Antimicrobial resistance in patients with suspected urinary tract infections in primary care in Assam, India

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Objectives: We investigated the prevalence and diversity of antimicrobial resistance in bacteria isolated from urine samples of community-onset urinary tract infection (UTI) patients in southern Assam, India.

Methods: Freshly voided midstream urine samples were collected from patients attending primary healthcare centres, with the patients' epidemiological data also recorded. Species identification was confirmed using a VITEK 2 compact automated system. Phenotypic confirmation of ESBLs was performed using the combined disc diffusion method (CLSI 2017) and carbapenemase production was phenotypically characterized using a modified Hodge test. Common ESBLs and carbapenem-resistance mechanisms were determined in Escherichia coli isolates using PCR assays. Incompatibility typing of the conjugable plasmids was determined by PCR-based replicon typing; the phylotypes and MLSTs were also analysed.

Results: A total of 301 (59.7%) samples showed significant bacteriuria along with symptoms of UTI and among them 103 isolates were identified as E. coli of multiple STs (ST3268, ST3430, ST4671 and others). Among them, 26.2% (27/103) were phenotypically ESBL producers whereas 12.6% (13/103) were carbapenemase producers. This study describes the occurrence of diverse ESBL genes—blaCTX-M-15, blaSHV-148, blaPER-1 and blaTEM—and two E. coli isolates carrying the blaNDM-1 carbapenemase gene. ESBL genes were located within transconjugable plasmids of IncP and IncF type whereas blaNDM-1 was carried in an IncFrepB type plasmid.

Conclusions: This study illustrates the high rate of MDR in E. coli causing UTI in primary care in rural Assam. UTIs caused by ESBL- or MBL-producing bacteria are very difficult to treat and can often lead to treatment failure. Thus, future research should focus on rapid diagnostics to enable targeted treatment options and reduce the treatment failure likely to occur with commonly prescribed antibiotics, which will help to combat antimicrobial resistance and the burden of UTIs.

Introduction

Urinary tract infections (UTIs) are one of the most frequent infectious diseases worldwide and the burden of UTIs is a substantial global health problem as approximately 150 million patients are diagnosed worldwide each year. Depending on the site of the infection, UTI is classified as urethritis (an infection of the urethra), cystitis (inflammation of the bladder) or pyelonephritis (infection of the kidneys) or may develop into a bloodstream infection causing urosepsis. In India, the prevalence of UTIs in the population varies from 21.8% to 31.3%. Medical treatment of UTIs usually is provided through primary care, which in India is delivered through the close to 38 000 primary health centres (PHCs) across the country. UTIs often require antimicrobial therapy and in the Indian state of Assam medical staff in PHCs commonly prescribe nitrofurantoin as a first-line treatment for UTI. Unfortunately, the use of more potent antibiotics bought over the counter (OTC) is a widespread phenomenon in India, similar to other low- and middle-income countries (LMICs). The use of OTC antibiotics such as trimethoprim, sulfamethoxazole, fluoroquinolones and especially β-lactams to treat UTIs are long-suspected drivers of antimicrobial
resistance (AMR). ESBLs and carbapenemases are predominant β-lactamases widely distributed in India and across the globe. Among Enterobacteriales, uropathogenic *Escherichia coli* has been noted as one of the most common and significant ESBL or carbapenemase producers.** While MDR *E. coli* isolates were previously found mainly in healthcare settings, e.g. causing nosocomial infections, they are increasingly described in the community and primary care.** ESBLs are plasmid-mediated β-lactamases recognized for their ability to hydrolyse cephalosporins and monobactams whereas carbapenemases can degrade almost all β-lactams including carbapenems. In Assam, there is a lack of centralized, local surveillance data on the prevalence of UTIs and, furthermore, there is a paucity of comprehensive data regarding ESBL and carbapenemase-producing strains in this part of India. Therefore, the present study was undertaken as part of the Indo-UK project Diagnostics for One Health and User Driven Solutions for AMR (DOSA, https://dosa-diagnostics.org/) to investigate the prevalence and diversity of AMR in bacteria isolated from patients presenting with UTI symptoms at two PHCs in southern Assam. A particular focus was placed on the diversity of ESBL- and carbapenemase-harbouring *E. coli* strains based on their phylotypes.

