Phenotypic Screen for ESBL-producing *Enterobacteriaceae* in the US Hospital Setting: Validation of CHROMagar ESBL versus HardyCHROM ESBL

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Introduction

Nearly 19% of healthcare-associated *Enterobacteriaceae* infections in the U.S are due to Extended Spectrum Beta Lactamase (ESBL)-producing strains.[1] Patients with a bloodstream infection (BSI) caused by ESBL-producers have longer hospital stays, higher exposure to improper antibiotic use, and are at a greater risk of mortality compared with BSI caused by non-ESBL *Enterobacteriaceae*.[1,3] Gastrointestinal colonization with an ESBL has been identified as a significant risk factor for subsequent infection with an ESBL-producing organism[3].

In the healthcare setting, guidelines for ESBL surveillance and how to handle patients with known ESBL infection/colonization upon admission are not uniform[2]. At the NIH Clinical Center, patients from whom an ESBL has been isolated are placed on contact isolation; and there is currently no active surveillance procedure in place to remove these patients from isolation or to assess carriage in new inpatients. The aim of this study was to develop a method for ESBL surveillance direct from perirectal swabs, and to compare the performance of two chromogenic agars available in the US: CHROMagar™ ESBL (CESBL) and HardyChrom™ ESBL (HESBL) (Fig. 1) . This study was conducted in three phases:

- Phase I: Performance of CESBL and HESBL using characterized Cephalosporin susceptible or resistant isolates
- Phase II: Performance of CESBL and HESBL using perirectal swabs from previously positive ESBL patients
- Phase III: Real-time validation of CESBL and HESBL during a whole-house perirectal surveillance.

Phase I: Characterization of Described Isolates

Table 1. Phenotypic Characterization of Isolates with Known Antimicrobial Susceptibility

	CHROMagar ESBL		HardyCHROM ESBL	
Organism & Susceptibility	**24h**	**48h**	**24h**	**48h**
Escherichia coli				
non-ESBL	--	--	--	--
ESBL	PINK	PINK	PINK	PINK
Klebsiella pneumoniae				
non-ESBL	--	--	--	--
ESBL	BLUE	BLUE	BLUE	BLUE
Proteus mirabilis				
Sensitive	--	--	--	--
Resistant	--	--	--	--
Enterobacter cloacae complex				
Sensitive	BLUE	BLUE	BLUE	BLUE
Resistant	BLUE	BLUE	BLUE	BLUE
Citrobacter freundii complex				
Sensitive	--	--	BLUE*	BLUE*
Resistant	BLUE	BLUE	BLUE	BLUE
Morganella morganii				
Sensitive	--	--	--	--
Resistant	--	--	--	--
Pseudomonas aeruginosa				
Sensitive	--	--	--	--
Resistant	YELLOW	YELLOW	OPAQUE	OPAQUE
Acinetobacter sp.				
Sensitive	--	--	CREAM**	CREAM**
Resistant	CREAM	CREAM	CREAM	CREAM

ESBL – Extended Spectrum Beta Lactamase; non-ESBL – susceptible to all 3rd generation cephalosporins; Sensitive –all susceptible to all 3rd generation cephalosporins; Resistant – resistant to at least two 3rd generation Cephalosporins *four colonies, **seven colonies

Phase I: Description of Study

10µL of a 0.5 McFarland suspension of each organism was plated onto CESBL, HESBL, and sheep blood agar (SB) as control. Plates were incubated at 35°C, 5% CO_2 and evaluated at 24 and 48h for growth and pigmentation.

