Journal of Global Antimicrobial Resistance 30 (2022) 115–122

Contents lists available at ScienceDirect

Journal of Global Antimicrobial Resistance

journal homepage: www.elsevier.com/locate/jgar

Phenotypic and genotypic distribution of ESBL, AmpC β-lactamase and carbapenemase-producing Enterobacteriaceae in community-acquired and hospital-acquired urinary tract infections in Sri Lanka

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ARTICLE INFO

Article history:
Received 1 February 2021
Revised 25 March 2022
Accepted 25 May 2022
Available online 3 June 2022

Editor: Stefania Stefani

Keywords:
Extended-spectrum β-lactamase (ESBL)
AmpC β-lactamase
Carbapenemase
Enterobacteriaceae
Urinary tract infections (UTI)
Sri Lanka

ABSTRACT

Objectives: Although Sri Lanka belongs to a region with a high prevalence of extended-spectrum β-lactamase (ESBL), AmpC β-lactamase and carbapenemase-producing Enterobacteriaceae, data regarding antimicrobial resistance (AMR) is limited. We studied the prevalence and diversity of β-lactamases produced by Enterobacteriaceae urinary pathogens from two hospitals in the Western Province of Sri Lanka.
Methods: ESBL, AmpC β-lactamase and carbapenemase production was detected by phenotypic testing followed by genotyping.
Results: The species responsible for urinary tract infections (UTI) were *Escherichia coli* (69%), *Klebsiella pneumoniae* (16%) and *Enterobacter* sp (6%). The prevalence of ESBL (50%), AmpC β-lactamase (19%) and carbapenemase (11%) phenotypes was high, and greater in hospital-acquired (HA-UTI) (75%) than in community-acquired UTI (CA-UTI) (42%). Identification of CA-UTI caused by carbapenemase-producing Enterobacteriaceae (5%) is alarming. Only one ESBL gene, $bla_{\text{CTX-M-15}}$, was detected. AmpC β-lactamase genes found in *E. coli* and *K. pneumoniae* were $bla_{\text{CMY-2}}$, $bla_{\text{CMY-42}}$ and $bla_{\text{DHA-1}}$, while *Enterobacter* sp. carried $bla_{\text{ACT-1}}$. Carbapenemase genes were $bla_{\text{NDM-1}}$, $bla_{\text{NDM-4}}$, $bla_{\text{OXA-181}}$ and $bla_{\text{OXA-232}}$, while bla_{KPC}, bla_{IMP} and bla_{VIM} were absent. Co-occurrence of multiple *bla* genes, with some isolates harbouring six different *bla* genes, was common. Carbapenem-resistant isolates without carbapenemase genes displayed mutations in the outer membrane porin genes, *ompF* of *E. coli* and *ompK36* of *K. pneumoniae*. Factors associated with UTI with β-lactamase-producing Enterobacteriaceae were age $\geq$50 years, previous hospitalization, presence of an indwelling urinary catheter, history of diabetes mellitus or other chronic illness and recurrent urinary tract infections.
Conclusion: This study adds to the currently scarce data on AMR in Sri Lanka.

1. Introduction

Antimicrobial resistance (AMR) in Gram-negative bacteria, particularly Enterobacteriaceae, is increasing globally. This is mainly due to the dissemination of strains producing extended-spectrum β-lactamases (ESBLs), AmpC β-lactamases and carbapenemases. β-lactamase-producing strains of Enterobacteriaceae are a frequent cause of both community-acquired and hospital-acquired infections, especially urinary tract infections (UTI). Common enzymes found in these isolates include the ESBLs CTX-M, SHV and TEM, AmpC β-lactamases CMY, ACT and DHA and carbapenemases KPC, NDM, OXA-48 [1]. In addition, decreased expression of major porins, such as OmpK35 and OmpK36 in *Klebsiella pneumoniae*

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https://doi.org/10.1016/j.jgar.2022.05.024

and OmpF and OmpC in *Escherichia coli*, in combination with β-lactamase production is known to confer carbapenem resistance in Enterobacteriaceae [2]. Studies on the prevalence and distribution of β-lactamase mediated resistance in local settings is important to decide on empiric antibiotic treatment and infection prevention and control measures.

Although Sri Lanka belongs to a geographical region with a high prevalence of ESBL, AmpC β-lactamase and carbapenemase-producing Enterobacteriaceae, epidemiological data regarding antimicrobial resistance (AMR) in Sri Lanka is limited. Most studies conducted on ESBL and carbapenemase production in pathogenic Enterobacteriaceae in Sri Lanka identified only the β-lactamase phenotypes [3–7], and only two characterized the genes coding for these β-lactamases [8,9]. These two studies, conducted on collections of carbapenemase-producing *K. pneumoniae* isolates from Sri Lanka, showed that the predominant β-lactamase genes were the ESBL gene CTX-M-15 and the carbapenemase genes OXA-181 and NDM-1. In addition, the study by Hall et al. identified mutations in the *omp*36 gene [8].

