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Evaluation of the NG-Test® CTX-M MULTI for the Detection of CTX-M from Enterobacterales

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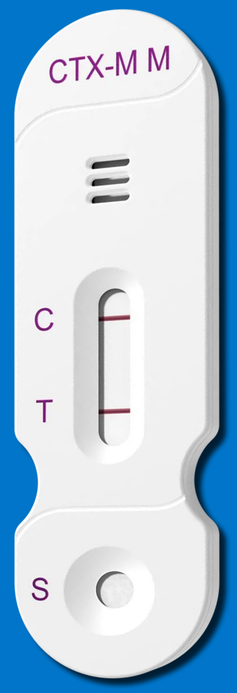
Revised Abstract

Introduction: The CDC considers extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales a serious threat in the United States. Among the plasmid-mediated class A ESBLs encountered in a clinical setting, CTX-M is the most widespread enzyme. CTX-M enzymes are classified into five major groups: 1, 2, 8, 9, and 25. Group 1 is the most prevalent, as it includes the most commonly encountered CTX-M-15 variant. Detection of CTX-M and other ESBL-producing organisms primarily relies on traditional or rapid antimicrobial susceptibility testing (AST) methods, molecular techniques, the CLSI ESBL Disk or MIC Test, and/or alternative screening culture media. NG-Test® CTX-M MULTI is an *in vitro* rapid and visual immunochromatographic assay for the qualitative detection of CTX-M enzymes (groups 1, 2, 8, 9, and 25) from pure colonies of Enterobacterales.

Methods: Enterobacterales isolates (*n*=51) with one or more CTX-M genes were evaluated in triplicate with the NG-Test® CTX-M MULTI from tryptic soy agar with 5% sheep blood (blood agar), MacConkey agar, and HardyCHROM™ ESBL (HC ESBL) agar. Non-target Enterobacterales isolates (*n*=58) with other ESBL or AmpC genes (TEM, SHV, ACT, CMY, DHA, FOX) were evaluated with the NG-Test® CTX-M MULTI immunoassay from blood, MacConkey, and HC ESBL agar. AST and ESBL phenotype testing via the CLSI ESBL Disk Test was performed prior to testing isolates with the NG-Test® CTX-M MULTI.

Results: 50/51 (98%), 51/51 (100%), and 50/50 (100%) of the target organisms were positive on the NG-Test® CTX-M MULTI from blood, MacConkey, and HC ESBL agar, respectively. 55/58 (95%) non-target organisms were negative on the NG-Test® CTX-M MULTI from blood and MacConkey agar, and 50/53 (94%) non-target organisms were negative from HC ESBL agar. Isolates with false positive results were molecularly characterized to harbor TEM and/or SHV genes. These three non-target isolates will be submitted for additional analysis via whole genome sequencing to confirm the presence or absence of CTX-M genes.

Conclusions: The NG-Test® CTX-M MULTI immunoassay is a robust method for the rapid detection of CTX-M enzymes from Enterobacterales colonies grown on blood, MacConkey, and HC ESBL agar. The availability of this test will allow laboratories to access and implement an antimicrobial resistance detection method in-house at a low cost and without the need for specialized equipment, reagents, or extensive training.



Introduction

An estimated 198,000 hospitalizations and an estimated 9,100 deaths in 2017 were attributed to ESBL-producing Enterobacterales. However, the current methods of ESBL detection have many limitations and are somewhat burdensome. The manual methods have a lower accuracy than the molecular methods and are only appropriate for a few species of Enterobacterales. The ideal test for detection of ESBLs in clinical laboratories should be accurate, applicable to a wide range of species, be rapid, cost-effective, and simple to use. According to cumulative data from SENTRY (JMI Labs) for US isolates collected from 2013 to 2021, 86.2% of ESBL isolates had CTX-M genes, 12.8% of ESBL isolates had SHV genes, and less than 1% of ESBL isolates had TEM genes. Similar percentages are observed for the global prevalence from SENTRY data, with a slight increase for CTX-M (88.8%) and slight decrease for SHV (9.4%) gene prevalence over the same time period. Thus, although it is not the only ESBL mechanism, CTX-M is far more prevalent and likely to contribute to infections caused by ESBL-producing Enterobacterales.

