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

Poster Number:

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Abstract

nd: 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 Enteropacteriaceae 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 toop, stoot specimens were inocutated onto Harray-CHROWITE 538, into 158 with 1gg/mL ceftacidime, and into 158 with 1gg/mL ceftotaxine and incubated overnight. After incubation, both TSB enrichment broths were subcultured to MacConkey agar. Backerid isolates from both reference and chromogenic agar were confirmed for identification and resistance using an FDA-deared 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 or matory brown — solution for the control of the c 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 Hardy-CHROMIM ESBL recovered ESBL-producing E. coli, K. pneumoniae, and K. oxytoca, and 88 specimens where HardyCHROMIM ESBL recovered Enterobacteriaceae which are non-susceptible to 3rd generation cephalosporins, while the reference method

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 lactama by their susceptibility to beta lactamase inhibitors such as davulanic acid.

HardvCHROM™ 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

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 syst



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

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 Hardy-CHROMT 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%	· a
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	2851	52	1897	97.7	94.7	99.0	86.9	85.5	88.3

alse positives consisted of mainly 3rd generation cephalosporin non-susceptible Enterobacteriaceae and one landida tropicalis species that grew as hiny pink colonies. Of the five false negatives, 3 were *E. coli* susceptible to the antimicrobial agent present in the media, but co firmed ESBL producing by other methods. Two were ESBL producing *E. coli* that grew white at 18-24 hours.

lardyCHROM™ ESBL for use in confirming the presence of 3rd generation cepholosporin non-interobacteriaces vs. confirmation of 3rd generation cepholosporin non-susceptible Enterobact om traditional culture.

Morphology vs. Confirmed Non-Susceptible

	Site	TP	FP	FN	TN	Sensitivity	95	% Q	Specificity	95%	q
ı	1	101	17	17	528	85.6	78.1	90.8	96.9	95.1	98.0
ı	2	90	25	11	679	89.1	81.5	93.8	96.4	94.8	97.6
ı	3	213	50	26	641	89.1	84.5	92.5	92.8	90.6	94.5
I	Overall	404	921	542	1848	88.2	84.9	90.9	95.3	94.2	96.1

h HardyCHROM ESBL. The other 17 isolates were confirmed to have M/Cs below the breakpoints for 3rd eneration cephalosporins, but above the concentration of the antimicrobial agent present in the media. Pholf 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 Number of confirmed positive isolates per method

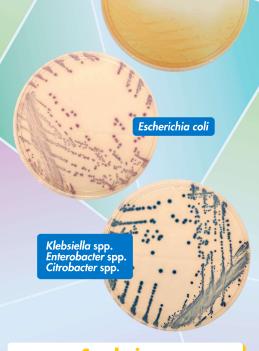
	t	SBL isolates		3 ^{rs} gen Ceph N5 isolates					
Method	TSB + CAZ	TSB + CTX	HC ESBL	TSB +CAZ	TSB + CTX	HC ESBL			
E. coli	115	107	141	142	142	175			
Site 1	25 (21.7%)	22 (20.6%)	24 (17.0%)	35 (24.6%)	31 (21.8%)	34 (19.4%			
Site 2	26 (22.6%)	23 (21.5%)	26 (18.4%)	34 (23.9%)	34 (23.9%)	34 (19.4%			
Site 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	61	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%)				
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%			

	Meth	od of De	tection		Cho	racteristi	cs of Iso	ates reco	vered on	HC ESBL		
	TS8 + CAZ	TSB + CDX	HC ESBL	NS to CAZ	1511 by 12/121	NS to CIX	ESRL by CT/CTL	ESSL by both	NS to FEP	NS to FOX	NS to IPM	NS to MEM
E. coli	115	107	141	135	120	171	125	104	102	88	12	- 8
Site 1	25 (21.7%)	120.6%	(17.0%)	26 (19.3%)	(19.2%)	(19.3%)	(16.8%)	20 [19.2%]	25 (24.5%)	[27.3%]	กล้าย	10%)
Site 2	(22.6%)	23 (21.5%)	26 (18.4%)	27 (20%)	21 (17.5%)	34 (19.9%)	24 (19.2%)	[18.3%]	23 (22.5%)	(27.3%)	(16.7%)	(03)
Site 3	(55.7%)	62 (57.9%)	91 (64.5%)	(60.7%)	76 (63.3%)	104 (60:8%)	90 [64%]	62.5%)	(52.9%)	60 (68.2%)	(66.7%)	(100h
К. ахучеса	6	6	7	8	5	9	6	6	- 4	5	3	2
Site 1	(66.7%)	(50%)	(57.1%)	62.5%I	(60%)	(55.6%)	(66.7%)	166.7%	(75%)	(40%)	(66.7%)	150%
Site 2	(33.3%)	33.3%	(28.6%)	125%I	(40%)	3 [33.3%]	(33.3%)	(33.3%)	(25%)	2 40%	(0)(1	(031)
Site 3		[16.7%]	(14.334)	(12.5%)	0 (0%)	(20%)	10%)	(0,°)	[035] 0	1 [20%]	1 [33.33]	J50%
K. pneumonios	61	62	81	90	48	91	71	58	73	50	27	27
Site 1	[31.13]	(30.6%)	(25.9%)	21 (23.3%)	(27.9%)	(22%)	123.9%)	15 (25.9%)	(31.5%)	(14%)	(11.1%)	(14.8)
Site 2	20 [32.8%]	18 (29.0%)	(27.2%)	121.133	18 (26.5%)	20	(26.8%)	[25.9%)	13 [17.8%]	(16%)	13.2%	17.4%
Site 3	22 [36.1%]	25 (40.3%)	39 (46.9%)	50 [55,6%)	31 (45.6%)	51 [56%]	35 (49.3%)	28 (48.3%)	(50.7%)	35 (70%)	(85.2%)	21 (77.81
Total EC/KP/KO	182	175	229	233	193	271	202	168	179	143	42	37
Site 1	(26.4%)	44 (25.1%)	49 (21,4%)	52 (22.3%)	(23.3%)	(21,4%)	(20.8%)	39 [23.2%]	51 [28.5%)	(16.1%)	116,7%	(13.5)
Site 2	48 (26.4%)	43 (24.6%)	50 (21.8%)	48 (20.6%)	41 [21.2%]	57 (21%)	(22.3%)	36 (21.4%)	37 (20.7%)	24 (16.8%)	(7.1%)	15.4%
Site 3	(47.3%)	88 (50.3%)	130 (56.7%)	133	107	156 (57.69)	(56.9%)	93 (55.4%)	91 (50.8%)	(67,1%)	32 (76.2%)	20 (81.1)

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

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Proteus Mirabilis



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 axytoca
- HardyCHROM™ ESBL showed an overall sensitivity of 88.2% and specificity of 95.3% for the detection of Enteropacteriaceae non-susceptible to 3rd generation
- HardyCHROM™ ESBL has shown to recover significantly larger amount of ESBL and Non-Susceptible strains in comparison to the selective enrichment method
- 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 ofter 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 resiste extended-spectrum beta lactamases and 3rd generation cephalosporins.