**Methods**

**Sample collection**
The study assesses two different community-based health centres located in two provinces with a geographic distance of approximately 50 km, i.e. peri-urban (PHC A) and rural (PHC B) areas. A total of 504 non-duplicate urine samples were collected from patients attending the PHCs A and B located in the southern part of Assam, India, between November 2018 and October 2019. On average, each day, approximately 80–100 patients visit PHC A while 50–60 patients attend PHC B. The study group included patients diagnosed with UTI and those who had at least one of the following clinical signs and symptoms: fever; burning and painful urination; the frequent urge to urinate; and oliguria with no other recognized cause. Several epidemiological factors for each patient were also recorded, including age, sex, pregnancy status, recurrent UTI (repeated UTI with a frequency of two or more UTIs in the last 6 months or three or more UTIs in the last 12 months), and history of antibiotic consumption.

**Bacterial strains and identification**
The urine samples were collected in a universal plastic container, the techniques employed for the collection of materials were made as per the lines of procedure by Mackie and Mccartney, 14th edition, and after collection the samples were kept in an insulated container with a cooling gel pack and transferred to the laboratory. After being delivered to the laboratory (within a duration of 2–2.5 h), the samples were immediately processed and streaked on MacConkey agar, CLED agar and HiCrome Chromogenic Caliform Agar medium (Hi-media, Mumbai, India) using a calibrated loop (which can hold approx. 2 μL of sample) and the plates incubated at 37 °C. After overnight incubation, the plates were observed for bacterial growth. The samples that showed significant growth were processed to identify the bacteria using a VITEK 2 compact automated system (bioMérieux, Marcy- l’Etoile, France).

**Phenotypic characterization of resistance mechanisms**

**Detection of ESBLs**
The detection of ESBL production was performed according to CLSI guidelines, with an initial screening followed by phenotypic confirmation by the combined disc diffusion method. The phenotypic screening of the ESBL-producing isolates was done using ceftazidime and cefazidime at a concentration of 1 mg/L in Mueller–Hinton agar by the agar dilution method. All isolates that screened positive were tested using combined disc diffusion tests against ceftazidime and cefazidime with and without clavulanic acid. An increase in zone diameter of ≥5 mm for either antimicrobial tested in combination with clavulanic acid versus its zone when tested alone confirms ESBL production.

**Detection of carbapenemases**
The isolates were subjected to a modified Hodge test (MHT), which uses *E. coli* ATCC 25922 as an indicator organism, and the presence of a clover leaf-like indentation after overnight incubation was interpreted as positive for carbapenemase production. Rapidec Carbo NP tests (bioMérieux) were also performed as per the manufacturer’s guidelines to identify the carbapenem-resistant bacterial isolates.

**Molecular characterization of resistance determinants**
Characterization of ESBL genes was done for the phenotypically ESBL-producing isolates by performing two sets of multiplex PCRs targeting *bla*TEM, *bla*SHV, *bla*CTX-M, *bla*OXA-2, *bla*OXA-10, *bla*PER, *bla*GES and *bla*OXA (Table 1) using a previously published protocol. Reactions were performed under the following conditions: initial denaturation 95 °C for 5 min, 32 cycles of 95 °C for 1 min, 54 °C (first multiplex), 50 °C (second multiplex) for 1 min, 72 °C for 1 min and final extension at 72 °C for 7 min. The phenotypically positive carbapenemase-producing isolates were analysed using PCR assays for the detection of carbapenem resistance determinants, including different classes of carbapenemase genes belonging to class A (*bla*KPC, *bla*NDM, *bla*IMI, *bla*OXA-58), class B (*bla*PER, *bla*IMP, *bla*VIM) and class D (*bla*CTX-M) as described previously. PCR was also performed targeting the AmpC genes i.e. CIT, DHA, ACC, FOX, MOX and EBC using the primers listed in Table 1. PCR amplification was performed using 30 μL of total reaction volume and the reactions were run under the following conditions: initial denaturation at 95 °C for 2 min, 34 cycles of 95 °C for 15 s, 51 °C for 1 min, 72 °C for 1 min and final extension at 72 °C for 7 min. The amplified product of the resistance determinant was purified using MinElute PCR Purification Kit (Qiagen, Hilden, Germany) and then sequenced to confirm the gene variant of the resistance determinant (data not shown).

**Antimicrobial susceptibility testing**
Strain identification and MIC determination for the *E. coli* isolates was done using a VITEK 2 compact automated system (bioMérieux) against a set of antibiotics, and results were interpreted as per CLSI and EUCAST guidelines.

