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Performance of Phenotypic Tests to Detect β-Lactamases in a Population of β-Lactamase Coproducing *Enterobacteriaceae* Isolates

Vindya Perera[1,2] Nelun de Silva[2,4] Kushlani Jayatilleke[3] Sara de Silva[3] Enoka Corea[1]

[1] Faculty of Medicine, Department of Microbiology, University of Colombo, Sri Lanka
[2] Faculty of Medicine, Department of Microbiology, Sabaragamuwa University of Sri Lanka, Ratnapura, Sri Lanka
[3] Sri Jayewardenapura General Hospital, Nugegoda, Sri Lanka
[4] Neville Fernando Teaching Hospital, Malabe, Sri Lanka

Address for correspondence Vindya Madushika Perera, BVSc, (Hons), Department of Microbiology, Faculty of Medicine, Sabaragamuwa university of Sri Lanka, P.O. Box 01, Hidellana, Ratnapura 70012, Sri Lanka (e-mail: v.perera@med.sab.ac.lk).

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Abstract

Objectives This study aimed to evaluate the performance of routinely used phenotypic tests to detect β-lactamase production in isolates coproducing multiple β-lactamase types.

Methods Commonly used phenotypic tests for the detection of extended spectrum β-lactamases (ESBL), AmpC β-lactamase, and carbapenemases were compared with detection and sequencing of β-lactamase genes (as the reference test) in 176 uropathogenic *Enterobacteriaceae* coproducing multiple β-lactamases from two hospitals in the Western Province of Sri Lanka.

Results Majority of the isolates (147/176, 83.5%) carried β-lactamase genes with (90/147, 61%) harboring multiple genes. The Clinical and Laboratory Standards Institute screening method using cefotaxime (sensitivity [Se], 97; specificity [Sp], 93; accuracy [Ac], 94) and ceftriaxone (Se, 97; Sp, 91; Ac, 93) was the most effective to detect ESBLs. The modified double disc synergy test (Se, 98; Sp, 98; Ac, 97) and combined disc test (Se, 94; Sp, 98; Ac, 96) showed good specificity for confirmation of ESBLs. Cefoxitin resistance (Se, 97; Sp, 73; Ac, 85) and the AmpC disc test (Se, 96; Sp, 82; Ac, 86) were sensitive to detect AmpC β-lactamase producers coproducing other β-lactamases but showed low specificity, probably due to coproduction of carbapenemases. Meropenem was useful to screen for New Delhi metallo β-lactamases and OXA-48-like carbapenemases (Se, 97; Sp, 96; Ac, 96). The modified carbapenem inactivation method showed excellent performance (Se, 97; Sp, 98; Ac, 97) in identifying production of both types of carbapenemases and was able to distinguish this from carbapenem resistance due to potential mutations in the porin gene.

Conclusion Microbiology laboratories that are still depend on phenotypic tests should utilize tests that are compatible with the types of β-lactamase prevalent in the region and those that are least affected by coexisting resistance mechanisms.

Keywords
- AmpC β-lactamases
- β-lactamase coproducers
- carbapenemases
- *Enterobacteriaceae*
- extended spectrum β-lactamases (ESBL)
- phenotypic tests
- Sri Lanka

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Introduction

Optimal use of the clinical microbiology laboratory is crucial in the surveillance of antibiotic-resistant pathogens. It is critical that β-lactamase production (extended spectrum β-lactamases [ESBL], AmpC β-lactamases and carbapenemases) in *Enterobacteriaceae* is detected accurately to inform antimicrobial policies. Although higher-income countries are moving toward automation of diagnostic tests to detect antimicrobial resistance mechanisms in pathogens, developing countries still depend on the phenotypic tests due to the cost.[1] Failure of clinical microbiology laboratories in developing countries in antimicrobial resistance testing will contribute to the global spread of resistant pathogens. False results due to coproduction of multiple enzyme groups result in adverse impacts on patient care.[2,3] This study aimed to evaluate the performance of routine phenotypic tests to detect β-lactamase production in isolates coproducing multiple β-lactamase types.

Subjects and Methods

Sample Size

Sample size at the required absolute precision level of 90% for sensitivity and specificity was calculated using Buderer's formula.[4] The prevalence rate of ESBL production was taken as 40% based on a previous study.[5] As the prevalence of AmpC β-lactamase and carbapenemase production was unknown, a prevalence of 50% was assumed. The calculated minimum sample size required was 89.

