Evaluation of HardyCHROM™ ESBL as a detection method of ESBL-producing *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*: a multi-centric study

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Abstract

Background: HardyCHROM™ ESBL is a chromogenic medium designed to screen for Extended-Spectrum-Beta-Lactamase (ESBL) producing *E. coli*, *K. pneumoniae*, and *K. oxytoca* from fecal specimens. Based on the colony color, this medium differentiates *E. coli* (pink) from *K. pneumoniae/Kl. oxytoca* (blue).The medium can also be used to recover and identify other Enterobacteriaceae that are non-susceptible to 3rd generation cephalosporins.

Methods: HardyCHROM™ ESBL was compared to traditional culture methods utilizing parallel TSB enrichments at three different hospital laboratories. Using a standard loop, stool specimens were inoculated onto HardyCHROM™ ESBL, into TSB with 1µg/mL ceftazidime, and into TSB with 1µg/mL cefotaxime and incubated overnight. After incubation, both TSB enrichment broths were subcultured to MacConkey agar. Bacterial isolates from both reference and chromogenic agar were confirmed for identification and resistance using an FDA-cleared system. CLSI recommendations were used for susceptibility result interpretation.

Results: A total of 1,737 fecal specimens were tested on HardyCHROM™ ESBL in parallel with the TSB enrichments. There were 216 target ESBL organisms recovered from the reference method and 230 target ESBL organisms recovered by colony color on HardyCHROM™ ESBL after 18 hours of incubation. A total of 211 isolates were recovered by both methods simultaneously. HardyCHROM™ ESBL was 97.7% sensitive for the screening of ESBL-producing *E. coli*, *K. pneumoniae*, and *K. oxytoca*, and 86.9% specific. The low specificity was due to the growth of microorganisms resistant to broad spectrum cephalosporins that did not contain ESBL phenotype (such as AmpC or KPC production), which produced the same color pattern as ESBL producing organisms. HardyCHROM™ ESBL was 88.2% sensitive and 95.3% specific for the recovery of *Enterobacteriaceae* which were non-susceptible to 3rd generation cephalosporins. In this case, the low sensitivity was due to some *Enterobacteriaceae* species not producing a color reaction. There were 24 specimens where HardyCHROM™ ESBL recovered ESBL-producing *E. coli*, *K. pneumoniae*, and *K. oxytoca*, and 88 specimens where HardyCHROM™ ESBL recovered *Enterobacteriaceae* which are non-susceptible to 3rd generation cephalosporins, while the reference method did not.

Conclusion: HardyCHROM™ ESBL is reliable for the selective screening of ESBL-producing microorganisms, as well as for the confirmation of the presence of *Enterobacteriaceae* which are non-susceptible to 3rd generation cephalosporins.

Introduction

According to Centers for Disease Control and Prevention (CDC), Extended-Spectrum-Beta-Lactamases (ESBLs) refer to a variety of enzymes which confer resistance to third generation cephalosporins and monobactams. ESBLs are widespread among *Enterobacteriaceae* and have no effect on carbapenems or cephamycins. ESBLs are generally distinguished from AmpC type beta lactamases by their susceptibility to beta lactamase inhibitors such as clavulanic acid.

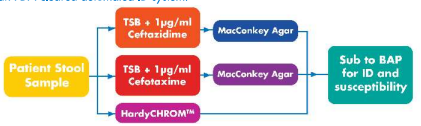
HardyCHROM™ ESBL is a selective and differential chromogenic medium containing a broad-spectrum beta-lactam intended for use as a screening medium for *K. pneumoniae*, *K. oxytoca*, and *E. coli* that produce an extended-spectrum beta-lactamase (ESBL). HardyCHROM™ ESBL can also be used as a confirmatory medium for the detection and isolation of *Enterobacteriaceae* non-susceptible to 3rd generation cephalosporins.

The selective components in HardyCHROM™ ESBL are designed to inhibit the growth of yeast, Gram-positive bacteria, and Gram-negative bacteria sensitive to broad spectrum beta-lactams (3rd generation cephalosporins). Chromogenic substrates in the medium allow for differentiation of *Enterobacteriaceae* non-susceptible to 3rd generation cephalosporins or are ESBL-producing, as bacteria that can grow and utilize the chromogens produce a colored colony. ESBL-producing *Klebsiella* spp. produce large, dark blue colonies. ESBL-producing *Escherichia coli* produce colonies that are rose to magenta in color, with darker pink centers. Other *Enterobacteriaceae* not susceptible to 3rd generation cephalosporins will produce colonies of varying size that are pink, blue, blue with pink halos, and yellow/gold colonies.