**Phylogroup analysis**
The *E. coli* phylogenies were determined targeting *chuA*, *yjaA*, *tpSE*C2, *arpApG*, and *tpnAgPC* based on the Clermont method with the following reaction conditions: initial denaturation at 95 °C for 3 min followed by 32 cycles of denaturation at 95 °C for 25 s, annealing at 50 °C for 40 s, extension at 72 °C for 1 min and final extension at 72 °C for 5 min. The oligonucleotides used for the phylogroup analysis are mentioned in Table 1.

**Determination of horizontal transferability**
Conjugation assays were performed to determine the horizontal transferability of the resistance determinants using the clinical strains harbouring the resistance gene as the donor and azide-resistant *E. coli* J53 as a recipient. Both the donor and recipient cells were cultured in LB broth (Hi-Media) to reach an OD of 0.8–0.9 at 600 nm (OD600). Cells were mixed at a ratio of 1:100.
| Serial no. | Target     | Primer pairs  | Sequence (5’-3’)                         | Product size (bp) |
|-----------|------------|--------------|-----------------------------------------|------------------|
| 1         | CTX-M      | CTX-M-F      | 5'-CGCTTTGCGATGTGCAG-3'                 | 550              |
|           |            | CTX-M-R      | 5'-ACCGGATAATTGGGT-3'                   |                  |
| 2         | SHV        | SHV-F        | 5'-AGGAATGACTGCTTTT-3'                  | 392              |
|           |            | SHV-R        | 5'-ATTTTGTGATTTCGCTG-3'                 |                  |
| 3         | OXA-2      | OXA-2-F      | 5'-AAAGAAAGCCTACTGCTGC-3'               | 478              |
|           |            | OXA-2-R      | 5'-CAACCTAACATCTACCTACC-3'              |                  |
| 4         | OXA-10     | OXA-10-F     | 5'-TCAACAAATGCGCAGAGAAG-3'              | 276              |
|           |            | OXA-10-R     | 5'-TCCACACCAAGAAACCGAG-3'               |                  |
| 5         | TEM        | TEM-F        | 5'-ATGAGATTTACACATTTCC-3'               | 110              |
|           |            | TEM-R        | 5'-CTGACAGTTCACAATGCTTA-3'              |                  |
| 6         | PER        | PER-F        | 5'-AATTGGGCTTGGGCAGAAGA-3'              | 920              |
|           |            | PER-R        | 5'-ATGAATGACCTTAAAAAACG-3'              |                  |
| 7         | VEB        | VEB-F        | 5'-CATTCCCAATGCAAAAGGT-3'               | 650              |
|           |            | VEB-R        | 5'-CAGACTTTCTTTGACTCTG-3'               |                  |
| 8         | GES        | GES-F        | 5'-AGTGCGCTAGACCGGAAAG-3'               | 863              |
|           |            | GES-R        | 5'-TTTTGCGTGTCCAGGAT-3'                 |                  |
| 9         | KPC        | KPC-F        | 5'-CACTAAAGGGGCTTTGCTGTCG-3'            | 538              |
|           |            | KPC-R        | 5'-ACGACCGCATAGTCTTTG-3'                |                  |
| 10        | IMI/NMC    | IMI/NMC-F    | 5'-CCATTACCCATCACCAAC-3'                | 440              |
|           |            | IMI/NMC-R    | 5'-CTACCCGATAATCATTTTGC-3'              |                  |
| 11        | SME        | SME-F        | 5'-AACGCGTCTATTTTTGTTAG-3'              | 831              |
|           |            | SME-R        | 5'-GCTTCCGCAATAGTTTTATCA-3'             |                  |
| 12        | NDM        | NDM-F        | 5'-GAGGCTGCTTTCCCAACAGG-3'              | 476              |
|           |            | NDM-R        | 5'-GTATGCTGACTGTGCGCAT-3'               |                  |
| 13        | VIM        | VIM-F        | 5'-GATGGGATTGTGTGGTCG-3'                | 390              |
|           |            | VIM-R        | 5'-CGAATGCCACACCCAG-3'                  |                  |
| 14        | IMP        | IMP-F        | 5'-TGACACTCCATTTACG-3'                  | 139              |
|           |            | IMP-R        | 5'-GATGGAACAAATGCAACAC-3'               |                  |
| 15        | OXA-23     | OXA-23-F     | 5'-GATCCGGATTTGGAACACAGA-3'             | 501              |
|           |            | OXA-23-R     | 5'-ATTTTGTGATTTCGCTG-3'                 |                  |
| 16        | OXA-48     | OXA-48-F     | 5'-GATATTGCGAATGCTGCGG-3'               | 845              |
|           |            | OXA-48-R     | 5'-CATCAAGGGCAGACGATCC-3'               |                  |
| 17        | OXA-58     | OXA-58-F     | 5'-CGATGAGGTGGTTTGGTCG-3'               | 529              |
|           |            | OXA-58-R     | 5'-ACGATTCCCCCTGTCGCG-3'                |                  |
| 18        | DHA        | DHA-F        | 5'-GTATGCAACTAAGCAAGATTCC-3'            | 997              |
|           |            | DHA-R        | 5'-GCTTTGACTCTTTTGGGT-3'                |                  |
| 19        | EBC        | EBC-F        | 5'-CGTAATACGCGATGTTGCAG-3'              | 683              |
|           |            | EBC-R        | 5'-AGCCTAAACCCCTGATAC-3'                |                  |
| 20        | CIT        | CIT-F        | 5'-CGTAATTACCACCCCTC-3'                 | 538              |
|           |            | CIT-R        | 5'-AGCCTAAACCCCTGATAC-3'                |                  |
| 21        | ACC        | ACC-F        | 5'-CACCTCCACGGCAATGTTGCAG-3'            | 346              |
|           |            | ACC-R        | 5'-GTAGCCAGACATCACGTACC-3'              |                  |
| 22        | MOX        | MOX-F        | 5'-GCAAACACGACATCATATCTC-3'             | 895              |
|           |            | MOX-R        | 5'-GGTATGCGGTAATACCTCCCAA-3'            |                  |
| 23        | FOX        | FOX-F        | 5'-CTACATTGCCGCGGTTT-3'                 | 162              |
|           |            | FOX-R        | 5'-CTATTGCGCGCCGATG-3'                  |                  |
| 24        | chuA       | chuA.1b      | 5'-ATGATCCGGAGCAGAACAC-3'               | 288              |
|           |            | chuA.2b      | 5'-TGGCGCCAGATGCAAACGAC-3'              |                  |
| 25        | yjaA       | yjaA.1b      | 5'-CAAAGTGAAAGGTGCGAGG-3'               | 211              |
|           |            | yjaA.2b      | 5'-AATGCGTCTCTCAACCTTG-3'               |                  |
| 26        | TspE4.C2   | TspE4C2.1b   | 5'-CAACCTCGAAAGTGTCGAG-3'               | 152              |
|           |            | TspE4C2.2b   | 5'-AGTTTATCGCAGGCTGGTGTCG-3'            |                  |
| 27        | arpA       | ArpA1.F      | 5'-AAGCCATTCGCCAGCTTG-3'                | 400              |
|           |            | ArpA1.R      | 5'-TCTCCCCATACCGTACGTA-3'               |                  |
M-15 (Sequence typing analysis of these 27 Gram-negative rods. We identified 103 isolates as harbour the AmpC (em (1 mg/L) and sodium azide (100 mg/L).22

