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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—$bla_{CTX\text{-}M\text{-}15}$, $bla_{SHV\text{-}148}$, $bla_{PER\text{-}1}$ and bla_{TEM}—and two *E. coli* isolates carrying the $bla_{NDM\text{-}1}$ carbapenemase gene. ESBL genes were located within transconjugable plasmids of IncP and IncF type whereas $bla_{NDM\text{-}1}$ was carried in an $IncF_{repB}$ 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.[1,2] 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%.[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.[4,5] 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.[6] 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).[7] The use of OTC antibiotics such as trimethoprim, sulfamethoxazole, fluoroquinolones and especially β-lactams to treat UTIs are long-suspected drivers of antimicrobial

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resistance (AMR). ESBLs and carbapenemases are predominant β-lactamases widely distributed in India and across the globe.[8] Among Enterobacterales, uropathogenic *Escherichia coli* has been noted as one of the most common and significant ESBL or carbapenemase producers.[9,10] 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.[11] 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)[12] 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,[13] 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 Coliform 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'Étoile, 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 cefotaxime and ceftazidime 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 cefotaxime and ceftazidime 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.[14]

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.[15] Rapidec Carba 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_{VEB} and bla_{GES} (Table 1) using a previously published protocol.[16] 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}, $bla_{IMI/NMC}$, bla_{SME}), class B (bla_{NDM}, bla_{IMP}, bla_{VIM}) and class D (bla_{OXA-23}, bla_{OXA-48}, bla_{OXA-58}) as described previously.[17,18] PCR was also performed targeting the AmpC genes i.e. CIT, DHA, ACC, FOX, MOX and EBC[19] 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.[14,20]

Phylogroup analysis

The *E. coli* phylotypes were determined targeting *chuA*, *yjaA*, *tspE4C2*, *arpAgpE*, and *tnpAgpC* based on the Clermont method[21] 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 (OD_{600}). Cells were mixed at a ratio of

Table 1. Details of target genes, primers and amplicons used in this study

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′-ACCGCGATATCGTTGGT-3′	
2	SHV	SHV-F	5′-AGGATTGACTGCCTTTTT-3′	392
		SHV-R	5′-ATTTGCTGATTTCGCTCG-3′	
3	OXA-2	OXA-2-F	5′-AAGAAACGCTACTCGCCTGC-3′	478
		OXA-2-R	5′-CCACTCAACCCATCCTACCC-3′	
4	OXA-10	OXA-10-F	5′-TCAACAAATCGCCAGAGAAG-3′	276
		OXA-10-R	5′-TCCCACACCAGAAAAACCAG-3′	
5	TEM	TEM-F	5′- ATGAGTATTCAACATTTCCG-3′	867
		TEM-R	5′- CTGACAGTTACCAATGCTTA-3′	
6	PER	PER-F	5′-AATTTGGGCTTAGGGCAGAA-3′	920
		PER-R	5′-ATGAATGTCATTATAAAAGC-3′	
7	VEB	VEB-F	5′-CATTTCCCGATGCAAAGCGT-3′	650
		VEB-R	5′-CGAAGTTTCTTTGGACTCTG-3′	
8	GES	GES-F	5′-AGTCGGCTAGACCGGAAAG-3′	863
		GES-R	5′-TTTGTCCGTGCTCAGGAT-3′	
9	KPC	KPC-F	5′-CATTCAAGGGCTTTCTTGCTGC-3′	538
		KPC-R	5′-ACGACGGCATAGTCATTTGC-3′	
10	IMI/NMC	IMI/NMC-F	5′-CCATTCACCCATCACAAC-3′	440
		IMI/NMC-R	5′-CTACCGCATAATCATTTGC-3′	
11	SME	SME-F	5′-AACGGCTTCATTTTTGTTTAG-3′	831
		SME-R	5′-GCTTCCGCAATAGTTTTATCA-3′	
12	NDM	NDM-F	5′-GGGCAGTCGCTTCCAACGGT-3′	476
		NDM-R	5′-GTAGTGCTCAGTGTCGGCAT-3′	
13	VIM	VIM-F	5′-GATGGTGTTTGGTCGCATA-3′	390
		VIM-R	5′-CGAATGCGCAGCACCAG-3′	
14	IMP	IMP-F	5′-TTGACACTCCATTTACDG-3′	139
		IMP-R	5′-GATYGAGAATTAAGCCACYCT-3′	
15	OXA-23	OXA-23-F	5′-GATCGGATTGGAGAACCAGA-3′	501
		OXA-23-R	5′-ATTTCTGACCGCATTTCCAT-3′	
16	OXA-48	OXA-48-F	5′-GATTATCGGAATGCCTGCGG-3′	845
		OXA-48-R	5′-CTACAAGCGCATCGAGCATCA-3′	
17	OXA-58	OXA-58-F	5′-CGATCAGAATGTTCAAGCGC-3′	529
		OXA-58-R	5′-ACGATTCTCCCCTCTGCGC-3′	
18	DHA	DHA-F	5′-TGATGGCACAGCAGGATATTC-3′	997
		DHA-R	5′-GCTTTGACTCTTTCGGTATTCG-3′	
19	EBC	EBC-F	5′-CGGTAAAGCCGATGTTGCG-3′	683
		EBC-R	5′-AGCCTAACCCCTGATACA-3′	
20	CIT	CIT-F	5′-CGGTAAAGCCGATGTTGCG-3′	538
		CIT-R	5′-AGCCTAACCCCTGATACA-3′	
21	ACC	ACC-F	5′-CACCTCCAGCGACTTGTTAC-3′	346
		ACC-R	5′-GTTAGCCAGCATCACGATCC-3′	
22	MOX	MOX-F	5′-GCAACAACGACAATCCATCCT-3′	895
		MOX-R	5′-GGGATAGGCGTAACTCTCCCAA-3′	
23	FOX	FOX-F	5′-CTACAGTGCGGGTGGTTT-3′	162
		FOX-R	5′-CTATTTGCGGCCAGGTGA-3′	
24	*chuA*	chuA.1b	5′-ATGGTACCGGACGAACCAAC-3′	288
		chuA.2b	5′-TGCCGCCAGTACCAAAGACA-3′	
25	*yjaA*	yjaA.1b	5′-CAAACGTGAAGTGTCAGGAG-3′	211
		yjaA.2b	5′-AATGCGTTCCTCAACCTGTG-3′	
26	TspE4.C2	TspE4C2.1b	5′-CACTATTCGTAAGGTCATCC-3′	152
		TspE4C2.2b	5′-AGTTTATCGCTGCGGGTCGC-3′	
27	*arpA*	ArpA1.F	5′-AACGCTATTCGCCAGCTTGC-3′	400
		ArpA1.R	5′-TCTCCCCATACCGTACGCTA-3′	