The present study was carried out to identify the prevalence and diversity of β-lactamases produced by Enterobacteriaceae urinary pathogens from two hospitals in the Western Province of Sri Lanka.

2. Materials and methods

A total of 422 consecutive, clinically significant, urinary isolates of Enterobacteriaceae from adults treated for UTI at the outpatient departments, medical and surgical units and intensive care units of two hospitals in the Western Province of Sri Lanka, Sri Jayawardenapura General Hospital, Nugegoda and the Neville Fernando Teaching Hospital, Malabe, were collected between 2015 and 2016. Community-acquired UTI (CA-UTI) and hospital-acquired UTI (HA-UTI) were differentiated based on CDC/National Healthcare Safety Network (NHSN) criteria [10]. Infections were sporadic with no outbreaks reported. Bacteria isolated from midstream urine samples, in pure growth of $>10^5$ CFU/mL (colony-forming units per millilitre), were taken as clinically significant isolates in CA-UTI. A urine culture with no more than two species of organisms, at least one of which had a colony count of $>10^5$ CFU/mL, was taken as significant in patients with HA-UTI [10].

Identification, up to species level, was done using colony morphology on cysteine lactose electrolyte deficient (CLED) agar, Gram stain appearance, oxidase test, biochemical testing (IMViC: indole, methyl red, Voges-Proskauer, citrate) and the Rap ID One system Enterobacteriaceae identification kit (Remel, Thermo Scientific).

2.1. Phenotypic detection of ESBLs, AmpC β-lactamases and carbapenemases

ESBL production was determined by a combination of the Clinical and Laboratory Standards Institute (CLSI) screening method using cefpodoxime, ceftazidime, aztreonam, cefotaxime and ceftriaxone discs, the CLSI confirmatory combination disc test [11] and the modified double disc diffusion test [12]. Screening for AmpC β-lactamase production using cefoxitin and cefotetan discs [13] was followed by confirmation with the AmpC disc test [14]. Carbapenem resistance was detected by the disc diffusion test, using CLSI cut-off values for imipenem, meropenem and ertapenem [11], followed by the following confirmatory tests: modified Hodge test [15], double disc synergy test with 0.5M EDTA [16] and the carbapenem inhibitory method [11].

Quality control was maintained using *E. coli* ATCC 25922 (negative ESBL control) and *K. pneumoniae* ATCC 700603 (positive ESBL control) for the ESBL phenotypic tests; in-house AmpC positive and AmpC negative strains of *K. pneumoniae* for the AmpC phenotypic tests; *K. pneumoniae* ATCC BAA-1705 (positive control for KPC type carbapenemase), an in-house *K. pneumoniae* strain as positive control for NDM and OXA-48-like carbapenemases and *K. pneumoniae* ATCC BAA-1706 (negative control) for the carbapenemase phenotypic tests.

2.2. Determination of bla gene types and diversity of bla genes

The isolates that demonstrated ESBL, AmpC β-lactamase or carbapenemase production by any phenotypic method ($n = 216$) were subjected to molecular characterization of β-lactamase gene type by conventional polymerase chain reaction (PCR). Isolates were subcultured on blood agar and incubated at 37°C in air for 24 h to obtain single colony growth. A suspension of bacteria was made in ultrapure water to a density of McFarland standard 2.0 and heated at 95°C for 10 min and centrifuged at 13 000 × *g* for 1 min to pellet cell debris. The supernatant was used as the template for subsequent PCR assays. Simplex PCR was used to detect the presence of genes coding for ESBL, AmpC β-lactamase and carbapenemase using the primers and annealing temperatures described in Table 1 [17–21].

A representative subset of 175 randomly picked amplified *bla* genes (80 from isolates causing CA-UTI and 95 from isolates causing HA-UTI) were sequenced by Sanger sequencing to identify β-lactamase gene variants (Table 1). PCR products were purified and sequenced in both directions using the same primer pairs used for PCR amplification. Sanger sequence service was provided by Macrogen, Korea. The sequences were analysed using the SeqMan (Lasergene 6) software tool and subjected to homology search using BLASTn (http://www.ncbi.nlm.nih.gov/) for determination of identities [22].

2.3. Molecular analysis of omp genes coding for outer membrane porin proteins (OMPs)

Isolates of *E. coli* ($n = 7$) and *K. pneumoniae* ($n = 7$) that showed reduced susceptibility to carbapenems but only harboured ESBL and/or Amp C β-lactamase genes without any of the main carbapenemase genes (bla_{NDM}, bla_{OXA-48}-like, bla_{KPC}, bla_{IMP} and bla_{VIM}) were further analysed for *omp* mutations that may signal porin loss. PCR was performed to amplify the genes coding for major OMPs [2]. The resulting *ompC, ompF, ompK35* and *ompK36* genes were sequenced by Sanger sequencing. The sequences were analysed using the SeqMan software tool and subjected to homology search using BLASTn for determination of identities [22]. Deduced protein sequences for OMPs were aligned against the reference sequences using the ClustalW sequence alignment software to identify variations.