4 o f7 with a bacterial load of total at two PHCs, 301 (59.7%) samples had significant bacteriuria and cloudy urine. From the 504 non-duplicate samples collected in 100 patients visited, with an average of two patients diagnosed at the PHCs, on a typical day during the study, approximately 80–

The ST of the isolates was determined by MLST, and it was performed targeting the internal fragments of seven conserved housekeeping genes of E. coli. Later the STs were assigned based on the existing E. coli database19 and were analysed using the Center for Genomic Epidemiology website (https://cge.cbs.dtu.dk/services/MLST/).

Results

At the PHCs, on a typical day during the study, approximately 80–100 patients visited, with an average of two patients diagnosed with UTI symptoms such as painful micturition, burning sensation and cloudy urine. From the 504 non-duplicate samples collected in total at two PHCs, 301 (59.7%) samples had significant bacteriuria with a bacterial load of $\geq 10^5$ cfu/mL of urine with symptoms of UTI, 32 (6%) samples had $< 10^5$ cfu/mL of urine and 171 (34%) samples had no bacterial growth. From the 301 bacteriuria samples, 198 isolates were identified as Gram-negative bacilli consisting of members of the Enterobacteraceae order and non-fermenting Gram-negative rods. We identified 103 isolates as E. coli, of which 43 isolates grew on screening agar plates that contained a third-generation cephalosporin antibiotic, i.e. either cefotaxime or ceftazidime. Among these, 27 (26.2%) isolates were found to be inhibited by clavulanic acid and confirmed as ESBL producers. Four different ESBL genes were harboured within the isolates: blaCTX-M-15 ($n = 6$), blaSHV-148 ($n = 3$), blaPER-1 ($n = 2$) and blaTEM ($n = 1$).