Phase II: Screen of Previous ESBL-Positive Patients

Table 2. Summary of Phase II Study, per patient specimen

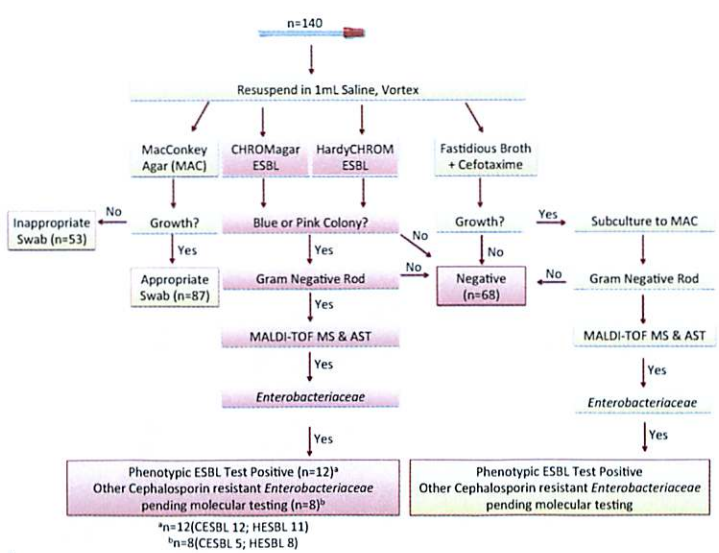
	Total	CESBL	HESBL
Total Swabs Evaluated (# patients = 20)	42		
Negative Swabs (# patients = 11)	18		
Positives Swabs (# patients = 13)	24		
Swabs with Confirmed ESBL (# patients = 12)	20		
Confirmed ESBL Organisms in this Cohort	20	18	17
Possible ESBL Organisms for Molecular Confirmation*	5	2	4

*Resistant to at least two 3rd generation cephalosporins

Phase II: Description of Study

For Phase II, discarded perirectal swabs (paired swabs <8 days old) from previously positive ESBL patients were resuspended in 1mL saline. Two drops were placed in fastidious broth containing 4µg/mL Cefotaxime (FBCEF), CESBL, and HESBL. After overnight incubation at 35°C, 5% CO_2, turbid FBCEFs were subcultured onto MacConkey agar (FBCEF-MAC). Growth and colony pigmentation of CESBL/HESBL cultures were documented, and gram negative rods (GNRs) from blue or pink colonies were identified by MALDI-TOF MS (Bruker Daltonics) and antimicrobial susceptibility testing (AST) was performed (BD Phoenix) which included an on-board phenotypic clavulanic acid ESBL screen. All colonies growing from FBCEF-MAC were identified and AST was performed; FBCEF-MAC findings served as the gold standard for our studies.

Overview of Phase III: Clinical Screen



Phase III: Clinical Screen

Table 3. Summary of Phase III Study, per patient specimen

	Total	CESBL	HESBL
Total Swabs Evaluated (# patients = 125)	140		
Total Inappropriate Swabs (# patients = 51)	53		
Total Appropriate Swabs (# patients = 79)	87		
True Negatives	68		
Positive Swabs	17		
Swabs Not Included*	2		
Swabs with Confirmed ESBL (# patients = 8)	9		
Confirmed ESBL Organisms in this Cohort	12	12	11
Possible ESBL Organisms for Molecular Confirmation**	8	5	8

*potential contamination, **Resistant to at least two 3rd generation cephalosporins

Phase III: Description of Study

For Phase III, perirectal swabs submitted for routine whole-house surveillance were resuspended in 1mL saline. Two drops were placed in FBCEF, CESBL, HESBL, and MacConkey agar (MAC) to control for appropriate specimen collection. Isolates were worked up as described in Phase II. Non-pigmented colonies representative of *Pseudomonas* sp. and *Acinetobacter* sp. were not further characterized. FBCEF-MAC served as the experimental gold standard.

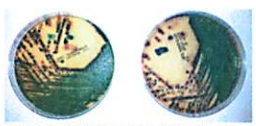


Figure 1. Paired perirectal culture on CHROMagar ESBL (left) and HardyCHROM ESBL (right). *Klebsiella pneumoniae* (blue), *Escherichia coli* (pink).

Conclusions

- Our preliminary findings do not show significant differences in performance of either CESBL or HESBL; however, further analysis on a large cohort of specimens is ongoing.
- Molecular detection of ESBLs are pending for 5 isolates from Phase II, and 8 isolates from Phase III. These isolates include cephalosporin resistant *Enterobacter* & *Citrobacter* sp.
- A surprising number of "inappropriate swabs" were generated during Phase III clinical surveillance (53/140, 37.9%), and may indicate a need for increased education regarding specimen collection technique.
- Additionally, we are investigating the utility of ESBL chromogenic agar for the pre-emptive detection of ESBL-colonization as a tool to direct early empiric therapy in cases of subsequent infection.
- ESBL media may be useful for proof of decolonization to remove patients from prolonged isolation. (Colonization with an ESBL-producing organism not detected in 8 previously positive ESBL patients in Phase II.)

References
[1] Antibiotic Resistance Threats in the United States, 2013. Centers for Disease Control and Prevention.
[2] Woerther, P.-L. et al., 2013. Clin Microbiol Rev, 26(4):744-758.
[3] Reddy, P. et al., 2007. Clin Infect Dis, 45:846-852.

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