Bacterial Isolates

A total of 176, nonduplicate, clinically significant, urinary isolates of *Enterobacteriaceae* from adults with urinary tract infection (UTI) managed at the outpatient department, medical and surgical units, and intensive care units of two hospitals in the Western Province of Sri Lanka (Sri Jayawardenapura General Hospital and the Neville Fernando Teaching Hospital, Malabe) in 2015 and 2016 were included in the study. Written informed consent was obtained from the patients when collecting urine samples. Community-acquired UTI and hospital acquired UTI were categorized based on criteria listed by CDC/National Healthcare Safety Network.[6] Speciation of the isolates was done using colony morphology on cysteine lactose electrolyte deficient agar, Gram stain appearance, biochemical testing Indole, Methyl Red, Voges-Proskauer, Citrate (IMViC),[7] and the use of a commercial identification kit Thermo Scientific™ RapID™ ONE System.

PCR to Detect ESBLs, AmpC β-Lactamases, and Carbapenamase Genes

Isolates were subcultured on blood agar and incubated at 37° C in air for 24 hours to obtain single colony growth. A suspension of bacteria was made in ultrapure water to McFarland standard 2.0 and heated at 95°C for 10 minutes and centrifuged at 13,000 g for 1 minute to pellet cell debris. The supernatant was used as the template for subsequent polymerase chain reaction (PCR) assays. All PCR assays were performed at the Faculty of Medicine, University of Colombo, using validated assays.

Conventional simplex PCR was used to detect the presence of ESBL genes (*bla*$_{TEM}$, *bla*$_{SHV,}$ *bla* $_{CTX-M}$, *bla*$_{OXA}$ types), AmpC β-lactamase genes (*bla*$_{CMY}$, *bla*$_{DHA}$, *bla*$_{FOX}$, *bla*$_{MOX}$, *bla*$_{ACC}$, *bla*$_{MIR}$, *bla*$_{ACT}$), and carbapenamase genes (*bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{IMP}$, *bla*$_{NDM}$, *bla*$_{OXA-48-like}$).[8] PCR was considered negative if it failed to amplify the target of expected size after at least three attempts. PCR products were sequenced by Sanger sequencing to identify the different β-lactamase gene variants. PCR products were purified and sequenced in both directions using the same primer pairs as used for PCR amplification. Sanger sequence service was provided by Macrogen, Korea. The sequences were analyzed using the SeqMan (Lasergene 6) software tool and subjected to homology search using BLASTn (http://www.ncbi.nlm.nih.gov/) (NCBI, United States).[12].

Phenotypic Tests to Detect ESBLs, AmpC β-Lactamases, and Carbapenamases

The phenotypic tests used to detect ESBL production were the Clinical and Laboratory Standards Institute (CLSI) screening method, the combined disc test (CDT),[13] and the modified double disc synergy test (MDDST).[3] Tests used to detect AmpC β-lactamase production were the cefoxitin screening test and cefotetan screening test,[14] AmpC induction test,[15] and AmpC disc test.[16] Tests used to detect carbapenemase production were the CLSI screening method,[13] modified Hodge test (MHT),[17] the ethylenediamine tetraacetic acid (EDTA)/double-disc synergy test (EDTA-DDST),[18] and the modified carbapenem inactivation method (mCIM).[13]

Quality control was maintained using *Escherichia coli* ATCC 25922 (negative ESBL control) and *Klebsiella pneumoniae* ATCC 700603 (positive ESBL control) for the ESBL phenotypic tests, an in-house AmpC β-lactamase positive strain and an in-house AmpC β-lactamase negative strain of *K. pneumoniae* for the AmpC β-lactamase phenotypic tests, *K. pneumoniae* ATCC BAA- 1705 as the positive control for KPC type carbapenemase, an in-house *K. pneumoniae* strain as the positive control for New Delhi metallo β-lactamase (NDM) and OXA-48 like carbapenemases and *K. pneumoniae* ATCC BAA- 1706 as the negative control for all the carbapenemase phenotypic tests.

Evaluation of Phenotypic Laboratory Tests to Detect ESBLs, AmpC β-Lactamases, and Carbapenamases

The performance (sensitivity [Se], specificity [Sp], positive predictive value [PPV], negative predictive value [NPV], and accuracy [Ac]) of the phenotypic tests was evaluated using PCR as the gold standard. Statistical analysis was done following the methods described by Marchiaro et al,[21] Jansonius[22] and Bayes' theorem.