Methods

Performance of HardyCHROM™ ESBL was evaluated at three geographically diverse hospitals with fresh stool specimens. The recovery of ESBL-producing *K. pneumoniae*, *K. oxytoca*, and *E. coli* on HardyCHROM™ ESBL was compared to routine culture, defined as the selective enrichment of microorganisms in Tryptic Soy Broth (TSB) containing either 1 µg/mL ceftazidime or 1 µg/mL cefotaxime, followed by subculture to MacConkey Agar. Organisms that grew on MacConkey Agar were identified using an FDA-cleared automated ID systems. Quality control was performed in parallel every day of testing. Results from days of QC failure were excluded from the analysis. Stool specimens were kept refrigerated for a maximum of 7 days if not immediately processed.

Confirmation of ESBL production and 3rd generation cephalosporin non-susceptibility was performed using traditional Kirby-Bauer AST following the device manufacturer's instructions. ID of organisms that grew on HardyCHROM™ ESBL was confirmed using an FDA-cleared automated ID system.



Participating study sites

Wake Forest Baptist Medical Center Winston-Salem, NC	504 Total Specimens enrolled
Medical College of Wisconsin Milwaukee, WI	554 Total specimens enrolled
New York-Presbyterian/Columbia University Medical Center, New York, New York	625 Total specimens enrolled

Results

A total of 1,737 fecal specimens were tested on HardyCHROMTM ESBL in parallel with the TSB enrichments. There were 216 target ESBL organisms recovered from the reference method and 230 target ESBL organisms recovered by colony color on HardyCHROM™ ESBL after 18 hours of incubation. A total of 211 isolates were recovered by both methods simultaneously.

HardyCHROM™ ESBL used to screen for ESBL-producing *K. oxytoca*, *K. pneumoniae*, and *E. coli* vs. confirmation of ESBL-resistance from traditional culture

Morphology vs. Confirmed ESBL

Site	TP	FP	FN	TN	Sensitivity	95% CI		Specificity	95% CI	
1	48	70	1	459	98.0	89.3	99.6	86.8	83.6	89.4
2	48	67	0	564	100.0	92.6	100.0	89.4	86.7	91.6
3	115	148	4	471	96.6	91.7	98.7	76.1	72.6	79.3
Overall	211	285[1]	5[2]	1897	97.7	94.7	99.0	86.9	85.5	88.3

[1]False positives consisted of mainly 3rd generation cephalosporin non-susceptible *Enterobacteriaceae* and one Candida tropicalis species that grew as tiny pink colonies.

[2]Of the five false negatives, 3 were *E. coli* susceptible to the antimicrobial agent present in the media, but confirmed ESBL producing by other methods. Two were ESBL producing *E. coli* that grew white at 18-24 hours.

HardyCHROM™ ESBL for use in confirming the presence of 3rd generation cephalosporin non-susceptible *Enterobacteriacae* vs. confirmation of 3rd generation cephalosporin non-susceptible *Enterobacteriacae* from traditional culture.

Morphology vs. Confirmed Non-Susceptible

Site	TP	FP	FN	TN	Sensitivity	95% CI		Specificity	95% CI	
1	101	17	17	528	85.6	78.1	90.8	96.9	95.1	98.0
2	90	25	11	679	89.1	81.5	93.8	96.4	94.8	97.6
3	213	50	26	641	89.1	84.5	92.5	92.8	90.6	94.5
Overall	404	92[1]	54[2]	1848	88.2	84.9	90.9	95.3	94.2	96.1

[1]False positives consisted of 75 isolates that were not recovered by the reference method, but were recovered on HardyCHROM ESBL. The either 17 isolates were confirmed to have MICs below the breakpoints for 3rd generation cephalosporins, but above the concentration of the antimicrobial agent present in the media.

[2]Half of the false negatives were *Enterobacteriaceae* species that did not grow Pink, Blue, or Yellow/Gold on HardyCHROM ESBL. The other half were susceptible to the antimicrobial agent present in the media, but confirmed non-susceptible to other 3rd generation cephalosporins.

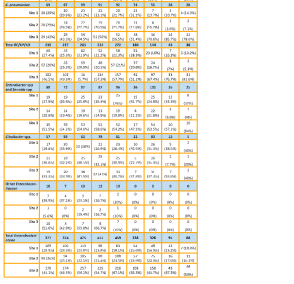
Number of confirmed positive isolates per method

