 61:e00179-17. https://doi.org/10.1128/AAC.00179-17
9. Harrison LB, Hanson ND. 2017. High-resolution melting analysis for rapid detection of sequence type 131 *Escherichia coli*. Antimicrob Agents Chemother 61:e00265-17. https://doi.org/10.1128/AAC.00265-17
10. Matsumura Y, Yamamoto M, Nagao M, Tanaka M, Machida K, Ito Y, Takakura S, Ichiyama S. 2014. Detection of extended-spectrum-β-lactamase-producing *Escherichia coli* ST131 and ST405 clonal groups by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 52:1034–1040. https://doi.org/10.1128/JCM.03196-13
11. Szijártó V, Lukasiewicz J, Gozdziewicz TK, Magyarics Z, Nagy E, Nagy G. 2014. Diagnostic potential of monoclonal antibodies specific to the unique O-antigen of multidrug-resistant epidemic *Escherichia coli* clone ST131-O25b:H4. Clin Vaccine Immunol 21:930–939. https://doi.org/10.1128/CVI.00685-13

12. Holland MS, Nobrega D, Peirano G, Naugler C, Church DL, Pitout JDD. 2020. Molecular epidemiology of *Escherichia coli* causing bloodstream infections in a centralized Canadian region: a population-based surveillance study. Clin Microbiol Infect 26:1554. https://doi.org/10.1016/j.cmi.2020.02.019
13. Peirano G, Chen L, Nobrega D, Finn TJ, Kreiswirth BN, DeVinney R, Pitout JDD. 2022. Genomic epidemiology of global carbapenemase-producing *Escherichia coli*, 2015-2017. Emerg Infect Dis 28:924–931. https://doi.org/10.3201/eid2805.212535
14. Peirano G, Lynch T, Matsumara Y, Nobrega D, Finn TJ, DeVinney R, Pitout JDD. 2020. Trends in population dynamics of *Escherichia coli* sequence type 131, Calgary, Alberta, Canada, 2006-2016. Emerg Infect Dis 26:2907–2915. https://doi.org/10.3201/eid2612.201221
15. Peirano G, Matsumara Y, Nobrega D, DeVinney R, Pitout J. 2021. Population-based epidemiology of *Escherichia coli* ST1193 causing blood stream infections in a centralized Canadian region. Eur J Clin Microbiol Infect Dis. https://doi.org/10.1007/s10096-021-04373-5
16. Peirano G, Pitout JDD. 2014. Fluoroquinolone-resistant *Escherichia coli* sequence type 131 isolates causing bloodstream infections in a Canadian region with a centralized laboratory system: rapid emergence of the *H*30-Rx sublineage. Antimicrob Agents Chemother 58:2699–2703. https://doi.org/10.1128/AAC.00119-14
17. Peirano G, van der Bij AK, Freeman JL, Poirel L, Nordmann P, Costello M, Tchesnokova VL, Pitout JDD. 2014. Characteristics of *Escherichia coli* sequence type 131 isolates that produce extended-spectrum β-lactamases: global distribution of the *H*30-Rx sublineage. Antimicrob Agents Chemother 58:3762–3767. https://doi.org/10.1128/AAC.02428-14
18. Peirano G, van der Bij AK, Gregson DB, Pitout JDD. 2012. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum β-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. J Clin Microbiol 50:294–299. https://doi.org/10.1128/JCM.06025-11
19. Moehling TJ, Choi G, Dugan LC, Salit M, Meagher RJ. 2021. LAMP diagnostics at the point-of-care: emerging trends and perspectives for the developer community. Expert Rev Mol Diagn 21:43–61. https://doi.org/10.1080/14737159.2021.1873769
20. Hill J, Beriwal S, Chandra I, Paul VK, Kapil A, Singh T, Wadowsky RM, Singh V, Goyal A, Jahnukainen T, Johnson JR, Tarr PI, Vats A. 2008. Loop-mediated isothermal amplification assay for rapid detection of common strains of *Escherichia coli* . J Clin Microbiol 46:2800–2804. https://doi.org/10.1128/JCM.00152-08
21. Ullah N, Assawakongkarat T, Akeda Y, Chaichanawongsaroj N. 2023. Detection of extended-spectrum β-lactamase-producing *Escherichia coli* isolates by isothermal amplification and association of their virulence genes and phylogroups with extraintestinal infection. Sci Rep 13:12022. https://doi.org/10.1038/s41598-023-39228-w