PCR to Detect Omp Mutations in Isolates Giving a False Positive Result in Screening Tests for Carbapenemase

One isolate of *E. coli* and four isolates of *K. pneumoniae* that showed resistance to carbapenems in one or more phenotypic test but did not harbor any of the main carbapenemase

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genes were further analyzed for *omp* mutations. PCR was performed to amplify the genes coding for major outer membrane proteins (OMPs) and the resulting *omp*C, *omp*F, *omp*K35, and *omp*K36 genes were sequenced. The sequences were analyzed using the SeqMan software tool and subjected to homology search using BLASTn for the determination of identities (NCBI, United States).[23] Deduced protein sequences for OMPs were aligned against the reference sequences using the ClustalW sequence alignment software to identify variations.[24]

Results

PCR and Sequencing of Genes Coding for ESBLs, AmpC β-Lactamases, and Carbapenamases

Genes coding for ESBLs, AmpC β-lactamases or carbapenamases were present in 147 of the 176 isolates (83.5%). ESBL genes, all belonging to the CTX-M15 type, were identified in 131 isolates (76.7%), AmpC β-lactamase genes, CMY ($n=60$), DHA ($n=10$), and ACT ($n=6$) types, in 75 (42%) isolates and carbapenemase genes, NDM ($n=24$) and OXA-48-like ($n=11$) in 34 (19%) isolates. The majority (90/147, 61%) were present in combinations of two or more types of β-lactamase genes (►**Table 1**).

Table 1 β-lactamase genes identified in *Enterobacteriaceae* isolates ($n=147$)

Bacterial species	β-lactamase genes	No. of isolates
Escherichia coli	**ESBL only**	
	$bla_{CTX\text{-}M}$	43
Klebsiella sp.	$bla_{CTX\text{-}M}$	5
E. coli	**AmpC β-lactamase only** bla_{CMY}	6
	$bla_{CMY}+bla_{DHA}$	1
Klebsiella sp.	bla_{CMY}	2
	$bla_{CMY}+bla_{DHA}$	2
Enterobacter sp.	bla_{ACT}	1
E. coli	**ESBL + AmpC β-lactamase**	
	$bla_{CTX\text{-}M}+bla_{CMY}$	33
	$bla_{CTX\text{-}M}+bla_{CMY}+bla_{DHA}$	2
	$bla_{CTX\text{-}M}+bla_{DHA}$	2
Klebsiella sp.	$bla_{CTX\text{-}M}+bla_{CMY}$	4
	$bla_{CTX\text{-}M}+bla_{DHA}$	1
Enterobacter sp.	$bla_{CTX\text{-}M}+bla_{ACT}$	5
Other Enterobacteriaceae	$bla_{CTX\text{-}M}+bla_{CMY}$	4
	$bla_{CTX\text{-}M}+bla_{DHA}$	2
E. coli	**ESBL + AmpC β-lactamase + carbapenemase**	
	$bla_{CTX\text{-}M}+bla_{CMY}+bla_{NDM}$	1
Klebsiella sp.	$bla_{CTX\text{-}M}+bla_{CMY}+bla_{NDM}$	1
	$bla_{CTX\text{-}M}+bla_{CMY}+bla_{OXA\text{-}48\text{-}like}$	1
	$bla_{CTX\text{-}M}+bla_{DHA}+bla_{OXA\text{-}48\text{-}like}$	1
Enterobacter sp.	$bla_{CTX\text{-}M}+bla_{ACT}+bla_{NDM}$	1
	$bla_{CTX\text{-}M}+bla_{ACT}+bla_{NDM}+bla_{OXA\text{-}48\text{-}like}$	1
E. coli	**ESBL + carbapenemase**	
	$bla_{CTX\text{-}M}+bla_{NDM}$	5
	$bla_{CTX\text{-}M}+bla_{OXA\text{-}48\text{-}like}$	3
Klebsiella sp.	$bla_{CTX\text{-}M}+bla_{NDM}$	11
	$bla_{CTX\text{-}M}+la_{OXA\text{-}48\ like}$	2
Other Enterobacteriaceae	$bla_{CTX\text{-}M}+bla_{NDM}$	2
	$bla_{CTX\text{-}M}+bla_{OXA\text{-}48\ like}$	1
E. coli	**AmpC β-lactamase +carbapenemase**	
	$bla_{CMY}+bla_{DHA}+bla_{NDM}$	2
Klebsiella sp.	$bla_{CMY}+bla_{DHA}+bla_{OXA\text{-}48\text{-}like}$	1
Enterobacter sp.	$la_{ACT}+bla_{OXA\text{-}48\text{-}like}$	1
		147

Abbreviation: ESBL, extended spectrum β-lactamases.

