Evaluation of HardyCHROM™ ESBL as a detection method of ESBL-producing Enterobacteriaceae, 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 isolates from K. pneumoniae/K. oxytoca. The medium can also be used to recover and identify 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, TSB with 1 mg/l ampicillin, and onto TSB with 1 mg/l ampicillin and incubated overnight. After incubation, both TSB enrichment broth were subcultured to MacConkey agar. Bacterial isolates from both reference and chromogenic agar were confirmed for identification and resistance using an FDA cleared system. Coli recommendations were used for susceptibility result interpretation.

Results: A total of 1,727 fecal specimens were tested on HardyCHROM™ ESBL in parallel with the TSB enrichment. 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 but did not contain ESBL genes (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 the case, the low sensitivity was due to some Enterobacteriaceae species not producing a color reaction. There were 26 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.

Conclusions: 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.

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 identification of organisms in MacConkey Yeast Broth (MYB) broth containing either 1 µg/ml ampicillin or 1 µg/ml ampicillin, followed by subculture to MacConkey agar. Organisms that grew on MacConkey Agar were confirmed using an FDA cleared automated ID system. 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.

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

Results

A total of 1,727 fecal specimens were tested on HardyCHROM™ ESBL in parallel with the TSB enrichment. 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 E. coli, K. pneumoniae, and K. oxytoca. 211 of 230 (91.7%) target ESBL organisms recovered from the reference method and 230 target ESBL organisms recovered by colony color on HardyCHROM™ ESBL were compared to routine culture, defined as the identification of organisms in MacConkey Yeast Broth (MYB) broth containing either 1 µg/ml ampicillin or 1 µg/ml ampicillin, followed by subculture to MacConkey agar. Organisms that grew on MacConkey Agar were confirmed using an FDA cleared automated ID system.

<table>
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<th>Method</th>
<th>TSB + Amp</th>
<th>TSB + Amp</th>
<th>MYB + Amp</th>
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<tr>
<td>K. oxytoca</td>
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</table>

Total EC/KP/KO 182 175 229 219 217 283

Conclusions

- HardyCHROM™ ESBL showed an overall sensitivity of 97.7% and specificity of 86.9% for the detection of ESBL producing Enterobacteriaceae, 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 carbapenems which often owing to 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.