One isolate was found co-harbouring both TEM-1 and SHV-148 (Figure 1). On performing antimicrobial susceptibility testing with a VITEK 2 compact automated system, 13 isolates were found to be resistant to at least one of the carbapenems tested (imipenem, meropenem and ertapenem). Further, MHT and Carba NP tests also confirmed these 13 isolates as carbapenem producers, and for two isolates blaNDM-1 genes could be identified by PCR. By performing PCR assay for AmpC genes, four isolates were found to harbour the blaCTT gene. Further variant analysis revealed that the sequences are identical to blaCMY-42. Apart from blaCTT, the two isolates PCR amplification and sequencing analysis revealed the presence of blaDHA-1. Interestingly, in both the PHCs single or multiple combinations of ESBLs (blaCTX-M-15, blaPER-1, blaTEM, blaSHV-148), AmpC (blaCMY-42, blaDHA-1) and carbapenemase genes (blaNDM-1) were detected, which are mentioned in detail in Figure 1. Sequence typing analysis of these 27 E. coli isolates identified nine different STs such as ST167 ($n = 4$), ST328 ($n = 3$), ST3430 ($n = 2$), ST4671 ($n = 3$), ST304 ($n = 3$), ST361 ($n = 3$), ST10 ($n = 2$) and a single isolate each of ST219 and ST3492, whereas unknown STs (no match with the MLST database) were observed in the case of 5 isolates (Figure 1). Susceptibility testing of ESBL producers revealed resistance to ampicillin (100%), cefuroxime (100%), ceftriaxone (93%), cefepime (87%), ciprofloxacin (80%) and nitrofurantoin (59.3%) and susceptibility to carbapenems (100%), colistin (100%) and trimethoprim/sulfamethoxazole (93%). Also, both the E. coli isolates carrying blaNDM-1 showed MDR phenotypes and were susceptible to only tigecycline and colistin. The details of the susceptibility profile of each isolate harbouring ESBL and carbapenemase genes are described in Figure 1. In the present study, we also analysed the phylogenetic type of these $\beta$-lactamase-harbouring E. coli isolates, as it is important to identify the presence of any novel group of bacteria carrying these resistant determinants within the community. Phylogenetic analysis indicated that most of the isolates belonged to group B1 (9/15) followed by the phylogroup A (6/15) which included all the isolates harbouring ESBL and carbapenem resistance determinants (Figure 1). Conjugation assays revealed that blaCTX-M-15, blaSHV-148, blaPER-1 and blaTEM could be conjugatively transferable in the recipient strain E. coli J53. Replicon typing of these transconjugable plasmids revealed that ESBL genes were carried within two different incompatibility groups: IncP and IncF. The blaNDM-1 Gene was found to be maintained within a plasmid of IncFrep type.

Discussion

This study aims to explore the prevalence and molecular diversity of AMR in UTI in a rural community in India, which is scarcely covered by surveillance but considered as significant to the emergence of AMR due to inappropriate antibiotic use. The study covers bacteria isolated from patients presenting with suspected UTI at two PHCs in southern Assam. Our study is based on a realistic scenario of patients presenting UTI at a PHC. We found mid-range positivity rates for bacterial growth in the urine samples as published in the literature for similar settings in India and other countries.25-28

As most UTIs are caused by E. coli and MDR E. coli carrying ESBLs or carbapenem-resistant Enterobacterales are considered a major health threat, we focused our study on this species, i.e. identified by selective culturing. According to WHO, E. coli is considered to be a significant threat as it is universally carried in the human gut and also because it is the commonest cause of UTI.29 The presence of ESBL genes within E. coli adds extra risk for treatment failure of community-onset UTIs as such isolates are frequently MDR.30 Until recently, it has been perceived that the production of $\beta$-lactamases such as ESBLs or carbapenemases was mainly described in nosocomial isolates.31 In the past few years, ESBLs and carbapenemases have been increasingly identified in community isolates and in the present study, ESBL-producing E. coli phenotypes were detected in 26% of the isolates and 5% of all samples taken. It is comparatively higher than the previous study reported from North India (21.4%).32 However, the prevalence of ESBL producers in our study was comparatively less than previous studies reported by Bajpai et al. (36.8%), Taneja et al. (40.2%), Gautam et al. (33%) and Kumar et al. (46.6%) from different parts of India. This increased prevalence of ESBL-producing E. coli isolates is a serious peril for healthcare as these organisms can cause many severe infections in humans even in countries with advanced public health and healthcare facilities.