Evaluation of Phenotypic Tests for Screening and Confirmation of ESBL Production

Of the 176 *Enterobacteriaceae* isolates, of which 131 harbored genes coding for ESBLs, 141 isolates (true positive,127; false positive,16; true negative, 29; false negative, 4), 125 isolates (true positive,120; false positive, 5, true negative, 40; false negative, 11), 130 isolates (true positive,127; false positive, 3; true negative, 42; false negative, 4), 131 isolates (true positive,127; false positive, 4; true negative, 41; false negative, 4), and 133 isolates (true positive,123; false positive, 10; true negative, 35; false negative, 8) were identified as ESBL producers in the CLSI screening test using cefpodoxime, ceftazidime, cefotaxime, ceftriaxone, and aztreonam discs respectively. The MDDST identified 129 isolates (true positive, 128; false positive,1; true negative, 44; false negative, 3) and the CDT identified 125 isolates (true positive, 124; false positive, 1; true negative, 44; false negative, 7) as ESBL producers, respectively.

The Se, Sp, PPV, NPV, and Ac for the five antibiotic discs in the CLSI screening test, MDDST and CDT in detecting ESBL production are shown in ►**Table 2**.

False positive results in one or more ESBL phenotypic tests were seen in 16 isolates and false negatives were seen in 23 isolates (►**Table 3**).

Evaluation of Phenotypic Tests for Screening and Confirmation of AmpC β-Lactamase Production

Of the 176 *Enterobacteriaceae* isolates, 75 harbored genes coding for AmpC β-lactamases. Hundred isolates (true positive, 73; false positive, 27; true negative, 74; false negative, 2), 65 isolates (true positive, 43; false positive, 22; true negative,79; false negative, 32), 54 isolates (true positive, 44; false positive, 10; true negative, 91; false negative, 32),

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Table 2 Performance parameters of phenotypic tests in detecting ESBL production in *Enterobacteriaceae*

	CLSI screening test					MDDST	CDT
	Cefpodoxime 10µg	Ceftazidime 30µg	Cefotaxime 30µg	Ceftriaxone 30µg	Aztreonam 30µg		
Se (%)	97	92	97	97	94	98	94
Sp (%)	65	88	93	91	78	98	98
PPV (%)	65	85	91	87	74	97	97
NPV (%)	97	94	98	98	95	98	96
Accuracy (%)	77	89	94	93	84	97	96

Abbreviations: CDT, combined disc test; CLSI, Clinical and Laboratory Standards Institute; ESBL, extended spectrum β-lactamases; MDDST, modified double disc synergy test; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

Table 3 Isolates giving false positive and false negative results in ESBL phenotypic tests