2.4. Determination of risk factors for UTI with β-lactamase-producing Enterobacteriaceae

The study protocol was approved by the Ethics Review Committee of the Faculty of Medicine, University of Colombo. Demographic and clinical data of patients, including age, sex, history of diabetes mellitus or other chronic illness, recurrent UTI (>3 episodes of UTI in the year preceding the present admission), recent hospitalization (within the last 1 year), household contact with a health care worker, frequent visits to hospitals or clinics, recent antibiotic exposure (exposure to antibiotics for longer than 24 hours within a 3-month period preceding the present admission), presence of an indwelling urinary catheter and type and duration of current antibiotic therapy were collected using an interviewer administered questionnaire. Data for variables were presented as percentages. Univariate analysis using the χ^2 test for discrete outcomes was

Table 1
Primers used for PCR and sequencing

	B-lactamase	Gene	Forward primers 5′-3′	Reverse primers 5′-3′	Amplicone size	Annealing temperature (°C)	Reference
1	ESBL	bla_{SHV}	AGCCGCTTGAGCAAATTAAAC	ATCCCGCAGATAAATCACCAC	713	60	Dallenne et al. [17]
2		bla_{TEM}	CATTTCCGTGTCGCCCTTATTC	CGTTCATCCATAGTTGCCTGAC	800	60	Dallenne et al. [17]
3		bla_{CTX-M}	ATGTGCAGYACCAGTAARGTKATGGC	TGGGTR AARTARGTSACCAGAAYCAGCGG	593	62	Fang et al. [18]
4		bla_{OXA}	ACACAATACATATCA ACTTCG C	AGT GTG TTT AGA ATGGTG ATC	813	62	Fang et al. [18]
5	AmpC	bla_{MOX}	GCTGCTCAAGGAGCACAGGAT	CACATTGACATAGGTGTGGTGC	520	64	Pérez and Hanson [19]
6		bla_{CMY}	TGGCCAGAACTGACAGGCAAA	TTTCTCCTGAACGTGGCTGGC	462	64	Pérez and Hanson [19]
7		bla_{DHA}	AACTTTCACAGGTGTGCTGGGT	CCGTACGCATACTGGCTTTGC	405	64	Pérez and Hanson [19]
8		bla_{ACC}	AACAGCCTCAGCAGCCGGTTA	TTCGCCGCAATCATCCCTAGC	346	64	Pérez and Hanson [19]
9		bla_{ACT}	TCGGTAAAGCCGATGTTGCGG	CTTCCACTGCGGCTGCCAGTT	302	64	Pérez and Hanson [19]
10		bla_{FOX}	AACATGGGGTATCAGGGAGATG	CAAAGCGCGTAACCGGATTGG	190	64	Pérez and Hanson [19]
11	Carbapenemase	bla_{KPC}	CGTCTAGTTCTGCTGTCTTG	CTTGTCATCCTTGTTAGGCG	798	52	Poirel et al. [20]
12		bla_{IMP}	GGAATAGAGTGGCTTAAYTC	TCGGTTTAAYAAAACAACCACC	232	52	Poirel et al. [20]
13		bla_{VIM}	GATGGTGTTTGGTCGCATA	CGAATGCGCAGCACCAG	390	52	Poirel et al. [20]
14		bla_{NDM}	GGTTTGGCGATCTGGTTTTC	CGGAATGGCTCATCACGATC	621	52	Poirel et al. [20]
15		bla_{OXA-48} like	TTGGTGGCATCGATTATCGG	GAGCACTTCTTTTGTGATGGC	743	57	Poirel et al. [21]

done using the SPSS 16 software. Odds ratios (ORs) with 95% confidence intervals (CIs) were calculated for target risk factors. Statistical significance was determined based on the confidence intervals and a P value of <0.05.

3. Results

3.1. Enterobacteriaceae urinary pathogens in CA-UTI and HA-UTI

Of 422 clinically significant Enterobacteriaceae isolates from UTI, 72% (302/422) were from CA-UTI and 28% (120/422) were from HA-UTI. The predominant isolates, in both CA-UTI and HA-UTI, were *E. coli* and *K. pneumoniae* (Fig. 1).

3.2. β-lactamase phenotypes in Enterobacteriaceae causing HA-UTI and CA-UTI

The overall prevalence rates of ESBLs, Amp C β-lactamases and carbapenemase phenotypes in Enterobacteriaceae uropathogens in our study were 50% (212/422), 19% (79/422) and 11% (46/422). The percentage of ESBL, AmpC β-lactamase and carbapenemase producing Enterobacteriaceae in CA-UTI was 40% (122/302), 16% (48/302) and 5% (15/302), respectively, and in HA-UTI were 75% (90/120), 26% (31/120) and 26% (31/120), respectively (Table 2).

Of the 302 Enterobacteriaceae from CA-UTI, 126 (42%) showed the presence of one or more β-lactamase phenotypes. Of 120 isolates from HA-UTI, 90 (75%) isolates showed the presence of one or more β-lactamase phenotypes. Multiple β-lactamases were seen in 25% (104/422), with 17% (51/302) of isolates from CA-UTI and 44% (53/120) of isolates from HA-UTI demonstrating these combinations (Fig. 2) (Supplementary Table S1).