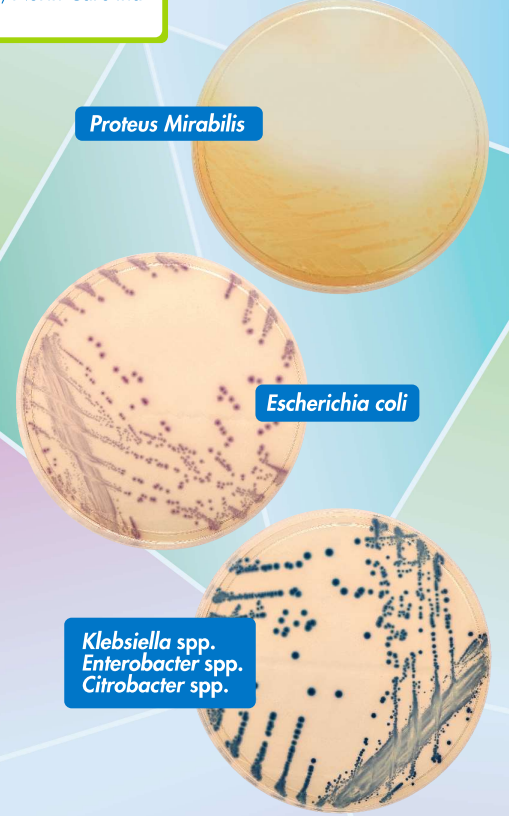
Method	ESBL isolates			3rd gen Ceph NS isolates		
	TSB + CAZ	TSB + CTX	HC ESBL	TSB +CAZ	TSB + CTX	HC ESBL
E. coli	**115**	**107**	**141**	**142**	**142**	**175**
Site 1	25 (21.7%)	22 (20.6%)	24 (17.0%)	35 (24.6%)	31 (21.8%)	34 (19.4%)
Site 2	26 (22.6%)	23 (21.5%)	26 (18.4%)	34 (23.9%)	34 (23.9%)	34 (19.4%)
Site 3	64 (55.7%)	62 (57.9%)	91 (64.5%)	73 (51.4%)	77 (54.2%)	107 (61.1%)
K. oxytoca	**6**	**6**	**7**	**8**	**8**	**9**
Site 1	4 (66.7%)	3 (50%)	4 (57.1%)	5 (62.5%)	4 (50%)	5 (55.6%)
Site 2	2 (33.3%)	2 (33.3%)	2 (28.6%)	3 (37.5%)	3 (37.5%)	3 (33.3%)
Site 3	0 (0%)	1 (16.7%)	1 (14.3%)	0 (0%)	1 (12.5%)	1 (11.1%)
K. pneumoniae	**69**	**62**	**81**	**69**	**67**	**99**
Site 1	19 (31.1%)	19 (30.6%)	21 (25.9%)	20 (29%)	20 (29.9%)	23 (23.2%)
Site 2	20 (32.8%)	18 (29.0%)	22 (27.2%)	20 (29%)	18 (26.9%)	22 (22.2%)
Site 3	22 (36.1%)	25 (40.3%)	38 (46.9%)	29 (42%)	29 (43.3%)	54 (54.5%)
Total EC/KP/KO	**182**	**175**	**229**	**219**	**217**	**283**
Site 1	48 (26.4%)	44 (25.1%)	49 (21.4%)	60 (27.4%)	55 (25.3%)	62 (21.9%)
Site 2	48 (26.4%)	43 (24.6%)	50 (21.8%)	57 (26%)	55 (25.3%)	59 (20.8%)
Site 3	86 (47.3%)	88 (50.3%)	130 (56.7%)	102 (46.5%)	107 (49.3%)	16 (5.7%)
***Enterobacter* spp and *Serratia* spp.**				**68**	**72**	**97**
Site 1				19 (27.9%)	19 (26.4%)	25 (25.8%)
Site 2				14 (20.6%)	14 (19.4%)	19 (19.6%)
Site 3				35 (51.5%)	39 (54.2%)	53 (54.6%)
***Citrobacter* spp.**				**57**	**59**	**82**
Site 1				17 (29.8%)	20 (33.9%)	23 (28%)
Site 2				21 (36.8%)	19 (32.2%)	25 (30.5%)
Site 3				19 (33.3%)	20 (33.9%)	34 (41.5%)
Other *Enterobacteriaceae*				**18**	**7**	**13**
Site 1				7 (38.9%)	4 (57.1%)	3 (23.1%)
Site 2				1 (5.6%)	0 (0%)	2 (15.4%)
Site 3				10 (55.6%)	3 (42.9%)	7 (53.8%)
Total *Enterobacteriaceae*				**377**	**374**	**475**
Site 1				109 (28.9%)	106 (28.3%)	113 (23.8%)
Site 2				98 (26%)	94 (25.1%)	105 (22.1%)
Site 3				170 (45.1%)	174 (46.5%)	257 (54.1%)