Bacterial species	β-lactamase genes	Phenotypic tests giving false positive results	No. of isolates
Escherichia coli	**AmpC β-lactamase + carbapenemase** $bla_{CMY} + bla_{DHA} + bla_{NDM}$	Screening with cefpodoxime, ceftazidime, cefotaxime, ceftriaxone and aztreonam	2
Enterobacter sp.	$bla_{ACT} + bla_{OXA\text{-}48\text{-}like}$	Screening with cefpodoxime; MDDST, CDT	1
Klebsiella sp.	**AmpC β-lactamase + *omp* mutation** bla_{CMY} + *omp36* mutation	Screening with cefpodoxime, ceftazidime, cefotaxime, ceftriaxone and aztreonam	1
E. coli	**AmpC β-lactamase** bla_{CMY}	Screening with cefpodoxime and aztreonam	4
	bla_{CMY}	Screening with cefpodoxime	2
	$bla_{CMY} + bla_{DHA}$	Screening with cefpodoxime, ceftriaxone and aztreonam	1
Klebsiella sp.	bla_{CMY}	Screening with cefpodoxime and aztreonam	2
	$bla_{CMY} + bla_{DHA}$	Screening with cefpodoxime	2
Enterobacter sp.	bla_{ACT}	Screening with cefpodoxime	1
Bacterial species	**β-lactamase genes**	**Phenotypic tests giving false positive results**	**No. of isolates**
	ESBL + AmpC β-lactamase		
E. coli	$bla_{CTX\text{-}M} + bla_{CMY}$	CDT	6
Enterobacter sp.	$bla_{CTX\text{-}M} + bla_{ACT}$	CDT	1
E. coli	**ESBL only** $bla_{CTX\text{-}M}$	Screening with cefpodoxime, ceftazidime, cefotaxime, ceftriaxone and aztreonam	3
		Screening with cefotaxime and ceftazidime	1
		Screening with ceftazidime and aztreonam	2
		Screening with ceftazidime	4
		Screening with ceftriaxone and aztreonam	1
		Screening with aztreonam	1
Klebsiella sp.	$bla_{CTX\text{-}M}$	Screening with ceftazidime and aztreonam	1
	$bla_{CTX\text{-}M}$	MDDST	2
	$bla_{CTX\text{-}M}$	Screening with cefpodoxime, MDDST	1

Abbreviations: CDT, combined disc test; ESBL, extended spectrum β-lactamases; MDDST, modified double disc synergy test.

and 95 isolates (true positive, 71; false positive, 24; true negative, 83; false negative,3) were identified as AmpC β-lactamase producers by screening with cefoxitin, screening with cefotetan, AmpC induction test, and with AmpC disc test, respectively.

The performance parameters of these phenotypic tests are presented in ►**Table 4**. False positive results in one or more AmpC β-lactamase phenotypic tests were seen in 33 isolates and false negative results were seen in 32 isolates (►**Table 5**).

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Table 4 Performance parameters of phenotypic methods to detect AmpC β-lactamase production in *Enterobacteriaceae*

	Screening with CLSI breakpoints		AmpC induction test	AmpC disc test
	Cefoxitin 30µg	Cefotetan 30µg		
Se (%)	97	66	57	96
Sp (%)	73	78	90	82
PPV (%)	78	75	85	80
NPV (%)	96	70	68	95
Accuracy (%)	85	72	74	86

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

Evaluation of Phenotypic Tests for Screening and Confirmation of Carbapenemase Production

Of the 176 *Enterobacteriaceae* isolates, 34 harbored genes coding for carbapenemases. Thirty-nine isolates (true positive, 29; false positive, 10; true negative, 132; false negative,5), 39 isolates (true positive, 33; false positive, 6; true negative, 136; false negative,1), and 48 isolates (true positive, 32; false positive, 16; true negative, 126; false negative, 2) were

Table 5 Isolates giving false positive and false negative results in AmpC β-lactamase phenotypic tests