3.3. β-lactamase genotypes in Enterobacteriaceae causing HA-UTI and CA-UTI

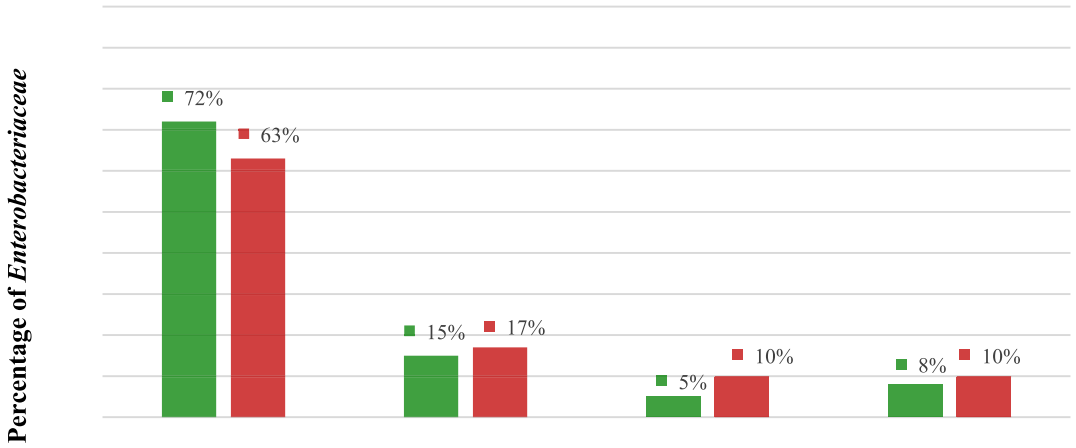
Four potential narrow spectrum β-lactamase/ESBL genotypes (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}), three AmpC β-lactamase genotypes (bla_{CMY}, bla_{DHA} and bla_{ACT}), and two carbapenemase genotypes (bla_{NDM}, bla_{OXA-48}-like) were identified. Overall prevalence of *bla* genes among Enterobacteriaceae uropathogens was bla_{TEM}: 39% (163/422), bla_{SHV}: 17% (73/422), bla_{CTX-M}: 46% (194/422), bla_{OXA}: 13% (54/422), bla_{CMY}: 15% (63/422), bla_{DHA}: 3% (13/422), bla_{ACT}: 2 (8/422), bla_{NDM}: 6% (24/422) and bla_{OXA-48}-like: 3% (11/422). Isolates of Enterobacteriaceae from CA-UTI (n = 302) and HA-UTI (n = 120) displayed these genotypes at diverse rates (Fig. 3). Co-occurrence of multiple *bla* genes, with some isolates harbouring up to six different *bla* genes, was common (Supplementary Table S2).

3.4. *bla* gene variants identified on Sanger sequencing

Several β-lactamase gene variants were identified in the representative subset of 175 *bla* genes that were subjected to Sanger sequencing. These included the narrow-spectrum β-lactamase genes bla_{TEM-1}, bla_{SHV-1}, bla_{SHV-2}, bla_{SHV-11}, bla_{SHV-28} and bla_{OXA-1}; the ESBL gene $bla_{CTX-M-15}$; the AmpC β-lactamase genes bla_{CMY-42}, bla_{CMY-2}, bla_{DHA-1}, blaACT$_{-1}$ and bla_{ACT-7} and the carbapenemase genes bla_{NDM-1}, bla_{NDM-4}, bla_{OXA-18} and $bla_{OXA-232}$. Gene variants identified in isolates from CA-UTI and HA-UTI are shown in Table 3.

3.5. Molecular analysis of genes coding for outer membrane porin proteins (OMPs) in carbapenem-resistant E. coli and K. pneumoniae isolates that did not harbour carbapenemase genes

The *omp* genes of the *E. coli* (n = 7) and *K. pneumoniae* (n = 7) isolates that were potential carbapenemase producers by pheno-



	E. coli	K. pneumoniae	Enterobacter sp.	Other
CA-UTI	72% (218/302)	15% (45/302)	5% (14/302)	8% (25/302)
HA-UTI	63% (75/120)	17% (20/120)	10% (12/120)	10% (13/120)
Total	69% (293/422)	16% (65/422)	6% (26/422)	9% (38/422)

Fig. 1. The prevalence of Enterobacteriaceae in CA-UTI and HA-UTI.