In addition, infections caused by ESBL-producing pathogens are problematic due to the potential for co-harbouring resistance
Figure 1. Features of MDR ESBL-producing *E. coli* isolates. Green shading indicates susceptibility of the isolates to the antibiotics. TGC, tigecycline; CST, colistin; SXT, trimethoprim/sulfamethoxazole; IPM, imipenem; ETP, ertapenem; MEM, meropenem; GEN, gentamicin; TZP, piperacillin/tazobactam; NAL, nalidixic acid; AMK, amikacin; NIT, nitrofurantoin; CRO, ceftiraxone; CIP, ciprofloxacin; AMC, amoxicillin/clavulanic acid; FEP, cefepime.

| PHC (Isolate ID) | ST     | ESBL, AmpC and carbapenemase genes | Phylotype | TGC | CST | SXT | IPM | ETP | MEM | NIT | CRO | CIP | AMC | TZP | FEP | AMK | GEN | NAL | Replicon type |
|------------------|--------|-----------------------------------|-----------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----------------|
| A (Ec-144)       | ST3430 | PER-1                             | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| A (Ec-154)       | ST304  | TEM                               | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncF           |
| A (Ec-155)       | ST361  | PER-1                             | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncF           |
| A (Ec-200)       | unknown| NDM-1                             | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncF<sub>rep</sub> |
| A (Ec-237)       | ST3430 | CTX-M-35 + DHA-1                   | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| A (Ec-252)       | unknown| CTX-M-15 + DHA-1                   | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| A (Ec-76)        | ST167  |                                   |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| A (Ec-106)       | ST361  |                                   | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| A (Ec-167)       | ST167  |                                   | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| A (Ec-218)       | unknown|                                   | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| A (Ec-244)       | ST10   |                                   | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| A (Ec-268)       | ST167  |                                   | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| A (Ec-284)       | ST361  |                                   | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| B (Ec-3)         | ST219  | SHV-148 + CMY-42                   | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| B (Ec-5)         | unknown| SHV-148                           | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| B (Ec-10)        | ST3268 | SHV-148                           | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| B (Ec-12)        | ST3492 | NDM-1 + CMY-42                     | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| B (Ec-89)        | ST4671 | CTX-M-15                           | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| B (Ec-125)       | ST3268 | TEM + SHV-148                      | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| B (Ec-146)       | ST304  | CTX-M-15                           | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| B (Ec-182)       | ST3268 | CTX-M-15 + CMY-42                  | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncP           |
| B (Ec-189)       | ST4671 | CTX-M-15                           | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | IncF           |
| B (Ec-272)       | ST10   | CMY-42                             | B1        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| B (Ec-36)        | ST167  |                                   |           |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| B (Ec-56)        | ST304  |                                   | B2        |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| B (Ec-132)       | unknown|                                   | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
| B (Ec-139)       | ST4671 |                                   | A         |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     | —              |
determinants to other antimicrobial agents, hence they have emerged as a major and severe challenge to public health practitioners due to the reduced treatment options and failure of therapy with broad-spectrum antibiotics. In recent years, the frequency of these β-lactamase-harbouring strains has dramatically increased globally, with the identification of many novel β-lactamases and many new variants of the existing enzymes.57 This increase may be due to the plasmid-mediated existence of these resistance determinants, which enables their horizontal transfer and spread from an MDR organism to another one with a susceptible phenotype, thus making it MDR.58 Similarly, in the present study all the ESBLs and the carbapenemase gene blaNDM-1 were also found to be carried within self-transferable plasmids. The detection of two carbapenemase genes among the 13 phenotype-specifically typical isolates may be due to presence of different types/varieties of resistance genes that could not be amplified with our target primers. Similar to our findings, plasmid-mediated spread of these β-lactamase genes was also previously reported worldwide.59 In the present study, it was observed that most of the isolates were carrying a single β-lactamase gene within one plasmid. In general, such transferable plasmids can carry multiple β-lactamase resistance determinants, and the co-existence of ESBL and carbapenemase genes within a single isolate may worsen the situation, leading to reduced therapeutic options and ultimately treatment failure.60

Recently, E. coli has been identified as the most frequently isolated ESBL-producing bacteria worldwide, with CTX-M being the most common ESBL type.61 Accordingly, in our study blaCTX-M-15 was found to be the predominant type. Less frequently, we found other ESBL types such as PER, TEM, VEB, GES and OXA, which corresponded with other previous studies.62,63 The high frequency of the B1 phylotype among the E. coli isolates in this study was in contrast to a previous study where the phylotype B2 was found to be the most common group among E. coli strains.64