Bacterial species	β-lactamase genes	Phenotypic tests giving false positive results	No. of isolates
Escherichia coli	**ESBL + carbapenemase** $bla_{CTX\text{-}M} + bla_{NDM}$	Screening with cefoxitin and cefotetan; AmpC disc test	5
	$bla_{CTX\text{-}M} + bla_{OXA\text{-}48\ like}$	Screening with cefoxitin, AmpC induction test	2
		Screening with cefoxitin, cefotetan	1
Klebsiella sp.	$bla_{CTX\text{-}M} + bla_{NDM}$	Screening with cefoxitin and cefotetan; AmpC disc test	9
	$bla_{CTX\text{-}M} + bla_{NDM}$	Screening with cefoxitin and cefotetan; AmpC induction test, AmpC disc test	2
	$bla_{CTX\text{-}M} + bla_{OXA\text{-}48\text{-}like}$	Screening with cefoxitin	2
Other *Enterobacteriaceae*	$bla_{CTX\text{-}M} + bla_{NDM}$	Screening with cefoxitin and cefotetan; AmpC disc test	2
	$bla_{CTX\text{-}M} + bla_{OXA\text{-}48\ like}$	Screening with cefoxitin	1
E. coli	**ESBL + *omp* mutation** $bla_{CTX\text{-}M}$ + *ompF* mutation	Screening with cefoxitin, cefotetan	1
Klebsiella sp.	$bla_{CTX\text{-}M}$ + *omp*36 mutation	Screening with cefoxitin, cefotetan	2
E. coli	**ESBL only** $bla_{CTX\text{-}M}$	AmpC induction test	2
Klebsiella sp.	$bla_{CTX\text{-}M}$	AmpC induction test	4
Bacterial species (n)	**β-lactamase genes**	**Phenotypic tests yielding false negative results**	**No. of isolates**
E. coli	**ESBL + AmpC β-lactamase** $bla_{CTX\text{-}M} + bla_{CMY}$	Screening with cefotetan; AmpC induction test	12
	$bla_{CTX\text{-}M} + bla_{DHA}$	Screening with cefoxitin, cefotetan; AmpC induction test, Amp C disc test	2
Klebsiella sp.	$bla_{CTX\text{-}M} + bla_{CMY}$	Screening with cefotetan; AmpC induction test	4
	$bla_{CTX\text{-}M} + bla_{DHA}$	Screening with cefotetan; AmpC induction test, Amp C disc test	1
Enterobacter sp.	$bla_{CTX\text{-}M} + bla_{ACT}$	Screening with cefotetan; AmpC induction test	3
Klebsiella sp.	**AmpC β-lactamase only** $bla_{CMY} + bla_{DHA}$	Screening with cefotetan; AmpC induction test, Amp C disc test	2
E. coli	bla_{CMY}	Screening with cefotetan; AmpC induction test	5
Klebsiella sp.	bla_{CMY}	Screening with cefotetan; AmpC induction test	2
Enterobacter sp.	bla_{ACT}	Screening with cefotetan; AmpC induction test	1

Abbreviation: ESBL, extended spectrum β-lactamases.

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Table 6 Performance parameters of phenotypic methods to detect carbapenemase production in *Enterobacteriaceae*

	Screening with CLSI breakpoints			mCIM in detecting NDM carbapenemase	MHT in detecting NDM carbapenemase	EDTA-DDST in detecting NDM detecting type carbapenemase	mCIM in detecting OXA-48 like carbapenemase	MHT in detecting OXA-48 like carbapenemase	EDTA- DDST in detecting OXA-48 like carbapenemase	mCIM in detecting both carbapenemase production	MHT in detecting both carbapenemase production	EDTA- DDST in detecting both carbapenemase production
	Imipenem (mm)	Meropenem (mm)	Ertapenem (mm)									
Se (%)	85	97	94	96	67	96	100	100	73	97	76	88
Sp (%)	93	96	90	98	94	97	98	94	97	98	94	97
PPV (%)	92	96	90	98	92	97	98	95	96	98	93	97
NPV (%)	86	97	94	96	74	96	100	100	78	97	80	89
Accuracy (%)	89	96	92	97	80	96	99	97	85	97	85	93

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; EDTA-DDST, ethylenediamine tetraacetic acid-double-disc synergy test; mCIM, modified carbapenem inactivation method; MHT, modified Hodge test; NPV, negative predictive value; PPV, positive predictive value; Se, sensitivity; Sp, specificity.

identified as carbapenemase producers by screening with the imipenem, meropenem, and ertapenem, respectively.

There were 24 isolates harboring bla_{NDM} genes. In the phenotypic tests to detect carbapenemases, 26 isolates (true positive, 23; false positive, 3, true negative, 138; false negative, 1), 24 isolates (true positive, 16; false positive, 8, true negative, 133; false negative, 8), and 27 isolates (true positive, 23; false positive, 4, true negative, 137; false negative, 1) were positive by mCIM, MHT, and EDTA-DDST test, respectively. There were 11 isolates harboring $bla_{OXA\text{-}48\ like}$ genes. In the phenotypic tests used to confirm carbapenemase production, 14 isolates (true positive, 11; false positive, 3, true negative, 139; false negative, 0), 19 isolates (true positive, 11; false positive, 8, true negative, 134; false negative, 0), and 12 isolates (true positive, 8; false positive, 4, true negative, 138; false negative, 3) were positive by mCIM, MHT, and EDTA-DDST test, respectively. Overall, 36 isolates (true positive, 33; false positive, 3, true negative, 139; false negative, 1), 34 isolates (true positive, 26; false positive, 8, true negative, 134; false negative, 8), and 34 isolates (true positive, 30; false positive, 4, true negative, 138; false negative, 4) were positive by mCIM, MHT, and EDTA-DDST test, respectively.