Table 2
ESBL, AmpC β-lactamase and carbapenemase phenotypes among Enterobacteriaceae causing HA-UTI and CA-UTI

Bacterial species (No. of isolates)	Prevalence of β-lactamase producing isolates ESBL (CA-UTI)	(HA-UTI)	AmpC β-lactamase (CA-UTI)	(HA-UTI)	Carbapenemase (CA-UTI)	(HA- UTI)
E. coli (n = 293)	82/218(38%)	52/75(69%)	33/218(15%)	18/75(24%)	4/218(2%)	11/75(15%)
K. pneumoniae (n = 65)	20/45(44%)	19/20(95%)	7/45(15.5%)	7/20(35%)	10/45(22%)	14/20(70%)
Enterobacter sp. (n = 26)	7/14 (50%)	10/12 (83%)	4/14(28%)	5/12 (42%)	1/14 (7%)	2/12 (17%)
Other (n = 38)	13/25 (52%)	9/13 (69%)	4/25 (16%)	1/13 (8%)	-	4/13 (30%)
Total (n = 422)	122/302(40%)	90/120(75%)	48/302(16%)	31/120(26%)	15/302(5%)	31/120(26%)

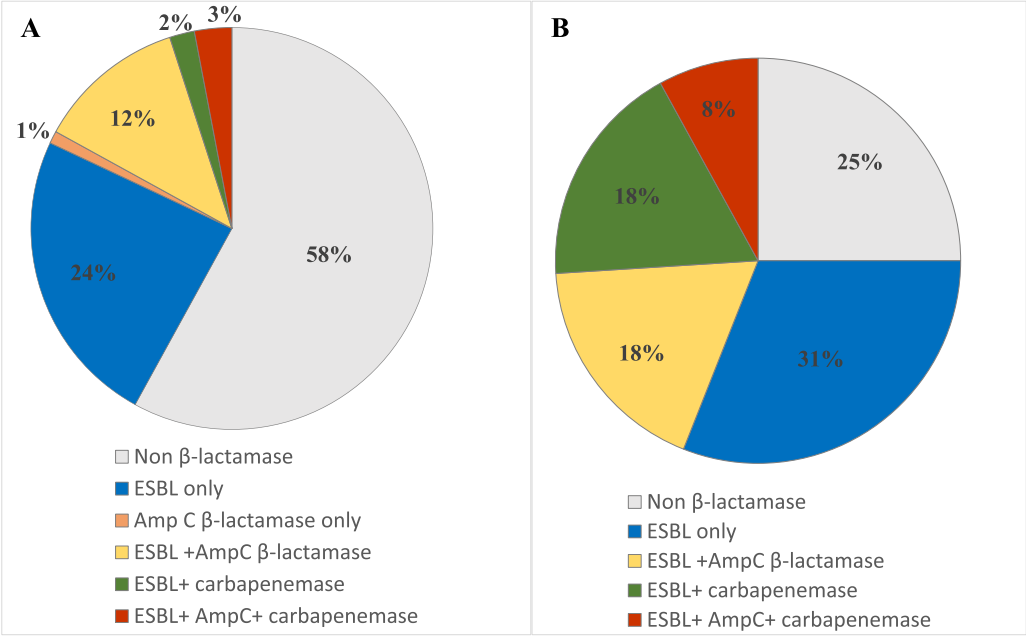


Fig. 2. Prevalence of β-lactamase phenotypes in Enterobacteriaceae in CA-UTI and HA-UTI. (A) Prevalence of β-lactamase phenotypes in Enterobacteriaceae in CA-UTI. (B) Prevalence of β-lactamase phenotypes in Enterobacteriaceae in HA-UTI.