Characteristics of ESBL isolates recovered on HardyCHROM ESBL

	Method of Detection			Characteristics of Isolates recovered on HC ESBL							
	TSB + CAZ	TSB + CTX	HC ESBL	NS to CAZ	ESBL by TZ/TL	NS to CTX	ESBL by CT/CL	ESBL by both	NS to FEP	NS to FOX	NS to IPM	NS to MOX
E. coli	115	107	141	135	120	171	125	104	102	88	12	8
Site 1	25 (21.7%)	22 (20.6%)	24 (17.0%)	24 (17.8%)	22 (19.2%)	23 (13.5%)	21 (16.8%)	20 (19.2%)	25 (24.5%)	16 (27.3%)	2 (16.7%)	2 (0%)
Site 2	26 (22.6%)	23 (21.5%)	26 (18.4%)	27 (20%)	27 (22.5%)	31 (18.1%)	24 (19.2%)	19 (18.3%)	23 (22.5%)	14 (27.3%)	2 (16.7%)	0 (0%)
Site 3	64 (55.7%)	62 (57.9%)	91 (64.5%)	84 (62.2%)	71 (63.3%)	117 (68.4%)	80 (64%)	65 (62.5%)	54 (52.9%)	58 (65.2%)	8 (66.7%)	6 (100%)
K. oxytoca	6	6	7	8	5	9	6	6	4	5	3	2
Site 1	4 (66.7%)	3 (50%)	4 (57.1%)	5 (62.5%)	3 (60%)	5 (55.6%)	4 (66.7%)	4 (66.7%)	3 (75%)	3 (60%)	2 (66.7%)	1 (50%)
Site 2	2 (33.3%)	2 (33.3%)	2 (28.6%)	2 (25%)	2 (40%)	3 (33.3%)	2 (33.3%)	2 (33.3%)	1 (25%)	2 (40%)	0 (0%)	0 (0%)
Site 3	0 (0%)	1 (16.7%)	1 (14.3%)	1 (12.5%)	0 (0%)	1 (20%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	1 (33.3%)	1 (50%)
K. pneumoniae	69	62	81	90	68	91	71	58	73	50	27	27
Site 1	19 (31.1%)	19 (30.6%)	21 (25.9%)	21 (23.3%)	19 (27.9%)	20 (22%)	17 (23.9%)	15 (25.9%)	19 (26.0%)	7 (14%)	3 (11.1%)	4 (14.8%)
Site 2	20 (32.8%)	18 (29.0%)	22 (27.2%)	17 (18.9%)	18 (26.5%)	20 (22%)	16 (22.5%)	14 (24.1%)	17 (23.3%)	8 (16%)	2 (6.7%)	2 (7.4%)
Site 3	22 (36.1%)	25 (40.3%)	38 (46.9%)	50 (55.6%)	31 (45.6%)	51 (56%)	38 (53.5%)	29 (50%)	37 (49.3%)	35 (70%)	23 (85.2%)	21 (77.8%)
Total EC/KP/KO	**182**	**175**	**229**	**233**	**193**	**271**	**202**	**166**	**179**	**143**	**42**	**37**
Site 1	48 (26.4%)	44 (25.1%)	49 (21.4%)	52 (22.3%)	45 (23.3%)	58 (21.4%)	42 (20.8%)	39 (23.5%)	51 (28.5%)	26 (18.2%)	7 (16.7%)	7 (18.9%)
Site 2	48 (26.4%)	43 (24.6%)	50 (21.8%)	48 (20.6%)	41 (21.2%)	54 (19.9%)	43 (21.3%)	35 (21.1%)	37 (20.7%)	24 (16.8%)	3 (7.1%)	2 (5.4%)
Site 3	86 (47.3%)	88 (50.3%)	130 (56.7%)	133 (57.1%)	107 (55.4%)	159 (58.7%)	117 (57.9%)	92 (56.9%)	91 (50.8%)	93 (65.1%)	32 (76.2%)	28 (75.7%)

Characteristics of isolates Non-susceptible to Cephalosporins and Carbapenems recovered on HardyCHROM ESBL





Conclusions

- HardyCHROM™ ESBL showed an overall sensitivity of 97.7% and specificity of 86.9% for the detection of ESBL producing *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca*.
- HardyCHROM™ ESBL showed an overall sensitivity of 88.2% and specificity of 95.3% for the detection of *Enterobacteriaceae* non-susceptible to 3rd generation cephalosporins.
- HardyCHROM™ ESBL has shown to recover significantly larger amount of ESBL and Non-Susceptible strains in comparison to the selective enrichment methods.
- All strains recovered by HardyCHROM™ ESBL presented some degree of resistance to cephalosporins regardless of mechanism (AmpC or ESBL).
- As predicted, a higher prevalence of resistant strains were recovered from the New York City study site, including strains resistant to carbapenem which often confer co-resistance to cephalosporins.
- Based on the data from this study, HardyCHROM™ ESBL can be reliably employed as initial screen for patients harboring *Enterobacteriaceae* conferring resistance to extended-spectrum beta lactamases and 3rd generation cephalosporins.

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