1:5 donor to recipient, and the inoculated plates were incubated overnight at 37 °C. Transconjugants were selected on LB medium containing imipenem (1 mg/L) and sodium azide (100 mg/L).[22]

Plasmid incompatibility typing

The incompatibility type of the conjugatively transferable plasmid carrying resistance determinants was determined by PCR-based replicon typing targeting 18 different replicon types, i.e. FIA, FIB, FIC, HI1, HI2, I1/Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA.[23] Plasmids encoding the transconjugants were extracted using QIAprep Spin Miniprep Kit (Qiagen) and used as a template for the reaction.

Sequence typing

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* database[19] 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 Enterobacterales 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: $bla_{CTX-M-15}$ ($n = 6$), $bla_{SHV-148}$ ($n = 3$), bla_{PER-1} ($n = 2$) and bla_{TEM} ($n = 1$). 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 carbapenemase producers, and for two isolates bla_{NDM-1} genes could be identified by PCR. By performing PCR assay for AmpC genes, four isolates were found to harbour the bla_{CIT} gene. Further variant analysis revealed that the sequences are identical to bla_{CMY-42}. Apart from bla_{CIT}, for two isolates PCR amplification and sequencing analysis revealed the presence of bla_{DHA-1}. Interestingly, in both the PHCs single or multiple combinations of ESBLs ($bla_{CTX-M-15}$, bla_{PER-1}, bla_{TEM}, $bla_{SHV-148}$), AmpC (bla_{CMY-42}, bla_{DHA-1}) and carbapenemase genes (bla_{NDM-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 ($n = 4$), ST3268 ($n = 3$), 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 bla_{NDM-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 β-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 $bla_{CTX-M-15}$ $bla_{SHV-148}$, bla_{PER-1} and bla_{TEM} 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 bla_{NDM-1} gene was found to be maintained within a plasmid of $IncF_{repB}$ 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[2,24] 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 UTIs.[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 β-lactamases such as ESBLs or carbapenemases was mainly described within 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.*[33] (36.8%), Taneja *et al.*[34] (40.2%), Gautam *et al.*[35] (33%) and Kumar *et al.*[36] (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

PHC (isolate ID)	ST	ESBL, AmpC and carbapenemase genes	Phylotype	Susceptibility pattern															Replicon type
				TGC	CST	SXT	IPM	ETP	MEM	NIT	CRO	CIP	AMC	TZP	FEP	AMK	GEN	NAL	
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																$IncF_{repB}$
A (Ec-237)	ST3430	CTX-M-15 + DHA-1	B1																IncP
A (Ec-252)	unknown	CTX-M-15 + DHA-1	B1																IncP
A (Ec-76)	ST167	—	B1																—
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																IncF
B (Ec-12)	ST3492	NDM-1 + CMY-42	A																$IncF_{repB}$
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	—	B1																
B (Ec-56)	ST304	—	B2																—
B (Ec-132)	unknown	—	A																—
B (Ec-139)	ST4671	—	A																—

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, ceftriaxone; CIP, ciprofloxacin; AMC, amoxicillin/clavulanic acid; FEP, cefepime.

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.[37] 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.[38] Similarly, in the present study all the ESBLs and the carbapenemase gene bla_{NDM-1} were also found to be carried within self-transferable plasmids. The detection of two carbapenemase genes among the 13 phenotypically positive isolates may be due to presence of different types/variants 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.[39] 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.[40]

Recently, *E. coli* has been identified as the most frequently isolated ESBL-producing bacteria worldwide, with CTX-M being the most common ESBL type.[41] Accordingly, in our study $bla_{CTX-M-15}$ 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.[42,43] 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.[44]

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 report[45] and, more worryingly, the bla_{NDM}-harbouring 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.[46,47]

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.

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