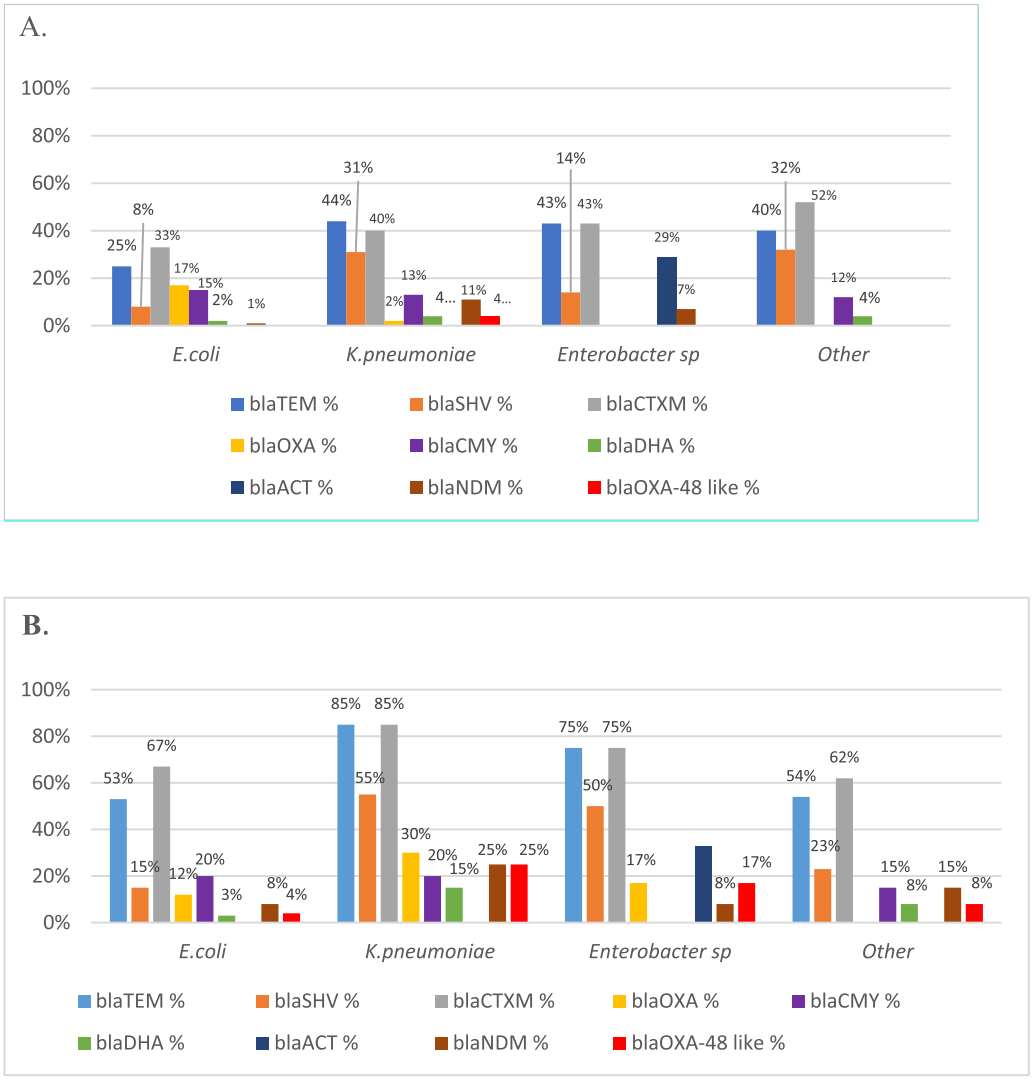


Fig. 3. The prevalence of *bla* genes in Enterobacteriaceae uropathogens from CA-UTI and HA-UTI. (A) Prevalence of *bla* genes in isolates from CA-UTI. (B) Prevalence of *bla* genes in isolates from HA-UTI.

typic tests, but only harboured ESBL and/or Amp C β-lactamase genes, showed amplicons of the expected size (approximately 1.1 kb). Alignment of the deduced protein sequences showed a normal OmpC sequence in the *E. coli* isolates and a normal OmpK35 sequence in the *K. pneumoniae* isolates. However, a large number of variations was observed in the *ompF* gene of one *E. coli* isolate, when compared to the wild-type *E. coli* K-12 strain and in the *ompK36* genes in four *K. pneumoniae* isolates, when compared with the wild-type *K. pneumoniae* strain ATCC 13883. These variations included insertions, deletions and substitutions resulting in corresponding variations in the deduced protein sequences. The *ompF* gene of the *E. coli* isolate showed a premature stop codon at 267 amino acid OmpF (compared to 362 amino acids in OmpF of the reference strain). The amino acid sequences of OmpK36 in the four *K. pneumoniae* isolates showed multiple variations including D49S, L58V, ins aa183_184 LPS, G189T, F198Y, V207L, A221S, T226L, ins aa 230_231S, Q231K, L233A, E237R, H240N, A285V, N304E, R345H and S346N.

3.6. Risk factors for β-lactamase mediated resistance in isolates from UTI

UTI with β-lactamase-producing Enterobacteriaceae was significantly associated with age ≥50 years (odds ratio [OR], 1.89; 95%, CI, 1.27–2.82; $P < .001$), previous hospitalization (OR, 2.21; 95% CI, 1.18–4.15; $P < .05$), presence of an indwelling urinary catheter (OR, 6.51; 95% CI, 3.81–11.12; $P < .001$), history of diabetes mellitus (OR, 3.45; 95% CI, 2.30–5.19; $P < .001$), presence of chronic illness (OR, 1.70; 95% CI, 1.16–2.50; $P < .001$) and a past history of recurrent UTI (OR, 2.33; 95% CI, 1.55–3.50; $P < .001$) (Table 4). Sex, frequent hospital visits and household contact with a health care worker was not associated with UTI with β-lactamase-producing Enterobacteriaceae. Adequate data describing antibiotic exposure could not be collected, and this risk factor was excluded from the final analysis.

4. Discussion

This study was conducted on 422 uropathogenic Enterobacteriaceae isolates [302 (72%) from CA-UTI and 120 (28%) from HA-UTI] collected from two hospitals in the Western Province of Sri Lanka during an 11-month period from 2015 to 2016. Similar to previous studies, the most common Enterobacteriaceae found in both CA-UTI and HA-UTI was *E. coli*, followed by *K. pneumoniae* and *Enterobacter* sp. [5,23]. The isolates displayed a variety of β-lactamase phenotypes (ESBL, AmpC β-lactamase and carbapenemase) and harboured a variety of β-lactamase genotypes. Of the

Table 3
bla gene variants identified by Sanger sequencing (n = 175)