The NG-Test® CTX-M MULTI is an *in vitro* rapid and visual immunochromatographic assay for the qualitative detection of CTX-M enzymes (groups 1, 2, 8, 9, and 25) from pure colonies of Enterobacterales. Colonies are added to 5 drops of an extraction buffer and vortexed. Once the sample is prepared, the assay is carried out by dispensing 100µL of the sample in the cassette sample well using a small pipette included with the kit. The sample migrates toward the conjugate pad and, if present, the CTX-M beta-lactamase reacts with labeled anti-CTX-M beta-lactamase monoclonal antibodies. Capillary action transports the complex through the nitrocellulose membrane, where it interacts with the corresponding immobilized anti-CTX-M beta-lactamase monoclonal antibodies. After 15 minutes, if the sample is positive for CTX-M enzymes, a red line will appear on the test line and on the control line of the membrane. If no CTX-M enzyme is detected, only the control line will appear.

Methods

Molecularly characterized Enterobacterales isolates (*n*=51) with one or more CTX-M genes were evaluated with the NG-Test® CTX-M MULTI to determine analytical reactivity. Species included in the analytical reactivity evaluation were *Citrobacter* species (*n*=1), *Enterobacter cloacae* (*n*=8), *Escherichia coli* (*n*=18), *Klebsiella oxytoca* (*n*=4), *Klebsiella pneumoniae* (*n*=13), *Kluyvera ascorbata* (*n*=1), *Proteus mirabilis* (*n*=2), and *Serratia marcescens* (*n*=1). CTX-M groups 1, 2, 8, and 9 were evaluated during the study. The CTX-M groupings were determined using the beta-lactamase database as a reference. In order to evaluate potential cross reactivity, molecularly characterized Enterobacterales isolates (*n*=58) with other ESBL or AmpC genes (TEM, SHV, ACT, CMY, DHA, FOX) were evaluated. Species included in the cross reactivity evaluation were *Citrobacter freundii* (*n*=2), *Enterobacter asburiae* (*n*=3), *E. cloacae* (*n*=8), *E. coli* (*n*=14), *K. oxytoca* (*n*=4), *K. pneumoniae* (*n*=16), *Morganella morganii* (*n*=1), *P. mirabilis* (*n*=7), and *S. marcescens* (*n*=3).

Each isolate was streaked for isolation onto a blood agar plate from frozen (-80°C) stock vials. Prior to incubation, a ceftriaxone (CRO) disk was placed onto the primary blood agar cultures to prevent spontaneous loss of plasmids carrying the CTX-M gene(s). After overnight incubation at 35°C, fresh colonies were subcultured to blood, MacConkey, and HC ESBL agar. After overnight incubation, the blood and MacConkey agar cultures were evaluated with the NG-Test® CTX-M MULTI. For the evaluation with HC ESBL agar, contrived stool specimens were prepared by spiking the target and non-target organisms that were able to grow on the HC ESBL media into ESBL-negative stool specimens. Microorganism suspensions equivalent to a 1 McFarland standard were prepared in tryptic soy broth from 18-24 hour cultures and spiked into ESBL-negative stool specimens (unpreserved and C&S Cary Blair) in a 1:10 ratio. After vortexing, spiked stool samples were streaked onto HC ESBL agar for isolation and were incubated overnight. After overnight incubation, HC ESBL agar cultures were evaluated with the NG-Test® CTX-M MULTI. Target strains were tested in triplicate, while those in the cross-reactivity study were tested once. A 1µL loop was used to touch three colonies, and the growth was inoculated into the extraction buffer. After mixing the colonies into the buffer and vortexing the samples, 100µL of each sample was added to the sample well. The operator was blinded to the AST and molecular characterization results when testing each organism.

AST was performed for all isolates from blood agar by inoculating Mueller Hinton agar following CLSI procedures. Disk diffusion was set up with cefotaxime (CTX), cefotaxime-clavulanic acid (CTX-CLA), ceftazidime (CAZ), ceftazidime-clavulanic acid (CAZ-CLA), ceftriaxone (CRO), cefpodoxime (CPD), aztreonam (ATM), and cefoxitin (FOX) disks. The CLSI ESBL Disk Test was performed for the applicable species (*E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis*) from blood agar following the procedures outlined in the CLSI M100, 33rd edition.