22. Yinur D, Moges B, Hassen A, Tessema TS. 2023. Loop mediated isothermal amplification as a molecular diagnostic assay: application and evaluation for detection of enterohaemorrhagic *Escherichia coli* (O157:H7). Pract Lab Med 37:e00333. https://doi.org/10.1016/j.plabm.2023.e00333
23. Saengsawang N, Ruang-Areerate P, Kaeothaisong N, Leelayoova S, Mungthin M, Juntanawiwat P, Hanyanunt P, Potisuwan P, Kesakomol P, Butsararattanagomen P, Wichaiwong P, Dungchai W, Ruang-Areerate T. 2023. Validation of quantitative loop-mediated isothermal amplification assay using a fluorescent distance-based paper device for detection of *Escherichia coli* in urine. Sci Rep 13:18781. https://doi.org/10.1038/s41598-023-46001-6
24. Maffert P, Reverchon S, Nasser W, Rozand C, Abaibou H. 2017. New nucleic acid testing devices to diagnose infectious diseases in resource-limited settings. Eur J Clin Microbiol Infect Dis 36:1717–1731. https://doi.org/10.1007/s10096-017-3013-9
25. Acharya KP, Subramanya SH, Pitout JDD. 2020. Inclusion of next-generation leaders and cost-effective precision diagnostic techniques are vital in combatting antimicrobial resistance in low- and middle-income countries. JAC Antimicrob Resist 2:dlaa032. https://doi.org/10.1093/jacamr/dlaa032
26. Johnson JR, Clabots C, Porter SB, Bender T, Johnston BD, Thuras P. 2022. Intestinal persistence of colonizing *Escherichia coli* strains, especially ST131-H30, in relation to bacterial and host factors. J Infect Dis 225:2197–2207. https://doi.org/10.1093/infdis/jiab638
27. Johnson JR, Davis G, Clabots C, Johnston BD, Porter S, DebRoy C, Pomputius W, Ender PT, Cooperstock M, Slater BS, Banerjee R, Miller S, Kisiela D, Sokurenko EV, Aziz M, Price LB. 2016. Household clustering of *Escherichia coli* sequence type 131 clinical and fecal isolates according to whole genome sequence analysis. Open Forum Infect Dis 3:ofw129. https://doi.org/10.1093/ofid/ofw129
28. Nicolas-Chanoine MH, Bertrand X, Madec JY. 2014. *Escherichia coli* ST131, an intriguing clonal group. Clin Microbiol Rev 27:543–574. https://doi.org/10.1128/CMR.00125-13
29. Nobrega D, Peirano G, Lynch T, Finn TJ, Devinney R, Pitout JDD. 2021. Spatial distribution of *Escherichia coli* ST131 C subclades in a centralized Canadian urban region. J Antimicrob Chemother 76:1135–1139. https://doi.org/10.1093/jac/dkab020
30. Bernasconi OJ, Campos-Madueno EI, Donà V, Perreten V, Carattoli A, Endimiani A. 2020. Investigating the use of bacteriophages as a new decolonization strategy for intestinal carriage of CTX-M-15-producing ST131 *Escherichia coli*: an *in vitro* continuous culture system model. J Glob Antimicrob Resist 22:664–671. https://doi.org/10.1016/j.jgar.2020.05.018
31. Forsyth JH, Barron NL, Scott L, Watson BNJ, Chisnall MAW, Meaden S, van Houte S, Raymond B. 2023. Decolonizing drug-resistant *E. coli* with phage and probiotics: breaking the frequency-dependent dominance of residents. Microbiology (Reading) 169:001352. https://doi.org/10.1099/mic.0.001352
32. Porter SB, Johnston BD, Kisiela D, Clabots C, Sokurenko EV, Johnson JR. 2022. Bacteriophage cocktail and microcin-producing probiotic *Escherichia coli* protect mice against gut colonization with multidrug-resistant *Escherichia coli* sequence type 131. Front Microbiol 13:887799. https://doi.org/10.3389/fmicb.2022.887799
33. Mathers AJ, Peirano G, Pitout JDD. 2015. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. Clin Microbiol Rev 28:565–591. https://doi.org/10.1128/CMR.00116-14
34. Enne VI, Aydin A, Baldan R, Owen DR, Richardson H, Ricciardi F, Russell C, Nomamiukor-Ikeji BO, Swart A-M, High J, Colles A, Barber J, Gant V, Livermore DM, O'Grady J. 2022. Multicentre evaluation of two multiplex PCR platforms for the rapid microbiological investigation of nosocomial pneumonia in UK ICUs: the INHALE WP1 study. Thorax 77:1220–1228. https://doi.org/10.1136/thoraxjnl-2021-216990