		No. of selected amplicons from isolates from CA-UTI (n = 80)			No. of selected amplicons from isolates from HA-UTI (n = 95)		
bla gene type	*bla* gene variant	*E. coli* (n = 32)	*K. pneumoniae* (n = 31)	*Enterobacter* sp. (n = 17)	*E. coli* (n = 36)	*K. pneumoniae* (n = 36)	Enterobacter sp. (n = 23)
TEM	bla_{TEM-1}	5	5	5	5	5	5
SHV	bla_{SHV-1} bla_{SHV-2} bla_{SHV-11} bla_{SHV-28}	5	3	2	5	3	5
		-	1	-	-	-	-
		-	2	-	-	1	-
		-	-	-	-	1	-
CTX-M	$bla_{CTX-M-15}$	5	5	5	5	5	5
OXA	bla_{OXA-1}	5	1	-	5	4	2
CMY	bla_{CMY-42} bla_{CMY-2}	5	5	-	4	4	-
		-	-	-	2	-	-
DHA	bla_{DHA-1}	5	2	-	2	3	-
ACT	bla_{ACT-1} bla_{ACT-7}	-	-	-	-	-	1
		-	-	4	-	-	3
NDM	bla_{NDM-1} bla_{NDM-4}	2	5	1	5	7	-
		-	-	-	-	-	1
OXA-48 like	$bla_{OXA-181}$	-	2	-	3	2	1
	$bla_{OXA-232}$	-	-	-	-	1	-

Table 4
Risk factors for β-lactamase mediated resistance in isolates from UTI

	β-lactamase producing isolates (n = 216) No. (%)	Non-β-lactamase producing Isolates (n = 206) No. (%)	OR (95% CI)	P value
Age ≥50 y	148 (68.5%)	110 (53.4%)	1.89 (1.27–2.82)	<0.001
Male	63(29.2%)	68 (33.0%)	0.84 (0.56- 1.26)	0.4
Previous hospitalization (within 1 y)	34 (15.7%)	16 (7.7%)	2.21(1.18- 4.15)	<0.05
Presence of indwelling urinary catheter	89 (41.2%)	20 (9.7%)	6.51(3.81–11.12)	<0.001
Diabetes mellitus	123 (56.9%)	57 (27.7%)	3.45 (2.30–5.19)	<0.001
Presence of chronic illnesses	123 (56.9%)	90 (43.7%)	1.70 (1.16–2.50)	<0.001
Recurrent UTI	102 (47.2%)	57 (27.7%)	2.33 (1.55–3.50)	<0.001
Regular hospital visits	53 (24.5%)	77 (37.4%)	0.545 (0.36- 0.83)	<0.05
Household contact with health care worker	17 (7.9%)	12 (5.8%)	1.381 (0.64- 2.97)	0.4

302 Enterobacteriaceae from CA-UTI, 126 (42%) showed the presence of one or more β-lactamase phenotypes, while 90 (75%) out of 120 isolates from HA-UTI showed the presence of one or more β-lactamase phenotypes.

The prevalence of ESBL production in Enterobacteriaceae in our study was 50% (212/422). This rate is higher than that reported by Tillekeratne et al. (40.2%) in 2016 [5] and, more recently, by Kumudunie et al. (30.8%) in 2020 [6]. However, in the latter study, the rate of cefotaxime/ceftriaxone resistance in Enterobacteriaceae was reported as 55.3%, and it is likely that co-production of AmpC β-lactamase and carbapenemase interfered with the phenotypic detection of ESBLs [12]. The rate of ESBL production in Sri Lankan isolates reported in this study is significantly higher than that reported in the UK and Europe (3% to 6%) and the United States (2%) [24]. On the other hand, the prevalence is much lower than that recorded in India, which is around 70% of clinical isolates [25,26].

As all the bla_{TEM}, bla_{SHV} and bla_{OXA-1} genes sequenced in a subset of isolates coded for narrow-spectrum β-lactamases, it is likely that almost all of the ESBL phenotypes resulted from the presence of the bla_{CTX-M} gene, specifically the gene variant, $bla_{CTX-M-15}$. This is compatible with previous studies on ESBL genotypes in Sri Lanka [5,8,9].

There is no previous data on AmpC β-lactamase production in Enterobacteriaceae in Sri Lanka. The prevalence rate of AmpC β-lactamase production in our study was 19%. This rate is much lower than that of our neighbour, India, with a prevalence rate of 40% [26], and the rate in China (26%) [27]. However, our rates are much higher than those reported in the United States and Europe, which are less than 10% [28, 29]. The Amp C β-lactamase genes found in *E. coli* and *K. pneumoniae*, in both CA-UTI and HA-UTI, were bla_{CMY} (bla_{CMY-2} and bla_{CMY-42}) and bla_{DHA} (bla_{DHA-1} and bla_{DHA-7}), while the only AmpC β-lactamase gene identified in *Enterobacter* sp. was bla_{ACT} (bla_{ACT-1} and bla_{ACT-7}).