Results

The NG-Test® CTX-M MULTI was positive for 50/51 (98%), 51/51 (100%), and 50/50 (100%) of the target organisms that were tested from blood, MacConkey, and HC ESBL agar, respectively. All tested CTX-M groups (1 [*n*=31], 2 [*n*=5], 8 [*n*=2], 9 [*n*=18]) were effectively detected by the device. One *K. pneumoniae* strain produced a positive NG-Test® CTX-M MULTI result from MacConkey and HC ESBL agar but was negative from blood agar. One *P. mirabilis* strain was not recovered on HC ESBL agar and was not included in the analytical reactivity study from HC ESBL only (Table 1).

Table 1. NG-Test® CTX-M MULTI Analytical Reactivity Results from Blood, MacConkey, and HardyCHROM™ ESBL Agar

Organism	CTX-M Variant	CTX-M Group	Disk Diffusion Zone Sizes (mm)[1]								CLSI ESBL Disk Test Result[2]	NG-Test® CTX-M MULTI Result		
			CTX	CTX-CLA	CAZ	CAZ-CLA	CRO	CPD	ATM	FOX		Blood Agar	MacConkey Agar	HC ESBL Agar[3]
Citrobacter species	CTX-M-15	1	6		6		6	6	6	6	N/A	Positive	Positive	Positive
Enterobacter cloacae	CTX-M-15	1	6		6		6	6	6	6	N/A	Positive	Positive	Positive
Enterobacter cloacae	CTX-M-15	1	6		9		6	6	8	6	N/A	Positive	Positive	Positive
Enterobacter cloacae	CTX-M-15	1	6		6		6	6	8	6	N/A	Positive	Positive	Positive
Enterobacter cloacae	CTX-M-9	9	6		6		6	6	8	6	N/A	Positive	Positive	Positive
Enterobacter cloacae	CTX-M-9	9	6		6		13	6	28	6	N/A	Positive	Positive	Positive
Enterobacter cloacae	CTX-M-9	9	6		6		9	6	24	8	N/A	Positive	Positive	Positive
Enterobacter cloacae	CTX-M-9-like	9	10		24		10	6	22	26	N/A	Positive	Positive	Positive
Enterobacter cloacae	CTX-M-15	1	6		6		6	6	16	6	N/A	Positive	Positive	Positive
Escherichia coli	CTX-M-15; CTX-M-40	1, 8	6	26	11	27	6	6	11	19	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-124	2	11	28	18	27	10	6	20	24	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-24	9	6	26	22	27	8	6	15	18	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-79	1	9	32	21	33	6	6	18	26	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-15	1	6	28	20	28	6	6	14	25	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-14	9	6	18	6	18	6	6	6	6	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-1	1	12	25	22	26	10	6	20	19	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-55	1	6	29	12	30	6	6	9	30	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-14	9	6	7	6	7	6	6	7	6	Indeterminate	Positive	Positive	Positive
Escherichia coli	CTX-M-14	9	15	32	29	33	13	8	28	29	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-55	1	6	19	6	15	6	6	6	12	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-14; CTX-M-55	1, 9	6	31	18	31	6	6	14	23	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-15	1	6	29	9	28	6	6	16	26	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-27	9	6	7	6	6	6	6	17	6	Indeterminate	Positive	Positive	Positive
Escherichia coli	CTX-M-3	1	6	6	6	6	6	6	16	6	Indeterminate	Positive	Positive	Positive
Escherichia coli	CTX-M-55	1	6	21	7	23	6	6	6	6	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-55	1	6	29	7	28	6	6	6	17	Positive	Positive	Positive	Positive
Escherichia coli	CTX-M-9; CTX-M-14	9	6	30	22	33	6	6	17	23	Positive	Positive	Positive	Positive
Klebsiella oxytoca	CTX-M-30; CTX-M-75	1, 2	12	30	23	29	6	6	22	26	Positive	Positive	Positive	Positive
Klebsiella oxytoca	CTX-M-22	1	15	12	8	14	14	8	8	6	Positive	Positive	Positive	Positive
Klebsiella oxytoca	CTX-M-15	1	6	28	10	28	6	6	10	16	Positive	Positive	Positive	Positive
Klebsiella oxytoca	CTX-M-14	9	6	15	16	16	6	6	6	6	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-14	9	6	23	6	21	10	6	6	10	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-64	1	6	26	15	26	6	6	16	25	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-38	9	22	33	27	31	20	14	28	26	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-22	1	15	29	20	26	13	8	19	18	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-12	1	14	29	22	28	13	6	21	25	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-74	2	12	25	8	22	13	6	20	13	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-40	8	16	29	27	30	17	6	29	25	Positive	Negative[4]	Positive	Positive
Klebsiella pneumoniae	CTX-M-124	2	6	25	16	24	6	6	11	20	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-14	9	13	27	23	29	11	6	20	23	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-15; CTX-M-14	1, 9	6	25	12	21	6	6	12	10	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-14	9	8	13	6	10	6	6	19	6	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-3	1	8	17	18	22	8	6	16	19	Positive	Positive	Positive	Positive
Klebsiella pneumoniae	CTX-M-14b; CTX-M-15	1, 9	6	8	6	8	6	6	6	6	Indeterminate	Positive	Positive	Positive
Kluyvera ascorbata	CTX-M-124	2	18		7		11	10	7	11	N/A	Positive	Positive	Positive
Proteus mirabilis	CTX-M-15	1	17	30	17	28	18	6	28	23	Positive	Positive	Positive	Positive
Proteus mirabilis	CTX-M-3	1	16	31	21	29	19	12	28	23	Positive	Positive	Positive	Positive
Proteus mirabilis	CTX-M-79	1	6	28	19	31	6	6	22	21	Positive	Positive	Positive	Positive
Proteus mirabilis	CTX-M-32	1	24	35	7	40	26	6	6	24	Positive	Positive	Positive	Positive
Proteus mirabilis	CTX-M-3	1	21	33	30	32	25	15	35	25	Positive	Positive	Positive	Not Tested
Serratia marcescens	CTX-M-15	1	6		6		6	6	6	13	N/A	Positive	Positive	Positive