The performance of these tests in detecting NDM type carbapenemases, OXA-48 like carbapenemases, and in detecting both type of carbapenemases is shown in ►**Table 6**. False positives in the phenotypic tests to detect carbapenemases were seen in 16 isolates and false negative results were seen in 15 isolates (►**Table 7**).

Discussion

Antimicrobial resistance in gram-negative bacteria, particularly *Enterobacteriaceae*, is increasing globally. This is mainly due to the dissemination of strains producing ESBLs, AmpC β-lactamases, and carbapenemases.

Detecting ESBL producers in the clinical microbiology laboratory by phenotypic testing is increasingly challenging due to geographic variation in ESBL type. Although cefotaxime, ceftriaxone, and cefpodoxime showed equal sensitivity (~97%) in the CLSI screening test for ESBLs, cefotaxime and ceftriaxone showed better performance in terms of the other parameters (Sp, PPV, NPV) in this population of CTX-M type ESBL producers. Cefotaxime has been shown, previously, to be a better substrate to detect CTX-M type ESBLs while ceftazidime was better at screening for TEM and SHV type ESBLs.[25,26] The lesser specificity of cefpodoxime has also been described previously.[25]

In this study, production of AmpC β-lactamases was shown to give rise to false positives in the CLSI screening test. This has been described previously, and it has even been recommended to use the CLSI screening test to screen for AmpC β-lactamases as well.[27] However, the MDDST and CDT demonstrated good accuracy for the confirmation of ESBL production and were able to distinguish the false positives identified on screening. Interference in the confirmatory tests due to AmpC β-lactamase coproduction was not as pronounced in this population as that described previously.[25] However, false negative results in the CDT

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Table 7 Isolates giving false positive and negative results in carbapenemase phenotypic tests

Bacterial species	β-lactamase genes	Phenotypic tests giving false positive results	No. of isolates
Escherichia coli	$bla_{CTX\text{-}M}$ + *omp*F mutation	Screening with ertapenem, imipenem; EDTA-DDST	1
Klebsiella sp.	$bla_{CTX\text{-}M}$ + *omp*36 mutation	Screening with imipenem, ertapenem, meropenem; EDTA-DDST, MHT	2
	bla_{CMY} + *omp*36 mutation	Screening with ertapenem, meropenem	1
	$bla_{CTX\text{-}M}$ + bla_{DHA} + *omp*36 mutation	Screening with ertapenem, imipenem, meropenem; MHT	1
E. coli	$bla_{CTX\text{-}M}$ + bla_{CMY}	Screening with meropenem, imipenem; mCIM	1
		Screening with ertapenem; mCIM, EDTA-DDST, MHT	1
		Screening with imipenem, ertapenem; mCIM	1
		Screening with ertapenem; MHT Screening with ertapenem	1 1
Klebsiella sp.	$bla_{CTX\text{-}M}$ + bla_{CMY}	Screening with imipenem, ertapenem, meropenem; MHT	1
		Screening with imipenem; mCIM	1
Other *Enterobacteriaceae*	$bla_{CTX\text{-}M}$ + bla_{CMY}	Screening with imipenem, ertapenem; MHT	1
E. coli	$bla_{CTX\text{-}M}$	Screening with ertapenem; mCIM	1
Klebsiella sp.	$bla_{CTX\text{-}M}$	Screening with imipenem, ertapenem; mCIM	1
Bacterial species	**β-lactamase genes**	**Phenotypic tests giving false negative results**	**No. of isolates**
E. coli	bla_{NDM}	MHT	3
	$bla_{OXA\text{-}48\text{-}like}$	Screening with imipenem	1
		EDTA-DDST	1
Klebsiella sp.	bla_{NDM}	MHT	4
		MHT, EDTA-DDST	1
		Screening with imipenem, ertapenem; mCIM	1
		Screening with imipenem, meropenem, ertapenem	1
		Screening with imipenem	1
	$bla_{OXA\text{-}48\text{-}like}$	EDTA-DDST	1
Other *Enterobacteriaceae*	$bla_{OXA\text{-}48\text{-}like}$	Screening with imipenem, EDTA-DDST	1

Abbreviations: EDTA-DDST, ethylenediamine tetraacetic acid-double-disc synergy test; mCIM, modified carbapenem inactivation method; MHT, modified Hodge test.