The prevalence of carbapenemases in Enterobacteriaceae in our study was 11%, which is comparable with the rates reported by Kumudunie et al. [6]. This rate, again, is much lower than the rates reported in India, i.e., 27%–65% [26] and China, i.e., 18% [30] but far higher than the prevalence in West European countries such as France, Germany and Switzerland (<1%) [31]. The carbapenemase genes found in this study were bla_{NDM} (bla_{NDM-1}, bla_{NDM-4}) and bla_{OXA-48}-like ($bla_{OXA-181}$, $bla_{OXA-232}$), while bla_{KPC}, bla_{IMP} and bla_{VIM} were absent. Similar to previous studies in Sri Lanka and reports from other countries in South Asia [23,25], the most prevalent carbapenemase gene was bla_{NDM}. The presence of bla_{OXA-48}-like carbapenemases has been reported previ-

ously in *K. pneumoniae* isolates from nosocomial infections in Sri Lanka [6,8,9], but this study reveals that bla_{OXA-48}-like genes are present in strains causing community-acquired infections and in hospital-acquired and community-acquired *E. coli* as well. Although bla_{NDM-1}, bla_{NDM-4} and $bla_{OXA-181}$ have been described previously in Sri Lanka, this is the first report of the $bla_{OXA-232}$ variant, even though it is common in India [32]. The bla_{KPC} gene was not identified in our study, although its presence in Sri Lanka has been recently reported [6]. bla_{KPC} is known to be uncommon in South and Southeast Asia [33].

As expected, the rate of ESBL production was higher in isolates from HA-UTI (75%) than CA-UTI (40%), both in *E. coli* (69% vs. 38%) and *K. pneumoniae* (95% vs. 44%). The rates determined in this study are much higher than those reported by Wijesooriya et al. [34] in isolates from Sri Lanka collected between 2012 and 2016 (HA-UTI, 68%; CA-UTI, 13%). The rapid increase in ESBL rates from 2012 to date, especially in CA-UTI, is a cause for concern.

Similarly, Amp C β-lactamase and carbapenemase production was much higher in isolates from HA-UTI (26% and 26%, respectively) than isolates from CA-UTI (16% and 5%, respectively) in both *E. coli* (Amp C β-lactamase 24% vs. 15%, carbapenemase 15% vs. 2%) and *K. pneumoniae* (Amp C β-lactamase 35% vs. 15.5%, carbapenemase 70% vs. 22%). The high prevalence of Amp C producers in CA-UTI and the occurrence of carbapenemases (i.e., bla_{NDM-1}, $bla_{OXA-181}$) in Enterobacteriaceae from CA-UTI, with 22% of *K. pneumoniae* from CA-UTI being carbapenemase producers, is a cause for alarm.

Enterobacteriaceae isolates producing multiple β-lactamase types are becoming increasingly common [1]. In our study, phenotypic testing revealed multiple β-lactamases in as many as 25% of isolates. Unsurprisingly, co-production of multiple β-lactamases was seen more commonly in HA-UTI (44%) than CA-UTI (17%). The co-occurrence of multiple ESBL, AmpC β-lactamase and carbapenemase genes in Sri Lanka has been reported previously only in *K. pneumoniae* [8,9]. Combinations of ESBL, AmpC beta-lactamase and carbapenemase genes was seen in *E. coli, K. pneumoniae* and *Enterobacter* sp., with some isolates harbouring up to six separate *bla* genes. Similar findings have been reported in India and other countries [35–37].

With regard to the isolates that showed potential carbapenemase production in the phenotypic tests but only harboured ESBL and/or AmpC β-lactamase genes, the mutation in the *ompF* gene of the *E. coli* isolate predicted a premature termination in the deduced protein OmpF. Premature stop codons resulting in carbapenemase resistance have been reported previously [38]. While some of mutations in the *ompK36* gene of the four *K. pneumoniae* isolates are well known [8], the isolates in our study showed several additional mutations. However, the common mutation associated with carbapenem resistance, Iaa134-135GD [8,38], was not found.

In this study, age ≥50 years, previous hospitalization, presence of an indwelling urinary catheter, a history of diabetes mellitus or other chronic illness and recurrent urinary tract infections were associated with UTI due to β-lactamase-producing Enterobacteriaceae. Similar findings have been documented in local and global studies [4,9,39].

5. Conclusion

Studies on the prevalence and characteristics of clinical isolates of Enterobacteriaceae with AMR are important to provide the data required to formulate local, national and international antibiotic guidelines and policies and to inform infection prevention and control guidelines to control the spread of resistance. This study adds to the currently scarce data on AMR in Sri Lanka and reveals that ESBL, AmpC β-lactamase and carbapenemase production is common in Enterobacteriaceae causing HA-UTI and CA-UTI in Sri Lanka.

Funding

This work was supported by grants from the National Research Council (grant number 14-45) and the University of Colombo (grant number AP/3/2/2018/SG/16).

Competing interests

None declared.

Ethical approval

Ethical approval for this study was obtained from the Ethics Review Committee of the University of Colombo, Faculty of Medicine (EC-14-143).

Acknowledgements

The authors thank the patients who took part in this study and the staff of the microbiology laboratories of the Sri Jayawardenapura General Hospital, Neville Fernando Teaching Hospital and Faculty of Medicine, University of Colombo for technical support.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.05.024.

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