Instead, the susceptibility profiles of our study isolates revealed high resistance rates to the cephalosporin and ciprofloxacin groups of drugs, which was similar to the recent GLASS report65 and, more worryingly, the blaNDM-harboring isolates were found to be resistant to all of the tested antibiotics except tigecycline and colistin. This high level of resistance could be attributed to previous inappropriate use of antibiotics, e.g. by inappropriate empirical treatment or self-medication with OTC antibiotics. This is supported by a former study carried out in India, which revealed that E. coli isolates have developed alarming levels of resistance to commonly prescribed antibiotics like fluoroquinolone, amoxicillin/clavulanic acid and trimethoprim/sulfamethoxazole, and it is a matter of concern as quinolones are the first-line drug in empirical therapy of community-acquired UTI. It has also been mentioned that the major forces in the development of these high resistance rates among uropathogens are maybe due to inadequate access to healthcare services, increased use and misuse of antibacterial drugs and OTC availability of antibacterial drugs.66,47

Conclusions
In conclusion, to our knowledge this is the first study providing evidence for a high rate of multidrug resistance in E. coli isolates from patients presenting with UTI in primary care in rural Assam. As the observed resistance rates are not immediately linked to the first-line antibiotics conventionally prescribed in primary care for this disease, likely dominating origins of these phenotypes are to be sought in the inappropriate antibiotic use through self-medication and AMR transmission in the community. Therefore, future research should focus attention on these links and ideally lead to interventions in the community, such as rapid diagnostics to enable targeted antimicrobial therapy, to both tackle the rise of AMR as well as the burden of UTI.

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Transparency declarations
None to declare.

References
1 Goel V, Kumar D, Kumar R et al. Community acquired enterococcal urinary tract infections and antibiotic resistance profile in North India. J Lab Physicians 2016; 8: 50-4.
2 Prakash D, Saxena RS. Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in urban community of Meerut City, India. ISRN Microbiol 2013; 2013: 749629.
3 George C, Norman G, Ramana GV et al. Treatment of uncomplicated symptomatic urinary tract infections: resistance patterns and misuse of antibiotics. J Family Med Prim Care 2015; 4: 416–21.
4 Ministry of Health and Family Welfare. National Health Policy 2017. https://www.nhp.gov.in/nhrpfies/national_health_policy_2017.pdf.
5 Ministry of Health and Family Welfare. Hospitals in the Country 2018. https://pib.gov.in/PressReleasePage.aspx?PRID=1539877.
6 Shakti L, Veeraraghavan B. Advantage and limitations of nitrofurantoin in multi-drug resistant Indian scenario. Indian J Med Microbial 2015; 33: 747–81.
7 Farooqui HH, Selvaraj S, Mehta A et al. Community level antibiotic utilization in India and its comparison vis-à-vis European countries: evidence from pharmaceutical sales data. PJLS One 2018; 13: e0204805.
8 Shen C, Badal RE, Hsueh PR. Distribution of extended-spectrum β-lactamases, AmpC β-lactamases, and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results of the study for monitoring antimicrobial resistance trends (SMART). Antimicrob Agents Chemother 2013; 57: 2981–8.
9 Alyamani EJ, Khiyami AM, Booq RY et al. The occurrence of ESBL-producing Escherichia coli carrying aminoglycoside resistance genes in
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20 UECAST. Breakpoint tables for Interpretation of MICS and Zone Diameters, Version 11.0, 2021. https://www.uecast.org/fileadmin/src/media/PDFs/UECAST_files/Breakpoint_tables/v_11_0_Breakpoint_Tables.pdf.

21 Clermont O, Christenson JK, Denarum E et al. The Clermont Escherichia coli phyla typing method revisited: improvement of specificity and detection of new phylo-groupos. Environ Microbiol Rep 2013; 5: 58-65.

22 Paul D, Chanda DD, Chakravarty A et al. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 2005; 63: 219-28.

23 Arora G, Kaur P, Agrawal D. Urinary tract infection in women of rural population of Haryana: a rising problem. Int J Reprod Contracept Obstet Gynecol 2016; 5: 4470-4.

24 Henry Oladeinde B, Omorogie R, Olley M et al. Urinary tract infection in a rural community of Nigeria. North Am J Med Sci 2011; 3: 75-7.