[1]CTX= cefotaxime; CTX-CLA= cefotaxime-clavulanic acid; CAZ= ceftazidime; CAZ-CLA= ceftazidime-clavulanic acid; CRO= ceftriaxone; CPD= cefpodoxime; ATM= aztreonam; FOX= cefoxitin
[2]N/A= not applicable; CLSI ESBL Disk Test only performed for *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis*.
[3]HC ESBL is intended for the qualitative and presumptive detection from stool specimens of: 1) Enterobacterales that are potentially non-susceptible to ceftazidime and cefpodoxime; and 2) ESBL-producing *E. coli*, *K. pneumoniae* and *K. oxytoca*.
[4]CTX-M-40 from *K. pneumoniae* was not detected from blood agar, but yielded the expected NG-Test® CTX-M MULTI result in triplicate from MacConkey and HC ESBL agar.

Conclusions

- 98-100% of the CTX-M groups evaluated were positive from blood, MacConkey, and HardyCHROM™ ESBL agar.
- 94-95% of the non-target organisms characterized for other types of ESBL, AmpC, and carbapenemase resistance mechanisms were negative from blood, MacConkey, and HardyCHROM™ ESBL agar.
- The NG-Test® CTX-M MULTI immunoassay is a rapid and robust method for the rapid detection of CTX-M enzymes from Enterobacterales colonies grown on blood, MacConkey, and HardyCHROM™ ESBL agar.

References:

1. CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.
2. Beta-Lactamase Database: Naas, T.; Oueslati, S.; Bonnin, R. A.; Dabos, M. L.; Zavala, A.; Dortet, L.; Retailleau, P.; Iorga, B. I., Beta-Lactamase DataBase (BLDB) – Structure and Function. J. Enzyme Inhib. Med. Chem. 2017, 32, 917-919.
3. SENTRY Online Public Dataset. https://sentry-mvp.jmilabs.com/

Results

The NG-Test® CTX-M MULTI was negative for 55/58 (95%) of the non-target organisms from blood and MacConkey agar and was negative for 50/53 (94%) of the non-target organisms from HC ESBL agar. There were three false positive results from all media. These three isolates were molecularly characterized to harbor TEM and/or SHV genes. These isolates will be submitted for whole genome sequencing analysis to confirm the presence or absence of CTX-M genes. Five non-target strains were not recovered on HC ESBL and were not included in the cross-reactivity study from HC ESBL only (Table 2).