were seen in a small number of isolates coproducing ESBLs and AmpC β-lactamases. Interestingly, a false positive result in both the MDDST and the CDT was seen in an isolate producing a combination of AmpC β-lactamase and OXA-48-like carbapenemase. This may be due to inhibition of the OXA-48-like carbapenemase by the β-lactamase inhibitors.[23]

The AmpC β-lactamase types detected in the study isolates were CMY, DHA, and ACT, with CMY and DHA as the most prevalent. Screening for AmpC β-lactamases using the cefotetan disc and the AmpC induction test showed poor performance. Although screening for AmpC using cefoxitin was highly sensitive (97%), specificity was extremely low (73%). The AmpC disc test also displayed a high (96%) sensitivity but low (82%) specificity. The low specificity of the cefoxitin screening test has been noted previously.[28,29] The lower specificity of the AmpC disc test in our study compared with previous reports[28] maybe due to the presence of carbapenemase producers in our collection of isolates as the false positives in both the cefoxitin screening and the AmpC disc test were mainly seen in isolates harboring combinations of ESBL and carbapenemase genes. A few isolates giving false positives in the cefoxitin screening test had *omp* mutations potentially resulting porin loss.[29]

Of the three carbapenem discs used to screen for carbapenemase production using CLSI breakpoints, meropenem showed relatively better performance followed by ertapenem and imipenem in this study featuring isolates with NDM type and OXA-48 like carbapenemases. Meropenem has been previously found to be the better substrate to detect these

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carbapenemase types.[30,31] False positive screening results for carbapenemases were seen for imipenem and meropenem in four, and for ertapenem in five, isolates that lacked any known carbapenemase genes but were found to have carry mutations in the *omp* gene that may have affected porin function.[29] False positives were also seen in isolates harboring combinations of ESBL and AmpC β-lactamase genes as previously described,[30] probably due to overexpression of AmpC β-lactamases. However, surprisingly, two isolates with only ESBL genes also gave false positive results in the CLSI screening test for carbapenemases, with ertapenem ($n = 2$) and imipenem ($n = 1$). Hydrolysis of ertapenem by CTX-M type ESBLs has been discussed by Wang et al.[32]

When considering the performance of the mCIM, MHT, and EDTA-DDST tests in detecting carbapenemases, mCIM and MHT had good test parameters in detecting OXA-48-like carbapenemases and mCIM and EDTA-DDST had good test parameters in detecting NDM type carbapenemases. However, MHT showed poor performance in detecting NDM carbapenemases and EDTA-DDST showed poor performance in detecting OXA-48-like carbapenemases. In addition, porin loss, combined with ESBL or AmpC β-lactamase production, has been previously shown to give false positive results in some phenotypic tests for carbapenemases, such as MHT.[33] Overall mCIM showed the best performance in identifying any carbapenemase and even the five isolates with *omp* mutations that were wrongly identified as carbapenemase producers in the CLSI screening tests were correctly identified by mCIM.

In conclusion, variation in the types of ESBLs, AmpC β-lactamases, and carbapenemases produced by *Enterobacteriaceae* affects the performance of phenotypic tests used to detect β-lactamases. When selecting a phenotypic test, it is important to select one that is least affected by coexisting β-lactamases and other resistance mechanisms prevalent in the particular geographic region. In this study population, consisting of isolates coproducing CTX-M ESBLs, AmpC β-lactamases and NDM and OXA-48-like carbapenemases, the MDDST was identified as the better test to identify ESBLs, screening with cefoxitin and AmpC disc test was found to have good sensitivity albeit low specificity in identifying AmpC production, meropenem was found to be a good substrate to screen for carbapenemases rather than ertapenem or imipenem, and mCIM was the better test to confirm carbapenemase production with minimal interference by other β-lactamases and porin changes.

Authors' Contributions

Both V.P. and E.C. contributed to performing the concepts design, definition of intellectual content, literature search, clinical studies, experimental studies, data analysis, statistical analysis, manuscript preparation, and manuscript editing. Both K.J. and N.d.S. contributed to concepts design, definition of intellectual content, and manuscript editing. S.d.S. contributed to data acquisition, data analysis, and manuscript preparation. All authors contributed to data acquisition and manuscript review.

Ethical Approval

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

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Conflict of Interest

E.C. reports all support from National Research Council of Sri Lanka Grant and University of Colombo Research Grant for Funding for consumables.

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