25 Orritt FA. Urinary tract infections in general practice in a rural community in South Trinidad. Saudi Med J 2001; 22: 537-40.

26 Winkens R, Nielssen-Atz S, Stoberingh E. Validity of the urine dipslide under daily practice conditions. Farn Pract 2003; 20: 410-2.

27 Verest LFHM, Van Esch WMJ, Van Ree JW et al. Management of acute uncomplicated urinary tract infections in general practice in the south of the Netherlands. Br J Gen Pract 2000; 50: 309-10.

28 Vila J, Sáez-López E, Johnson JR et al. Escherichia coli: an old friend with new tidings. JEMS Microbial Rev 2016; 40: 437-63.

29 Hassuna NA, Kheiralla AS, Farahat EM et al. Molecular characterization of extended-spectrum β-lactamase-producing E. coli recovered from community-acquired urinary tract infections in Upper Egypt. Sci Rep 2020; 10: 2772.

30 Abayneh M, Tesfaw G, Abdissa A. Isolation of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae from patients with community-onset urinary tract infections in Jimma University Specialized Hospital, Southwest Ethiopia. Can J Infect Dis Med Microbiol 2018; 2018: 4846159.

31 Datta P, Gupta V, Sidhu S. Extended spectrum beta lactamase positive uropathogenic E. coli - epidemiological factors and resistance. Br J Med Pract 2014; 7: 7-9.

32 Bajpai T, Pandey M, Varma M et al. Prevalence of extended spectrum β-lactamase producing uropathogens and their antibiotic resistance profile in patients visiting a tertiary care hospital in central India: implications on empiric therapy. Indian J Pathol Microbiol 2014; 57: 407-12.

33 Taneje N, Rao P, Arora J et al. Occurrence of ESBL & Amp-C β-lactamases & susceptibility to newer antimicrobial agents in complicated UTI. Indian J Med Res 2008; 127: 85–8.

34 Gautam V, Thakur A, Sharma M et al. Molecular characterization of extended-spectrum β-lactamases among clinical isolates of Escherichia coli & Klebsiella pneumoniae: a multi-centric study from tertiary care hospitals in India. Indian J Med Res 2019; 149: 208–15.

35 Kumar N, Chatterjee K, Deka S et al. Increased isolation of extended-spectrum β-lactamase-producing Escherichia coli from community-onset urinary tract infection cases in Uttarakhand, India. Cureus 2021; 13: e13837.

36 Shaikh S, Fatima J, Shalil S et al. Antibiotic resistance and extended spectrum β-lactamases: types, epidemiology and treatment. Saudi J Biol Sci 2015; 22: 90-101.

37 Vaidya VK. Horizontal transfer of antimicrobial resistance by extended-spectrum β-lactamase-producing Enterobacteriaceae. J Lab Physicians 2011; 3: 37–42.

38 Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbial Rev 2005; 18: 657–86.

39 Gajam R, Bhattacharjye A, Paul D et al. High prevalence of carbapenemase, AmpC β-lactamase and aminoglycoside resistance genes in extended-spectrum β-lactamase-positive uropathogens from Northern India. J Glob Antimicrob Resist 2020; 20: 197–203.

40 Brolund A. Overview of ESBL-producing Enterobacteriaceae from a Nordic perspective. Infect Ecol Epidemiol 2014; 4. doi:10.3402/iee.v4.24555.

41 Rohit A, Deekshit VK, Balaraj M et al. CTX-M type extended-spectrum β-lactamase in Escherichia coli isolated from extra-intestinal infections in a tertiary care hospital in south India. Indian J Med Res 2019; 149: 281–4.

42 Bonnet R. Growing group of extended-spectrum β-lactamases: the CTX-M enzymes. Antimicrob Agents Chemother 2004; 48: 1-14.

43 Jaurguy F, Landraud L, Posset V et al. Phylogenetic and genomic diversity of human bacteraemic Escherichia coli strains. BMC Genomics 2008; 9: 560.

44 WHO. Global Antimicrobial Resistance and Use Surveillance System (GLASS) Report: GLASS Country, Territory and Area Profiles, 2018. https://apps.who.int/gho/tableau-public/tpc-frame.jsp?id=2012.

45 Kolita D, Deka S, Sarma R et al. Bacteriologic profile and drug-resistance in urinary tract infection from a rural area of Northeast India. Int J Health Res Medico Leg Prac 2017; 3: 30-4.

46 Bajpey P, Singh VS, Virdi JS. Escherichia coli β-lactamases: what really matters. Front Microbiol 2016; 7: 417.