Table 2. NG-Test® CTX-M MULTI Cross Reactivity Results from Blood, MacConkey, and HardyCHROM™ ESBL Agar

Organism	Molecular Characterization	Disk Diffusion Zone Sizes (mm)[1]								CLSI ESBL Disk Test Result[2]	NG-Test® CTX-M MULTI Result		
		CTX	CTX-CLA	CAZ	CAZ-CLA	CRO	CPD	ATM	FOX		Blood Agar	MacConkey Agar	HC ESBL Agar[3]
Citrobacter freundii	CMY-49; KPC-2	19		17		15	6	11	6	N/A	Negative	Negative	Not Tested
Citrobacter freundii	TEM-OSBL(2b); CMY-41; IMP-8	6		6		7	6	16	6	N/A	Negative	Negative	Negative
Enterobacter asburiae	ACT-2; IMP-14	22		24		22	18	20	6	N/A	Negative	Negative	Negative
Enterobacter asburiae	MIR-8; OXA-48(c)	17		19		17	6	23	6	N/A	Negative	Negative	Negative
Enterobacter asburiae	TEM-OSBL(2b); VEB-2; NDM-1	6		6		6	6	6	6	N/A	Negative	Negative	Negative
Enterobacter cloacae	TEM-1(2b)	6		9		6	6	8	8	N/A	Positive[4]	Positive[4]	Positive[4]
Enterobacter cloacae	SHV-12(2be); TEM-OSBL(2b); ACT-type	14		6		16	6	7	6	N/A	Negative	Negative	Negative
Enterobacter cloacae	TEM-1; KPC-2	16		12		14	6	8	6	N/A	Negative	Negative	Negative
Enterobacter cloacae	ACT-type; IMP-1	6		6		6	6	10	6	N/A	Negative	Negative	Negative
Enterobacter cloacae	SHV-12(2be); TEM-OSBL(2b); ACT-32; IMP-8	6		6		10	6	6	6	N/A	Negative	Negative	Negative
Enterobacter cloacae	ACT-type; IMP-8; SHV-12(e); TEM-OSBL(b)	6		6		6	6	6	6	N/A	Negative	Negative	Negative
Enterobacter cloacae	DHA-1; OXA-1; SHV-12; SHV-12	10		7		18	7	6	6	N/A	Negative	Negative	Negative
Enterobacter cloacae	FOX-5; KPC-3; OXA-9	8		6		12	6	6	6	N/A	Negative	Negative	Negative
Escherichia coli	TEM-20(2be); CMY-2	6	6	6	7	6	6	6	6	Indeterminate	Negative	Negative	Negative
Escherichia coli	TEM-214(u)	7	28	11	26	8	6	11	24	Positive	Positive[4]	Positive[4]	Positive[4]
Escherichia coli	TEM-12(2be)	34	38	20	36	38	28	37	31	Positive	Negative	Negative	Negative
Escherichia coli	CMY-42	6	6	6	7	6	6	14	6	Indeterminate	Negative	Negative	Negative
Escherichia coli	TEM-1; CMY-2	12	14	6	7	8	6	15	6	Indeterminate	Negative	Negative	Negative
Escherichia coli	TEM-1; CMY-2	12	13	6	11	11	6	14	6	Positive	Negative	Negative	Negative
Escherichia coli	TEM-1(2b); OXA-48(c)	21	21	28	28	25	18	30	16	Indeterminate	Negative	Negative	Not Tested
Escherichia coli	TEM-OSBL(2b); CMY-42; NDM-5	6	6	6	6	6	6	17	6	Indeterminate	Negative	Negative	Negative
Escherichia coli	CMY-2; OXA-1	19	21	15	19	18	6	21	6	Indeterminate	Negative	Negative	Negative
Escherichia coli	OXA-1; OXA-30; CMY-42	6	6	6	6	6	6	12	6	Indeterminate	Negative	Negative	Negative
Escherichia coli	KPC-3; TEM-1	6	6	6	10	6	6	6	6	Indeterminate	Negative	Negative	Negative
Escherichia coli	NDM-1; TEM-1; SHV-12	11	18	6	15	10	6	7	17	Positive	Negative	Negative	Negative
Escherichia coli	TEM-10	26	33	6	26	25	6	6	15	Positive	Negative	Negative	Negative
Escherichia coli	TEM-6(2be)	23	26	6	21	23	10	10	18	Positive	Negative	Negative	Negative
Klebsiella oxytoca	TEM-52(2be)	19	30	18	28	22	13	20	26	Positive	Negative	Negative	Negative
Klebsiella oxytoca	SHV-7(2be)	18	31	6	27	20	8	7	20	Positive	Negative	Negative	Negative
Klebsiella oxytoca	SHV-5	14	30	9	27	18	8	10	23	Positive	Negative	Negative	Negative
Klebsiella oxytoca	TEM-OSBL(2b); ACC-1; NDM-1	6	9	6	6	6	6	27	6	Indeterminate	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-136(u); CMY-2	16	17	8	13	15	6	17	6	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-178(u); TEM-1(2b)	6	25	8	22	6	6	6	13	Positive	Positive[4]	Positive[4]	Positive[4]
Klebsiella pneumoniae	SHV-55(2be); TEM-1(2b)	16	30	8	24	17	12	11	23	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-90(2be); TEM-OSBL(2b)	13	30	6	25	15	6	9	23	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	TEM-1; SHV-11; KPC-2	6	13	6	8	6	6	6	10	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	TEM-1; SHV-7 SHV-11; KPC-2	16	31	6	23	14	6	6	15	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-11(2b); TEM-1(2b); OXA-48(c)	21	21	26	26	22	19	30	11	Indeterminate	Negative	Negative	Not Tested
Klebsiella pneumoniae	SHV-11(2b); TEM-1(2b); KPC-3	6	6	6	6	6	6	6	6	Indeterminate	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-11(2b); NDM-1	6	10	6	11	6	6	30	6	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-27(2b); IMP-8	8	8	6	7	8	6	31	6	Indeterminate	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-12(2be); TEM-OSBL(2b); KPC-3	10	21	6	12	11	6	6	16	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-2A(2be); KPC-12	6	8	6	7	6	6	6	6	Indeterminate	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-11(2b); TEM-11(2be)	29	30	18	26	28	22	28	24	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-133(u); VEB-1	20	29	6	25	23	16	12	19	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	SHV-83(2b); TEM-4(2be)	15	28	17	26	17	14	22	24	Positive	Negative	Negative	Negative
Klebsiella pneumoniae	KPC-3; SHV-12	9	10	6	7	8	6	6	10	Indeterminate	Negative	Negative	Negative
Morganella morganii	DHA-type	6		6		15	6	16	6	N/A	Negative	Negative	Negative
Proteus mirabilis	TEM-92(2be)	14	30	17	29	19	8	25	23	Positive	Negative	Negative	Negative
Proteus mirabilis	TEM-15(2be)	14	31	18	30	23	8	29	18	Positive	Negative	Negative	Negative
Proteus mirabilis	TEM-1; VEB-1a	17	36	10	32	24	12	22	23	Positive	Negative	Negative	Negative
Proteus mirabilis	TEM-24(2be)	31	33	19	28	31	29	33	25	Positive	Negative	Negative	Not Tested
Proteus mirabilis	TEM-1; KPC-3	21	23	21	23	22	15	25	17	Indeterminate	Negative	Negative	Not Tested
Proteus mirabilis	DHA-1; IMP-26	21	21	17	18	24	6	33	10	Indeterminate	Negative	Negative	Negative
Proteus mirabilis	TEM-OSBL(u); CMY-16; VIM-1	6	11	10	14	6	6	27	9	Positive	Negative	Negative	Negative
Serratia marcescens	TEM-1(2b); IMP-47	6		6		8	6	35	6	N/A	Negative	Negative	Negative
Serratia marcescens	FOX-5	19		6		22	6	26	6	N/A	Negative	Negative	Negative
Serratia marcescens	CMY-16	6		6		6	6	10	6	N/A	Negative	Negative	Negative

[1]CTX= cefotaxime; CTX-CLA= cefotaxime-clavulanic acid; CAZ= ceftazidime; CAZ-CLA= ceftazidime-clavulanic acid; CRO= ceftriaxone; CPD= cefpodoxime; ATM= aztreonam; FOX = cefoxitin
[2]N/A= not applicable; CLSI ESBL Disk Test only performed for *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *P. mirabilis*.
[3]HC ESBL is intended for the qualitative and presumptive detection from stool specimens of: 1) Enterobacterales that are potentially non-susceptible to ceftazidime and cefpodoxime; and 2) ESBL-producing *E. coli*, *K. pneumoniae* and *K. oxytoca*.
[4]Whole genome sequencing will be performed to confirm genotype.